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Abstracts
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WS01 – ALLERGY AND ASTHMA

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425 – WS01.1

IL-12 expression on allergen-containing VNP prevents Th2 induction in a humanized mouse model of mugwort allergy

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Purpose: Virus-like nanoparticles (VNP) can be loaded with allergens that are safely shielded inside them to achieve allergen-specific T-cell activation in the absence of sensitization. In addition, VNP can be decorated with functional cytokines on their surface. We have analyzed whether a combination of both approaches, i.e., surface decoration of allergen-laden VNP with IL-12, would improve their immunomodulatory properties.
Methods: The major mugwort pollen allergen Art v 1 was N-terminally fused to the viral matrix protein MAp15 of MoMLV for shielded expression, while the single-chain (p35::p40) mIL-12 was C-terminally fused to the minimal CD16b GPI-anchor acceptor sequence for surface expression. The immunomodulatory capacity of such VNP was analyzed with the help of a mugwort allergen-specific humanized mouse model *in vitro* and *in vivo*.
Results: Stimulation of splenocyte cultures of humanized allergy mice with allergen-expressing IL-12$^+$VNP induced the secretion of high levels of IFN-g, moderate levels of IL-10, and very low levels of IL-4, IL-5, IL-13 and IL-17A compared to recombinant allergen. This was accompanied by moderately inhibited T-cell proliferation but a significantly increased proportion of IFN-g$^+$CD4$^+$T-cells, which additionally co-expressed IL-10, indicative of the induction of a Th1/Tr1 T-cell response. To mimic a prophylactic approach *in vitro*, sorted naïve allergen-specific T-cells from humanized mice were incubated with allergen-expressing IL-12$^+$VNP in the presence of bone marrow-derived dendritic cells, resulting in a high proportion of IFN-g$^+$CD4$^+$Th1-cells, similarly obtained upon classical Th1 polarization. The IL-12-VNP-induced Th1-cells remained stable after secondary stimulation with recombinant allergen, demonstrating their strong and sustained polarization. The addition of IL-12$^+$VNP to pre-polarized Th2-cells (IL-4 and anti-IL-12/anti-IFN-g mAb) strongly inhibited the further expansion of allergen-specific IL-13$^+$Th2-cells *in vitro*. *In vivo*, prophylactic intranasal treatment with IL-12-decorated allergen-laden VNP before exposure to allergen aerosol as acute challenge reduced the expansion of IL-4$^+$ and IL-13$^+$CD4$^+$T-cells compared to control VNP decorated with an inactive form of IL-12.
Conclusion: Decoration of allergen-laden VNP with IL-12 improves their Th1-priming capabilities and therefore could be a useful new tool for the treatment of allergies in the future.

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1671 – WS01.2

Development of an in vivo model of IgE-mediated allergy

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Mast cells are central in IgE-mediated allergies. These hematopoietic cells reside in barrier tissues and bind allergen-specific IgE antibodies *via* the high-affinity Fc epsilon receptor (FcεRI) on their surface. Cross-linking of the receptor by IgE-binding to allergen induces their degranulation and the release of various factors, e.g. histamine, that cause allergic reactions. We sought to establish an *in vivo* model of IgE-mediated allergy by engrafting non-obese diabetic severely immunodeficient $\gamma c^{-/-}$ mice transgenic for human membrane-bound stem cell factor [NSG-Tg(hu-mSCF)] with human stem cells (HSC) in order to develop human mast cells.
$CD34^+$ HSC were isolated from cord blood mononuclear cells (CBMC) by positive selection with magnetic beads. Their purity and viability were assessed by flow cytometry. HSC were intravenously (i.v.) injected in NSG-Tg(hu-mSCF) mice. The percentage of human (hu) $CD45^+$ cells in blood was assessed by flow cytometry every 4-weeks. After 16-weeks, $huCD45^+$ cells and $Fc\varepsilon RI^+CD117^+$ mast cells were stained in bone marrow, peritoneal lavage, lung, and spleen. A serum pool from birch pollen allergic donors (50µl/mouse) containing >100 kU_A/ml Bet v 1-specific IgE was subcutaneously injected. After two-days, mice received intracutaneous injections of 30µl PBS containing recombinant (r) Bet v 1 in the belly skin and ears. Allergic reactions were detected by i.v. injection of Evans blue (0.5%) and local visualization, anaphylactic reactions by core body temperature measurements.
CBMC contained 0.52% HSC on average, which were enriched to >95% $CD34^+$ cells and injected ($0.5\text{-}1x10^5$) per NSG-Tg(hu-mSCF) mouse. The percentage of $huCD45^+$ cells in blood constantly increased. After 16-weeks, 55±4% (SEM) of alive $huCD45^+$ cells were found in bone marrow. At the same time, 19±4% of $Fc\varepsilon RI^+CD117^+huCD45^+$ cells were detected in bone marrow and peritoneal lavage, followed by 18±6% and 5±1% in lungs and spleen, respectively. NSG-Tg(hu-mSCF) mice passively sensitized with birch pollen-specific IgE reacted anaphylactic to 0.5µg rBet v 1 but not to PBS.
NSG-Tg(hu-mSCF) mice injected with HSC developed mast cells and anaphylactic reactions to allergen after sensitization with human allergen-specific IgE. This *in vivo* model of IgE-mediated allergy will now be used to study agents that block anaphylactic reactions.

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2131 – WS01.3

Impact of micro- and nanoplastic particles on allergic responses in BALB/c mice

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Abundant micro- and nano-plastic particles (MNPs) in the environment raise concerns about potential impacts on human health. Our study aimed to investigate the influence of MNPs on allergic responses, which might relate to the rising incidence and prevalence of allergic diseases over recent decades. We conducted experiments utilizing BALB/c mice co-administered intranasal ragweed pollen along with either manufactured polyethylene terephthalate (PET) or polypropylene (PP) MNP spheres (1-25 µm) to test our hypothesis. Assessments included evaluating allergen-specific responses such as airway inflammation, mucus secretion in the respiratory tract, and levels of allergen-specific immunoglobulin in sera. Our results revealed distinct effects of different MNPs on allergic responses. Specifically, PP MNPs exacerbated ragweed pollen-induced airway inflammation, indicating an adjuvant-like activity. In contrast, PET MNPs demonstrated an unexpected immunosuppressive effect, as evidenced by reductions in airway inflammation, mucus production, and allergen-specific immunoglobulin levels. These findings shed light on the potential divergent roles of PP and PET MNPs in modulating allergic responses, suggesting a need for further mechanistic investigations. Understanding the underlying mechanisms will be critical for elucidating the complex interplay between MNPs and allergic diseases, informing strategies for mitigating their adverse effects on human health.

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949 – WS01.4

Eosinophil responses in adaptive pulmonary type 2 immunity is locally regulated by interferon type 1 and -2

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Purpose: Eosinophils are considered terminally differentiated innate effector cells associated with both homeostatic and pathogenic immune responses. It is currently unclear how eosinophil function is shaped within the tissue, especially in eosinophilic afflictions like allergic asthma. Indeed, eosinophil-intrinsic decision-making during allergic inflammation is completely understudied and might explain their role in common acute allergic exacerbations to allergens and viruses.
Results: Surprisingly, we find that interferons intrinsically shape the function of eosinophils in type 2 immunity in the airways. Specifically, eosinophils infiltrating the lung during HDM exacerbations directly respond to interferon (IFN)α, resulting in robust ISG-transcriptomes and membrane expression of the type 1 interferon-sensitive markers. In fact, using interferon-reporter mice we show the local transcriptional reactivity of eosinophils in response to IFNα. This response is required for eosinophils to simultaneously respond to IFNγ, locally produced by activated NK cells under the control of the IL18-IL18BP-IL18R axis. Eosinophil-specific transgenic mice and BM chimera showed the intrinsic requirement of IFNAR1, IFNGR1 and STAT1 for this response. Importantly, the time-dependent dual interferon requirement of eosinophils is conserved in mice and humans, *in vitro* and *in vivo*. MultiNicheNet analysis on CITEseq data from the lungs of eosinophil-specific IFNAR1- or IFNGR1-KO mice showed the role of IFN in modulating eosinophil-mediated airway inflammation.
Conclusion: We find the surprising role of interferons in shaping the phenotype and function of eosinophils during acute allergic exacerbations. Indeed, type 2 inflammation in response to HDM allergen is preceded by an interferon-dominant type 1 immune response in the airways, which modulates local eosinophil responses. This novel interferon circuitry will advance our understanding of eosinophil functioning during asthma exacerbations, both allergic (HDM) and virally induced.

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1611 – WS01.5

Heterogeneous ILC responses in severe asthma patients undergoing anti-IL5/5Rα therapy

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Purpose: Historically, asthma has been defined as a type 2-driven disease. However, severe asthma (SA) is heterogeneous with additional non-type 2 features. Innate lymphoid cells (ILCs) are early effector cells with crucial roles in tissue homeostasis, yet ILC2s are also implicated in asthma pathology. There is limited understanding of the role of additional ILC subsets in SA. While type 2 biologics are effective at ameliorating disease in subsets of asthma patients, little is known regarding their impact upon ILC populations. This study sought to exhaustively characterise ILCs in SA relative to healthy controls (HC), and infer the impact of anti-IL5/5Rα therapy on these populations.
Methods: Patient demographics, spirometry and FeNO were prospectively collected with blood and induced sputum from HCs (n=20) and SA subjects pre and post-establishment on benralizumab/mepolizumab (n=26) for ≥3 months. 21-colour full-spectrum flow cytometry was used for ILC evaluation and proteomic profiling performed via the Olink Inflammation assay.
Results: SA was associated with increased ILC progenitors (ILCPs) in the blood and sputum, as well as elevated T-bet$^+$ ILC1s, GATA-3$^+$ ILC2s (including those with an IL-13$^+$ and c-Kit$^+$ pathological phenotype) and RORγt$^+$ ILC3s within the airways only of SA subjects. Moreover, increased sputum ILC1 and ILC2 populations correlated with clinical outcomes of disease (Asthma control questionnaire-7 score and FEV_1 % predicted). Anti-IL5/5Rα therapy did not alter ILC subset numbers in the blood or sputum, however there was a reduction in ILCs displaying type 2 features within the airways. Proteomic profiling highlighted a significant positive correlation between total ILCs within the airways of SA subjects and expression of type 2-associated cytokines, with all correlations absent following anti-IL5/5Rα therapy.
Conclusion: SA subjects exhibit a multifaceted ILC subset response in their airways that correlates with type 2 cytokines and clinical prognosis. Importantly, pathogenic airways ILC2 responses are selectively dampened following anti-IL5/5Rα therapy, perhaps contributing to the capacity of these biologics to ameliorate eosinophilic inflammation and instigate disease control.

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296 – WS01.6

Selective elimination of allergen-specific B cells in house dust mites allergy model

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Purpose: House dust mites (HDM) allergy is widely spread and over 50% of asthmatic individuals are sensitized to its allergens. Der p1 is one of the major allergens from the house dust mite *Dermatophagoides pteronyssinus* (Dpt). Thus Der p1-specific B cells play an important role in pathology as a producer of allergen-specific IgE antibodies and the specific elimination of these cells is a legitimate goal for ameliorating the allergic symptoms.
The targeted suppression of allergen-specific B cells could be accomplished by co-crosslinking of the human inhibitory Complement receptor 1 (CR1) and B cell receptor (BCR). This mechanism could be achieved by generation of protein-engineered chimeric molecules consisting of 3D9 monoclonal antibody against CR1 conjugated with proven epitope-carrying peptides from Der p1 molecule.
Methods: protein engineering, FACS, animal models, histology, ELISA
Results: The effectiveness of the chimeric molecule was assessed in a humanized mouse HDM allergy model by transferring human peripheral blood mononuclear cells (PBMCs) isolated from patients diagnosed with HDM allergy in immunodeficient Rag2- γc- mice. Intravenous injection of the chimeric molecule led to a reduction of Dpt-specific IgE antibodies in the serum and bronchoalveolar lavage fluid (BALF) compared to the control chimeric molecule consisting of the same antibody coupled with irrelevant peptide. The total protein level in BALF, as a marker of vascular permeability and the mast cell degranulation in the lungs evaluated by measuring β-hexosaminidase activity in BALF, were significantly reduced. Phenotyping of the lung cells revealed a tendency for reduction of the percentage of human CD45+ and CD4+ cells and increasing of CD8+ cells in the animals treated with chimera. Histology of mouse lungs showed decreased perivascular infiltration.
Conclusion: The present study proposed a new approach for the therapy of HDM allergy which aims specific elimination of allergen-specific B cells. Treatment with the protein-engineered chimeric molecules resulted in a reduction of Dpt-specific IgE antibodies and overall allergic inflammation. This effective approach could be further developed into a therapeutic agent for the treatment of patients and can help for a better understanding of the role of B cells in HDM allergy.

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WS02 – INNATE IMMUNE TRAINING

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1869 – WS02.1

Neutrophil-specific targeting of STAT3 impairs tumor progression via the expansion of cytotoxic CD8+ T cells

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Purpose: Neutrophils have been shown to play heterogeneous roles during cancer progression. However, in the clinical situation these cells are mainly associated with tumor progression and worse patient survival. Therefore, multiple pre-clinical and clinical trials targeting neutrophil migration or activity are ongoing, but without visible success to date. Previously, we and others could show that elevated STAT3 is associated with pro-tumoral activity of neutrophils. Here, we addressed the possibility to target STAT3 in neutrophils to provide therapeutic opportunity in cancer.
Methods: We have created neutrophil-specific Stat3 knockout mice (NStat3$^{-/-}$) and injected them with murine head and neck cancer (HNC) and melanoma cell lines, MOPC and B16, respectively. Tumor growth, progression and metastasis, anti-tumor T cell response, as well as basic and tumor-related neutrophil functions, were evaluated.
Results: We demonstrated that deletion of STAT3 in neutrophil-specific manner results in significantly impaired tumor growth and metastasis in both HNC and melanoma cancer models. Neutrophils from NStat3$^{-/-}$mice showed a robust bias towards anti-tumoral phenotype, with upregulation of immune co-stimulatory molecules. Multi-parameter analyses of tumors and TDLNs revealed significant expansion of strongly cytotoxic CD8^{+} T cell populations, but hardly affected CD4^{+} cells. Inhibiting STAT3 phosphorylation in neutrophils using a small molecule inhibitor induced the expansion and activation of tumor cytotoxic CD8^{+} T cells in patient-derived tumor explants (HNC) when treated with such neutrophils, but not in those treated with STAT3 sufficient cells. Moreover, targeting neutrophil-STAT3 *in vivo* with intratumoral injection of STAT3 antisense oligonucleotide (STAT3ASO) impaired the tumor growth and elevated cytotoxic T cell activity in mice bearing HNC.
Conclusion: These findings provide new insights in therapeutic avenues and suggest targeted neutrophil-STAT3 inhibition as a potential immunotherapy in cancer patients.

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891 – WS02.2

Pharmacological activation of free fatty acid receptor 2 in intestinal type 3 innate lymphoid cells ameliorates experimental autoimmune encephalomyelitis

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Innate lymphoid cells type 3 (ILC3) play a central role in maintaining intestinal homeostasis and regulating the balance between effector T cell populations and regulatory T cells (Treg) in the gut milieu. Dysregulation of this balance, which is strongly influenced by the gut microbiota and dietary components, is associated with autoimmune diseases such as multiple sclerosis (MS). Intestinal ILC3 are activated by dietary compounds like short-chain fatty acids (SCFA), which are produced by gut bacteria. SCFA, acting via the free fatty acid receptor 2 (FFAR2), stimulate ILC3 proliferation, IL-22 and IL-2 production in the small intestine. The secretion of IL-22 by ILC3 supports the integrity of the intestinal barrier and the balance of the immune system, while IL-2 enhances the activity of Treg and thus improves immune regulation in the intestine.
In this study, the efficacy of a FFAR2 agonist - Cpd1 as evaluated in a mouse model of chronic experimental autoimmune encephalomyelitis (EAE). Treatment with Cpd1 efficiently ameliorated EAE and showed significant attenuation of CNS inflammation and lessened infiltration of immune cells into the spinal cord. Flow cytometric analysis of immune cells in the spinal cord showed that the number of $CD4^+$ T cells, Th1, and Th17 cells decreased with Cpd1 treatment. In addition, Cpd1 treatment in EAE mice led to changes in the composition of immune cells in the lamina propria of the small intestine, which was characterized by an increased proportion of Treg and IL-22-producing ILC3 and a decrease in IL-17-expressing ILC3, while there was no difference in the proportion of IL-2-producing ILC3 cells between Cpd1-treated and control mice. Consistent with this, Cpd1 treatment also altered the composition of the microbiota in the EAE model.
In summary, these data indicate that activation of immune-regulatory, gut-resident ILC3 cells by FFAR2 agonists modulates the autoimmune response local to the small intestine as well as in distal tissues such as the spinal cord. These findings highlight the potential therapeutic use of FFAR2 agonists in the treatment of autoimmune diseases.

*Cpd1: compound 1 in patent no. WO 2011/073376 A1
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1995 – WS02.3

Early measles immunization in children before 12 month of age affects specific memory B cell responses

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Purpose: Children vaccinated against measles under 12 months of age show a reduced antibody response and waning of antibodies in the long-term. Whether this implies the loss of immune memory remains to be determined. We aim to investigate the presence and function of measles-specific memory B cells in such early vaccinated children.
Methods: Our study involves children that received an additional early dose of measles vaccine between 6 and 12 months alongside the routine vaccination at 14 months of age, and compared this to children who only received the routine vaccination at 14 months. Measles-specific neutralizing antibody concentrations were assessed up to 7 years post vaccination, to understand the influence of age at first vaccination on antibody persistence. To investigate effects on immune memory, we isolated B lymphocytes from blood and expanded them in vitro to generate antibody-producing plasma cells from single memory precursors. In addition to frequencies of measles-specific memory B cells, the functionality, avidity, and epitope specificity of these measles-specific antibodies at the long-term, up to 3 years after vaccination, are currently investigated.
Results: Measles-specific neutralizing antibody data indicated a negative correlation between the age at first vaccination and antibody concentrations later in life. Around 6-7 years of age, a considerable proportion of early-vaccinated children exhibited antibody concentrations which are considered below the level of protection. Preliminary analysis of memory B cells indicate lower frequencies of measles-specific B cells in the circulation. Further characterization of the specificity and function of the antibodies produced from these memory B cells is ongoing. This will provide better insight into the effect of early immunization with live attenuated measles vaccine on the development and maintenance of immune memory.

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1294 – WS02.4

IFN-γ induced trained immunity metabolically reprograms macrophage metabolism and promotes immunity to Mycobacterium tuberculosis.

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Background: Tuberculosis (TB) is a leading cause of death worldwide and a major global health concern due to increased incidence of multi-drug resistant infections. Host-directed therapies (HDT) are therapies which harness a patient's own immune system to fight infection, to support the efficacy of antibiotics. Trained immunity is a functional reprogramming of innate immune system whereby myeloid cells are metabolically and epigenetically reprogrammed, resulting in heightened responses to infection. This work aimed to determine if IFN-γ could induce trained immunity in human monocytes and airway macrophages and assess its potential as a HDT for TB.
Methods: We aimed to assess if a single exposure to IFN-γ could metabolically reprogram human monocytes or airway macrophages to induce trained immunity resulting in enhanced response to challenge with *Mycobacterium tuberculosis* (*M.tb)*. Monocytes were enriched from PBMC, or airway macrophages were isolated from bronchioalveolar lavage fluid (BALF), trained with IFN-γ for 24 hours, washed and allowed return to homeostasis and rest. On day 6, metabolic reprogramming was analysed using Seahorse extracellular flux analysis and by examining changes in the gene expression of key glycolytic enzymes following challenge with *M.tb*. Trained macrophages were stimulated for 24 hours with *M.tb* and cytokine and chemokine production, and the expression of cell surface markers associated with trained immunity were assessed.
Results: Macrophages trained with IFN-γ had an increased glycolysis and produced more IL-1β upon stimulation with *M.tb*, compared with untrained controls. Trained macrophages also showed increased expression of glycolytic enzymes, supporting the evidence that metabolic rewiring had occurred. Functionally, IFN-γ trained macrophages produced higher concentrations of TNF, IL-6, CXCL1, MIP-1α and IL-10 when stimulated with *M.tb*.
Conclusion: IFN-γ can induce trained immunity in human macrophages resulting in enhanced glycolytic metabolism and cytokine production which are associated with better *M.tb* clearance. Inhalable IFN-γ may therefore be beneficial as HDT for people in high-risk settings or in individuals at risk of recurrent TB disease.

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471 – WS02.5

Trained immunity drives neutrophil reprogramming providing protection against pneumococcal sepsis

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Purpose: Trained immunity reflects the capacity of the innate immune system to adapt to an initial challenge and mount an improved response to a secondary challenge. Trained immunity is associated with metabolic, epigenetic and functional reprogramming of stromal cells and myeloid cells. We reported that trained immunity protects mice from a wide range of bacterial infections. Surprisingly, while neutrophils play a key role in host defences, their role during training is poorly understood. We aimed to determine whether neutrophils are reprogrammed during training and to assess their role during streptococcal pneumoniae.
Methods: Mice were challenged with PBS (control) or β-glucan (training) given intraperitoneally. After one week, mice were sacrificed to collect organs and cells, or were challenged intranasally with *Streptococcus pneumoniae* with or without control or trained neutrophils. Control and trained neutrophils were labelled and co-injected intravenously in mice challenged intranasally with lipopolysaccharide (LPS). Samples were analysed by flow cytometry, scRNAseq and multiplex bead assay. Mouse morbidity and mortality were registered daily.
Results: Training increased bone marrow myeloid stem and progenitor cells, and immature and mature neutrophils in bone marrow, blood, spleen and lungs ($P<0.001$). scRNAseq pseudobulk analyses revealed an alteration of neutrophil developmental subsets in the bone marrow and spleen. The transcriptome of trained neutrophils was enriched in gene pathways related to cellular effector functions. In agreement, trained neutrophils showed increased *S. pneumoniae*-induced phagocytosis and cytokine production, and chemotaxis towards CXCL2 ($P<0.05$). Trained neutrophils migrated to a greater extent than control neutrophils in the lungs of LPS-challenged mice ($P<0.01$). Training decreased bacteremia and increased survival of mice with lethal pneumococcal pneumonia ($P<0.0001$). The protection was lost upon neutrophil depletion (0.0% *vs* 87.5% survival in neutrophil-depleted *vs* neutrophil-non-depleted trained mice, $P<0.001$). The adoptive transfer of trained neutrophils to naive mice increased their resistance to *S. pneumoniae* infection.
Conclusion: Training triggered central and extramedullary hematopoiesis and elicited a marked rewiring of the neutrophil compartment. Trained neutrophils had enhanced chemotactic and antibacterial functions and played a key role in protecting against lethal pneumococcal pneumonia. The molecular mechanisms underlying neutrophil reprogramming are under investigation.

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2210 – WS02.6

Sepsis-trained macrophages promote anti-tumoral tissue-resident T cells

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Purpose: Sepsis induces immune alterations, which last for months after the cure. The impact of this immunological reprogramming on the risk of developing cancer remains unclear.
Methods: We use scRNA-seq dataset from a publicly available database of immune cells from bronchoalveolar lavages of moderate or severe COVID-19 patients, RNA-seq datasets from publicly available databases of human tissue-resident T cells sorted from lung tumors or the dermis, national claims database, well-defined mouse model of lung metastasis and skin metastasis, adoptive transfer of immune cells into the lungs and high-throughput scRNAseq, TCRseq and ChIPseq analysis of the immune cells from murine lungs.
Results: Using a national claims database, we observed that sepsis survivors had a lower cumulative incidence of cancers than matched controls. We identified a chemokine network released from sepsis-trained resident macrophages that triggers tissue residency of T cells via CCR2 and CXCR6 stimulations as the immune mechanism responsible for this decreased risk of *de novo* tumor development after sepsis-cure. While non-septic inflammation did not provoke this network, laminarin injection could therapeutically reproduce this protective sepsis consequence. This chemokine network and CXCR6 tissue-resident T-cell accumulation were observed in humans with sepsis and were associated with prolonged survival in humans with cancer.
Conclusion: Here, we identified a novel and therapeutically relevant anti-tumor consequence of sepsis-induced trained immunity.

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WS03 – IMMUNOMODULATION BY DIET, EXERCISE AND HORMONES

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2056 – WS03.1

Sustained immune and metabolic effects of a traditional African diet and fermented beverage versus a Western diet: insights from a short dietary transition study in Kilimanjaro, Tanzania

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Purpose: Traditional diets of many African communities are being rapidly replaced by Western-style diets. African populations are underrepresented in nutrition immunology research, resulting in a scarcity of data on the immune and metabolic effects of traditional, largely plant-based African diets and fermented food products. This study aimed to investigate these effects through a short controlled dietary intervention in the Kilimanjaro region in Tanzania.
Methods:
We conducted a short-term dietary intervention study involving young men residing in urban and rural areas in Moshi district. Participants (n=22 per group) underwent two-week periods of transitioning between a traditional diet (TD) and a Western diet (WD), as well as consumption of a traditional fermented banana beverage (‘Mbege’) for one week by urban participants (n=22). Additionally, ten controls who maintained their habitual diet were also enrolled. Functional immune assays (cytokine production capacity) and omics analyses (targeted plasma proteomics, whole blood transcriptomics, untargeted plasma metabolomics) were employed to assess changes in immune and metabolic parameters before, immediately after, and four weeks post-intervention (follow-up).
Results:
Our findings demonstrate that transitioning from a TD to a WD induces a pro-inflammatory state at the level of circulating leukocytes, proteins, and gene expression, whereas transitioning from a WD to a TD and consuming the fermented beverage exerts an overall anti-inflammatory effect. In addition to traditional markers of inflammation and immune function, these effects encompassed alterations in circulating concentrations of proteins involved in cardiometabolic processes and the plasma metabolome, including lipid and amino acid metabolism. Importantly, these effects persisted at follow-up, suggesting sustained impacts on immune and metabolic profiles following the intervention.
Conclusion:
This study provides new insights into the potential health risks associated with abandoning traditional African diets and the potential health effects of consuming traditional fermented beverages. Our findings underscore the importance of preserving indigenous dietary practices amidst dietary transitions in Africa and highlight the role of nutrition transition in the rising burden of non-communicable diseases in urbanized areas across Sub-Saharan Africa.

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2102 – WS03.2

Regulatory T cells require IL-6 receptor alpha signaling to control skeletal muscle function and regeneration

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Tissue-residing Foxp3$^+$ regulatory T cells (Tregs) control local tissue integrity and function by exerting non-canonical functions which go beyond classic immune-modulation. These tissue Tregs interact with the local microenvironment they reside in and implement environmental and metabolic cues. Recently, a special subset of tissue Tregs was described in the muscle. However, the molecular interface that connects Treg-based regulation with muscle function and regeneration remained largely unexplored.
Here, we show that exercise (i.e. voluntary wheel running) fosters a stable induction of highly functional muscle-residing Tregs with increased expression of amphiregulin (sedentary vs. pre-exercised p=0.0064; exercised vs. pre-exercised p=0.0009), EGFR (sedentary vs. exercised p=0.0192, sedentary vs. pre-exercised p<0.0001, exercised vs. pre-exercised p<0.0001), ST2 (sedentary vs. pre-exercised p=0.0031, exercised vs. pre-exercised p=0.0002) and IL6Rα (sedentary vs. pre-exercised p=0.0064,exercised vs. pre-exercised p=0.0001).
Mechanistically, we find that mice lacking IL6Rα on T cells (TKO) show significant reductions in muscle Treg phenotypic maturation, deficits in numbers of satellite cells (IL6Rα floxed vs TKO p=0.046) and fibro-adipogenic progenitor cells (IL6Rα floxed vs TKO p=0.0021), which are required for proper muscle regeneration. Using exercise and additionally sarcopenia models, IL6Rα TKO mice demonstrate deficits in muscle Tregs, their functional maturation and a more pronounced decline in muscle function as assessed by grip strength tests (IL6Rα floxed vs TKO p=0.0146). Furthermore, a chemical muscle injury model shows that IL6Rα TKO mice have significant impairments in muscle regeneration 14 days post injury as indicated by reduced fiber cross sectional areas (IL6Rα floxed vs TKO p=0.0017). Importantly, Treg gain-of-function by Treg expansion restores the impaired muscle repair in IL6Rα TKO mice, highlighting the importance of Tregs for proper muscle function. Of note, pharmacological IL6R blockade in WT mice (as used to treat autoinflammatory disorders in the clinics) phenocopies impairments in muscle function (control InVivo mAb vs anti-IL6R InVivo mAb p=0.0014) identified in IL6Rα TKO mice, thereby underscoring the clinical implications of these findings.
Overall, the results of this study highlight the relevance of dissecting the molecular basis of muscle-specific immune regulation which will be of importance for the design of tailored precision medicines targeting niche-specific Tregs in the future.

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262 – WS03.3

Local thyroid hormone action in T cells and its role for protective immunity

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Purpose: Thyroid hormones (TH) are critical regulators of development, growth and physiological function of many tissues and cells among which the immune system has emerged as an important target. However, the effect of TH in T cells, which play a central role in protective immunity, is poorly understood. Therefore, we aimed to address local TH action in T cells and its role for protective immunity.
Methods: Using mice, which either lack the TH receptor α (TRα) or exclusively the canonical TRα action, we analyzed the impact of TH-TRα signaling on T cell immunity in health and disease.
Results: Our findings suggest a significant role of TRα-mediated TH signaling in T cell immunity. In naïve, female mice, lack of classical, canonical TRα action increased the frequency of regulatory T cells (Treg) and induced an activated and migratory Treg phenotype. Moreover, canonical TRα action reduced activation of the NFκB pathway previously shown to play a pivotal role in Treg differentiation and function. Similarly, during influenza A virus (A/PR8/34) infection body weight loss was attenuated in absence of canonical TRα action, whereas entire loss of TRα signaling aggravated disease as shown by increased viral loads in lung.
Conclusion: Taken together, our findings demonstrate for the first time that TRα impacts T cell differentiation and phenotype and thus may have an important role regulating protective immune responses during disease.

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1673 – WS03.4

Oleic acid improves clinical features in psoriasis mouse model: effects on immune cells

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Purpose: Psoriasis is an auto-immune disease that affects thousands of millions of people in the world. There is no cure for psoriasis and the treatments available focus on block the cytokines involved in the disease. Considering that diet is a trigger for the disease, we aimed to investigate the effects of oral supplementation with oleic acid (OA), the main fatty acid found in the Mediterranean diet, on psoriasis.
Methods: Male C57BL/6 mice were induced with psoriasis by applying a 5% imiquimod-based cream (IMQ) for 5 consecutive days while simultaneously receiving OA by gavage. We assessed skin thickness, body weight, water and food intake, and spleen area and weight, and gene expression of cytokines and markers of keratinocytes proliferation in the skin. We also performed flow cytometry and WB analyses. Statistical analyses were performed using one-way or two-way ANOVA followed by Bonferroni's post-test ($p < 0.05$).
Results: IMQ increased skin thickness, redness, and peeling on the dorsal skin compared to the control group. Treatment with OA reduced these clinical symptons. The gene expression of IL-17, IL-22, IL12p40, IL-23 were increased in IMQ and reduced in IMQ+OA. IMQ elevated the percentage of monocytes on dermis, and OA reduced it. The same pattern was observed for macrophages; IMQ increased the proliferation rate of skin macrophages while OA returned it to control rate. Performing dextran tracking experiments, we observed that IMQ reduced the migration of macrophages from blood to skin and OA reversed this effect. Considering the results obtained so far we investigated the effects on NLRP3/Caspase 1 pathway. By WB, we observed the increase in activated Caspase 1 in IMQ samples and a reduction in IMQ+OA.
Conclusion: OA has beneficial effects on psoriatic inflammation acting on immune cells, mainly monocytes and macrophages. OA seems to reduce the exacerbation of the skin inflammation inhibiting the proliferative response and reducing cell death. Altogether, these effects explain the improvemnet in the clinical aspects of psoriasis.

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347 – WS03.5

Hyperandrogenism cause tissue specific alterations of NK cells in a mouse model of polycystic ovary syndrome

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Immune dysfunction is linked to several features of polycystic ovary syndrome (PCOS), including reproductive failure and metabolic comorbidities. Uterine NK (uNK) cells are pivotal for a successful implantation, while immune cells residing in visceral adipose tissue (VAT) are central for glucose homeostasis. We have previously shown that hyperandrogenism, a hallmark of PCOS, render female mice insulin resistant and alters immune populations, with an increased number of uNK cells and a higher frequency of $CD69^+$ NK cells in VAT. However, it remains unknown if this corresponds to a disturbed NK cell function.
To better understand how hyperandrogenism affect NK cells, phenotypic markers were analyzed by flow cytometry in dihydrotestosterone-exposed mice (PCOS-mice). Co-treatment with flutamide, an androgen receptor (AR) antagonist, was used to study AR activation. Preliminary data show a clear reduction of mature $CD27^-CD11b^+$ NK cells in uteri of PCOS-mice, with a higher proportion of $CD27^+CD11b^-$ and $CD27^+CD11b^+$ NK cells. A similar but less pronounced effect was seen in VAT, spleen, and blood. Decreased frequencies of NK cells expressing the maturation marker KLRG1 was also found in all analyzed compartments. Moreover, PCOS mice displayed reduced frequencies of $NKG2A^+$ uNK cells. NKG2A has been shown to educate uNK cells and correlate with the expression of the activating molecule DNAM1. Indeed, a lower frequency of $DNAM1^+$ uNK cells was seen in PCOS mice, which may suggest alterations in uNK education. Surprisingly, a population of $PD1^+$ NK cells was found in uteri, which was reduced in PCOS-mice and absent in spleen and blood. In contrast, a higher frequency of NK cells co-expressing CD69 and CXCR6 was found in in VAT of PCOS-mice, indicating that tissue-resident NK cells in VAT are specifically affected by androgen exposure. Most effects were prevented by co-treatment with flutamide, indicating AR driven alterations.
Our results propose an inhibitory effect of androgens on NK cell maturation, while other alterations are tissue specific. Hyperandrogenism may distort uNK education, which could be detrimental for embryo implantation. In turn, tissue-resident NK cells in VAT seem specifically affected with an unknown effect on metabolic regulation.

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2037 – WS03.6

High glucose diet promotes development of cross-reactive intestinal IgA responses via the TLR4/TNF axis

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Intestinal microbiota controls multiple aspects of the body homeostasis including the development of many diseases. Microbiota composition is regulated by the host's immune system as well as by extrinsic factors, such as diet. Patients with type II diabetes (T2D) are characterized by impaired glucose tolerance, microbiota dysbiosis and enhanced amount of immunoglobulin A (IgA) antibodies in blood. However, the molecular mechanisms of how increased glucose levels affect humoral immune responses remain elusive. IgA is one of the major regulators of microbiota composition on the mucosal surfaces. Using a panel of monoclonal IgA antibodies cloned from the intestinal plasma cells, we found that some of them cross-reacted between microbial species and host tissues such as pancreas in steady state. Interestingly, high-glucose diet (HGD) in mice amplified the amount of cross-reactive IgA antibodies towards microbiota and pancreatic antigens. First of all, HGD increased *Akkermansia muciniphila* abundance in gut that was accompanied by the expansion of *Akkermansia*-specific IgA. Strikingly, *Akkermansia*-specific IgA cross-reacted with several pancreatic surface proteins on acinar cells as revealed by the competitive inhibition of binding to the tissue by bacterial antigens. Notably, treatment of mice with monoclonal IgA antibody cross-reactive to pancreas resulted in improved glucose tolerance. Mechanistically, such cross-reactive IgA response was controlled by TNF expression induced via TLR4 signaling during HGD. Altogether, our data reveal that glucose rich diet induced cross-reactive IgA production that ameliorated diabetes development.

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WS04 – GRANULOCYTE DIFFERENTIATION AND FUNCTION

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903 – WS04.1

Single-cell proteomics and transcriptomics resolve the development of eosinophils and the role of IL-5 in their lineage transit amplification.

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Eosinophils, specialized granulocytes initially recognized to accumulate in response to helminth infection, have also been attributed roles in immune homeostasis, microbial defense, metabolism, or anticancer protection. Despite their proposed beneficial functions, eosinophils are predominantly considered for their diagnostic value and implication in various type 2 immune disorders. "Anti-eosinophil" biologics targeting IL-5 or its receptor IL-5Rα, which drastically reduce circulating eosinophils, are now commonly used for the treatment of asthma and chronic rhinosinusitis. Yet, our understanding of the eosinophil lineage, and therefore of the mechanisms of action of targeted therapies, remains limited. We integrated single-cell proteomics and transcriptomics with a novel transgenic IL-5Rα reporter (IL5RAporter) mouse model to comprehensively resolve eosinophil development in human and mice. This approach reconciles human and murine eosinophilopoiesis and facilitates further exploration of eosinophilopoiesis at both cellular and molecular levels. We notably reveal that eosinophil lineage expansion is driven by IL-5-promoted transit amplification, which is characterized by increased cycling activity, prolonged proliferative capacity, and delayed maturation. Conversely, IL-5 deletion or neutralization attenuates eosinophil progenitor transit amplification without compromising maturation, challenging previous assumptions. Additionally, we demonstrate that Interferon Response Factor-8 is not inherently essential for eosinophilopoiesis, contrary to earlier hypotheses. The IL5RAporter strain also allows the identification of cells expressing IL-5Rα in mice, which include eosinophils, subpopulations of B cells, but not neutrophils or their progenitors. In addition, IL5RAporter mice indicate that IL5-Rα becomes expressed after the divergence of the murine basophil/mast cell and eosinophil lineages. This is unlike in humans, where IL-5Rα is expressed in common basophil/eosinophil progenitors and their progeny, which explains the impact of "anti-eosinophil" biologicals on both lineages. Overall, our study provides valuable resources, accessible methodologies, and novel insights into eosinophil ontogeny, the impacts of precision therapeutics, and the broader regulation of eosinophil development in health and disease.

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2091 – WS04.2

Neutrophils: not as ready-to-go as we thought?

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Neutrophil granulocytes (neutrophils) are first responders in inflammation, patrolling the bloodstream and ready to invade infected tissue. In recent years, the historical view of neutrophils as uniform and finally differentiated cell type has been challenged by increasing reports of heterogeneity in neutrophils. In the bone marrow, we find different stages of maturation, after which circulating neutrophils age over time, altering their appearance and functionality in the blood. This phenotypic diversity is also observed in different tissues upon recruitment.
We made use of a reporter mouse expressing an irreversibly photoconvertible protein (mKikumeGR) to track arrival time of neutrophils at the site of *Staphylococcus aureus (S. aureus)* infection. "Tissue-matured" neutrophils that had remained at the site of infection for several hours differed greatly from those "recently-recruited" from the blood. The phenotypic changes that accompany extravasation of circulating neutrophils into infected tissue further increased when the recently-recruited cells transformed into tissue-mature neutrophils over time. In particular, as CXCR2 and CD62L expression decreased, CD11b as well as CXCR4 increased.
Furthermore, behavioural differences were observed between the two subtypes in terms of phagocytic activity, which was much higher in tissue-matured neutrophils compared to recently-recruited cells. This was true towards bacteria at the site of infection, as well as beads inoculated into the infection site. Using a biosensor to measure bacterial proliferation *in vivo*, we were able to show that recently-recruited neutrophils harbour not only fewer bacteria, but that these bacteria also show higher proliferation. Finally, using intravital 2-photon microscopy, we were able to map the behaviour of neutrophils at the site of infection. We found that while recently-recruited neutrophils behave in a fairly uniform manner, tissue-matured neutrophils exhibit distinct morphodynamic changes depending on their proximity to *S. aureus.*
Taken together, we provide a dynamic map of how neutrophils unleash their full microbicidal potential following arrival at the site of infection.

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233 – WS04.3

Deciphering phenotype, dynamics, and function of early-life neutrophils using single-cell RNA sequencing and lineage tracing

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In the adult immune system neutrophils are considered the first responders to all types of acute tissue damage and infections. In line with the known ontogeny of other innate immune cells, neutrophils are first detected in circulation prior to birth at day 12.5 of embryonic development. Nevertheless, these cells have thus far not been subjected to the same rigorous scrutineering as other myeloid cells like macrophages. This project has hence aimed to consolidate this gap in knowledge of the prenatal and early-life innate immune system.
For the first time, we here provide a detailed look at the phenotype, kinetic dynamics, and functionality of neutrophils during late embryogenesis and postpartum. Using single-cell RNA sequencing of $CD45^+$ immune cells in the early immune compartments, we deciphered their transcriptomic landscape. Through *in silico* integration of published adult neutrophil transcriptome data, we were able to re-construct their journey from progenitor to mature immune cells.
We next analysed the kinetics and surface marker phenotype of neutrophil populations across haematopoietic tissues from embryogenesis until adulthood using multiplexed flow cytometry. Furthermore, we dissected their behaviour in relation to the circadian rhythm and used *in vitro* assays to validate characteristic neutrophil functions *ex vivo*. By utilizing state-of-the-art lineage tracing in combination with tissue clearing-aided 3D wholemount imaging, we were also able to study cellular location and distribution of the cells in their undisturbed environment.
Taken together, we provide a thorough, multimodal description of the phenotypic, kinetic, and functional landscape neutrophils during the crucial timeframe of embryogenesis and early life.

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2035 – WS04.4

Neutrophils acquire a peculiar phenotype in psoriatic arthritis patients

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Backgroud: Neutrophils (polymorphonuclear leukocytes: PMNs) are the most abundant subtype of white blood cells and the main actors in the inflammatory response. Psoriatic arthritis (PsA) is a chronic inflammatory disease that affects the axial and peripheral joints. Typically associated to psoriasis, can also affect multiple systems and organs, including nails and enthesis. PMNs are also involved in the pathogenesis of PsA but their role has not been fully understood, yet. This study aimed to investigate the role of neutrophils and neutrophil-related mediators in PsA patients.
Matherials and Methods: 30 PsA patients and 22 healthy controls (HCs) were prospectively recruited. PMNs were freshly purified from peripheral blood and *in vitro* stimulated with lipopolysaccharide (LPS), N-Formylmethionyl-leucyl-phenylalanine (fMLP), tumor necrosis factor alpha (TNF-α), phorbol 12-myristate 13-acetate (PMA) or control medium. Highly purified peripheral blood neutrophils (>99%) were evaluated for activation status, reactive oxygen species (ROS) production, phagocytic activity, granular enzymes, and Neutrophil Extracellular Trap (NETs) release. Serum levels of matrix metalloproteinase-9 (MMP-9), myeloperoxidase (MPO), TNF-α, interleukin 23 (IL-23) and interleukin 17 (IL-17) were measured by ELISA. Serum concentrations of Citrullinated histone H3 (CitH3) was measured as NET biomarker.
Results: activated PMNs from PsA patients displayed reduced activation profile, ROS production and reduced phagocytic activity upon stimulation with TNF-α, compared with HCs. Upon stimulation with TNF-α, PMNs from PsA patients displayed also reduced granular enzyme release (MPO) and NET release. PsA patients presented higher circulating levels of MMP-9, MPO, TNF-α, IL-23, IL-17 and CitH3 compared with HCs. CitH3 serum levels were positively correlated to MPO and TNF-α concentrations in PsA patients. Moreover, IL-17 concentrations showed a positive correlation with IL-23 levels in PsA patients. These findings show a reduced *in vitro* activation of neutrophils purified from peripheral blood of PsA patients upon activation with TNF-α, but increased levels of neutrophil-derived mediators (MMP-9, MPO, TNF-α, IL-23, IL-17 and CitH3) in serum of PsA patients.
Conclusions: Taken together, our findings suggest the acquisition of an "exhausted" phenotype of neutrophils in PsA and highlight their plasticity and multiple roles in PsA pathophysiology.

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836 – WS04.5

DEVELOPMENT OF A MAST CELL DEPLETING ANTIBODY FOR THE TREATMENT OF MASTOCYTOSIS

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Purpose: Mast cells (MCs) are key effector cells in allergic reactions, and express KIT receptor which is essential for MC maturation, growth and survival. Somatic mutations in *c-kit* cause hyper- or constitutively- active receptor signalling, leading to mastocytosis. An anti-KIT monoclonal antibody (mAb), CDX-0159, depletes normal MCs. This mAb blocks KIT signalling without engaging effector functions, due to its Fc Silent format. This, however, renders CDX-0159 likely unsuitable for mastocytosis treatment, as mutations in the KIT receptor are known to signal regardless of ligand binding or receptor homodimerization.
Here, the objective is to develop an Fc-Engineered anti-KIT mAb able to deplete normal and KIT mutant MCs through its effector function.
Methods: An anti-mKIT mAb, clone ACK2, blocks KIT signalling and is therefore a surrogate for CDX-0159. This mAb was produced in three different formats called Fc Silent, WT, and Fc-Engineered. C57BL/6 mice or KIT^{K512I} mice, containing a constitutively active KIT receptor, were treated with 30μg of anti-mKIT mAbs with differently engineered Fc portions on day 0 and 7. Subsequently, MC burden was assessed in several tissues using flow cytometry or histochemistry.
Post-depletion functional tests of MC-dependent anaphylaxis were carried out, specifically IgE mediated- or mrgprb2 mediated- passive systemic anaphylaxis. Core body temperature was measured as hypothermia is the gold standard read out of anaphylaxis in mice.
Results: An Fc-Engineered anti-mouse KIT mAb is most efficient at depleting normal MCs compared to other Ab formats in numerous tissues. Subsequently, this treatment significantly reduces the severity of MC-mediated anaphylaxis. The Fc-Engineered anti-KIT but not the Fc Silent mAb also depletes MCs in KIT^{K512I} mice.
Conclusion: The murine surrogate of CDX-0159 can reduce MC burden. Fc-Engineering of this mAb can increase the efficacy of MC depletion. Furthermore, the Fc-Engineered anti-KIT can deplete KIT mutant MCs, whereas the Fc Silent cannot. These results provide a proof of concept that an Fc-Engineered mAb against the KIT receptor would be effective in mastocytosis.

This work was carried out in collaboration with Argenx SE, biotechnology company. W.P.M.W was supported by fellowships from INSERM-Region Occitanie and the Fondation ARC.

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948 – WS04.6

Innovative approaches for neutrophil elastase imaging in vivo using low-molecular-weight NIR fluorescent probes in inflammatory disorders

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Purpose: Neutrophil Elastase (NE) plays a crucial role in inflammation by degrading bacterial components and extracellular matrix proteins, thus facilitating phagocytosis and tissue remodeling. Dysregulated NE activity is implicated in various inflammatory disorders such as chronic obstructive pulmonary disease, pancreatitis, inflammatory bowel disease, and acute lung injury. Furthermore, NE also cleaves extracellular matrix proteins such as elastin and collagen, promoting neutrophil migration and tissue remodeling during inflammation, e.g. lung damage at COVID or heart damage in a range of cardiovascular disorders. Uncontrolled and unregulated activity of NE can inflict adverse effects, degrading host tissues and exacerbating inflammatory responses. In this study, we aimed to develop innovative approaches for in vivo imaging of NE using low-molecular-weight near-infrared (NIR) fluorescent probes, with the goal of improving our understanding of NE dynamics in inflammatory processes and potentially aiding in the diagnosis and management of these disorders.
Methods: We synthesized squaraine-based fluorescent probes connected with peptides designed to specifically bind to or be cleaved by NE. These probes were used to develop both fluorescence resonance energy transfer (FRET)-based and non-FRET fluorescent NIR probes for NE detection. We evaluated the specificity, kinetics, and photostability of the probes in vitro and then applied them in models of chronic inflammatory conditions in vivo.
Results: Our findings revealed increased NE activity in a neutrophil-driven heart fibrosis model under both acute (isoproterenol) and chronic (angiotensin) inducers of fibrosis. The developed probes allowed us to visualize NE activity in various inflammatory settings, including MSU crystal-induced footpad inflammation and nanoparticle-induced air pouch inflammation. Furthermore, a non-FRET NIR probe enabled NE quantification at the cellular level using modified immunohistochemistry techniques.
Conclusion: Our study demonstrates the feasibility and utility of using low-molecular-weight NIR fluorescent probes for in vivo imaging of NE in inflammatory disorders. These probes offer a promising tool for studying NE dynamics, understanding the pathogenesis of inflammatory diseases, and potentially facilitating the development of targeted therapies.

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WS05 – DIVERSITY OF ANTIGEN RECOGNITION

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1697 – WS05.1

Composite peptides spanning PDGFRa and AAV5 virus trigger autoimmunity in systemic sclerosis patients

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Purpose: Systemic sclerosis (SSc) is characterized by an unknown etiopathogenesis**,** where platelet-derived growth factor receptors (PDGFRs) show an overexpression in patients with SSc. Since PDGFRα is the entry receptor for the adeno-associated virus type 5 (AAV5), here we analysed the prevalence of AAV5 in the lung of SSc patients with interstitial lung disease (ILD) and correlated the results to anti-PDGFRα immune responses.
Methods: The binding of the AAV5 capsid to the monomeric human PDGFRα was assessed by *in silic*o molecular docking, surface plasmon resonance (SPR), and using cells lacking PDGFRα through CRISPR/Cas9 technology. AAV5 was detected in SSc lung by *in situ* hybridization, immunohistochemistry, confocal microscopy, and molecular analyses of bronchoalveolar lavage (BAL). Immune responses to AAV5 and PDGFRα were evaluated by immunoprecipitation, SPR, liquid chromatography-tandem mass spectrometry (LC-MS/MS) with multiple reaction monitoring (MRM) of the composite peptides within the HLA class I immunopeptidomes in SSc peripheral blood mononuclear cells (PBMCs), and *in vitro* stimulation of PBMCs with peptides detected by MRM/MS.
Results: AAV5 was detected in the BAL of 41 out of 66 (62.1%) SSc patients and in 17 out of 66 controls with lung disorders (25.7%) ($p<0.001$). In SSc, AAV5 localized in type II pneumocytes and in lung interstitial cells. A molecular complex between AAV5 and PDGFRα was characterized. Composite peptides spanning specific fragments of huPDGFRα and AAV5 capsid were identified in HLA class I immunopeptidome of SSc PBMCs by MRM/MS. These peptides elicited *in vitro* a significant immune reactivity in PBMCs from SSc patients compared to controls.
Conclusions: The identification of the presence of AAV5 in lungs of SSc patients with ILD and the immune responses to AAV5 and PDGFRα composite peptides suggest a newly unrecognized role of AAV5 in the etiopathogenesis of SSc and of viruses in autoimmune disorders.

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373 – WS05.2

Exploring the germline diversity of various macaque B-cell receptor regions

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Macaque species are widely applied as a model for the study of infectious diseases and vaccine efficacy. Despite their importance, genomic characterization of some macaque immune regions remains limited, such as regions encoding the highly variable B-cell receptor (BCR). Specificity of each BCR is dictated by the rearrangement of variable (V), diversity (D), and joining (J) gene segments, the pairing of heavy and light chains, combinatorial and junctional diversity, and somatic hypermutations. The well-characterized human V(D)J segments are located on three chromosomes, forming distinct regions encoding the heavy chain (IGH) and two light chains (IGK, IGL). At an individual level, these BCR regions are highly diverse, featuring insertions, deletions, and allelic variations. The macaque BCR regions, also distributed across three chromosomes, seem to display more diversity, especially in IGK and IGL V segments.
To refine translatability of immunological research from macaques to humans, a deeper understanding of the macaque BCR layout is essential. In this study, long-read sequencing was performed using ONT and PacBio platforms to characterize complete BCR regions. Using a custom bioinformatic pipeline, macaque BCR clusters were assembled and annotated. Despite abundant repetitive sequences and highly similar segments, phased haplotypes were resolved for IGH, IGK and IGL regions. This allowed identification and mapping of previously reported and novel gene segments, including potentially functional and pseudo entities.
Our sequencing approach enabled a comprehensive characterization of the heavy and light chain BCR regions in macaques, revealing extensive allelic and structural variations. Further implementation of this strategy would generate accurate germline references of V, D, and J segments, which allows assessment of mutational rates and BCR affinity maturation in response to infection or vaccination, thereby refining the macaque model in biomedical research.

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386 – WS05.3

Dynamics of T cell and B cell receptor repertoires in response to obesity in a murine model of atherosclerosis

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Purpose: Atherosclerosis is caused by a buildup of lipid-rich plaques within arterial walls. While T cells and B cells are both implicated in the immune response associated with atherosclerosis, the specific alterations in their respective receptor repertoires in response to obesity remain poorly understood. Here, we investigate obesity-induced changes in the T cell receptor (TCR) and B cell receptor (BCR) repertoires in a mouse model of atherosclerosis.
Methods: For this study, we used ten Ldlr-/- Apob100/100 mice, of which half received high-fat diet, while the remaining five were fed standard chow diet. From each mouse, we collected four different tissue types: perivascular adipose tissue, aorta, spleen, and epididymal white adipose tissue. The same tissue type from all mice in the same diet group was combined into one pool, resulting in eight sample pools. Afterwards, the tissues were dissociated and aorta samples were enriched for CD45+ cells. Finally, we performed single-cell RNA-sequencing using a 5' gene expression and VDJ immune profiling protocol. By analyzing VDJ repertoires, we aimed to identify clones with shared motifs and inspect them in the context of their gene expression profiles.
Results: The TCR repertoires were markedly distinct between obese and non-obese mice, with a more pronounced overlap between tissues from obese mice, suggesting a potential difference in predominant epitopes between the two diet groups. TCR clones that were more abundant in adipose tissues and/or the aorta were also present in the spleen, albeit in smaller numbers. In contrast, in the BCR repertoires, there was more overlap between the different diet groups, and the largest overlap was again seen between tissues from obese mice. We also observed tissue-specific BCR clones, which, although present in both diet groups, were much more abundant in obese mice.
Conclusion: This study contributes to the broader effort of unravelling the immunological mechanisms underlying cardiovascular diseases. Understanding the effect of diet on the dynamics of TCR and BCR repertoires in various tissues may inform the development of therapeutic strategies aiming to mitigate the progression of atherosclerosis in genetically predisposed individuals.

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1213 -WS05.4

Broadly neutralizing HIV-1 antibodies have unusually high capacity to bind heme

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Purpose: An antibody able to recognize many structurally unrelated antigens is referred to as polyreactive. Polyreactive antibodies play an important role for neutralization of human immunodeficiency virus (HIV-1) and represent >60% of broadly neutralizing HIV-1 antibodies (bNAbs). Besides the naturally polyreactive antibodies, healthy humans contain a fraction of antibodies that can acquire polyreactivity upon contact with the cofactor molecule - heme. Although in normal condition it is intracellularly constrained, heme can be released *in vivo* in case of hemolytic disease and / or tissue damage. Previous data have demonstrated that HIV-1 is unusually sensitive to heme. However, it remains unclear whether heme can impact specificity and functions of HIV-1 bNAbs.
Methods: The presence of cryptic antigen-binding specificities in a panel of 37 HIV-1 bNAbs was assessed by various binding assays (ELISA, Western Blots, immunofluorescence) and was compared to a panel of 43 neutralizing Abs specific for influenza virus. The molecular mechanism of interaction of heme-induced polyreactive antibodies with HIV-1 gp120 and gp41 proteins was studied by site-directed mutagenesis and biophysical methods (SPR, absorbance and fluorescence spectroscopy). The functional activity of heme-bound bNAbs was studied using virus neutralization assay.
Results: Here, we show that a considerable number (>60%) of HIV-1 bNAbs are able to bind heme, which is not the case for the other panel of human antibodies. HIV-1 bNAbs bind to heme especially to regions of the protein molecule that are rich in aromatic and positively charged amino acid residues. In addition, we demonstrated that the binding of heme results in a dramatic extension of the spectrum of antigens recognized by these antibodies. Nevertheless, heme does not impact the recognition of envelope protein (gp120) and neutralization of a panel of HIV-1 strains.
Conclusion: These findings might have relevance for understanding the unique molecular properties and the binding mechanism of HIV-1-neutralizing Abs. While some of these HIV-1 bNAbs have been already evaluated in clinical trials with encouraging results, further work is needed to characterize the importance of their capacity to interact with heme in viral recognition and immune processes during HIV-1 infection. This may contribute to harnessing their full therapeutic potential.

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1829 – WS05.5

Surrogate light chain-deficient B cells expressing short and hydrophobic H-CDR3 loops escape from the bone marrow and expand in the periphery

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Purpose: In mice lacking the surrogate light chain proteins (SLC$^{-/-}$), antibody heavy chain selection at the pre-BCR checkpoint is defective, resulting in reduced numbers of precursor (pre) B, immature, mature and follicular B cells. In addition, B cell tolerance at the BCR checkpoint is also severely compromised, both in the bone marrow and in the periphery, resulting in overly autoreactive follicular B cells. Here we investigated whether the features of expressed heavy chains present in early bone marrow B cell precursors are also found in spleen and bone marrow plasma cells. We chose to analyse the molecular features associated with the H-CDR3 of the heavy chain repertoire because of its strong influence on antigen binding.
Methods: To better understand how the selection process occurs during (self-) antigenic activation, we have analysed high-throughput *IGH-VDJ* sequences from bone marrow progenitor (pro) B, preB and mature B cells as well as plasma cells from the spleen and bone marrow of mice with and without SLC deficiency.
Results: Our results show that certain plasma cell populations are highly expanded in *SLC*$^{-/-}$ mice and that they predominantly express heavy chains with short H-CDR3 loops, a feature that is already present at the preB cell stage. In addition, the use of *D* gene segment reading frames (RFs) is abnormal in the expanded plasma cell populations of *SLC*$^{-/-}$ compared to wild-type animals, with the otherwise "counter-selected" RFs II and III being favoured. Early bone marrow B cells and plasma cells from *SLC*$^{-/-}$ mice have an increased hydropathy index in the H-CDR3 loops compared to wild-type animals. PCA analysis based on H-CDR3 features shows that selection occurs in *SLC*$^{-/-}$ mice, but not to the same extent as in wild type.
Conclusion: The absence of SLC proteins severely compromises the *bona fide* selection process and thus the pre-B cell heavy chain repertoire, with changes being propagated to the next developmental stages, ultimately affecting the repertoire of plasma cells residing in the spleen or bone marrow.

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2138 – WS05.6

Recognition of SARS-CoV-2 spike sequence by HLA-E-restricted CD8+ T cells

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Some viruses can evade the immune system by downregulating the expression of HLA class Ia on infected cells. However, virus-mediated downregulation of HLA class Ia can result in upregulation of HLA-E, a class Ib molecule, on the surface of infected cells. In homeostatic conditions, HLA-E presents self-peptides derived from the leader sequence of HLA class Ia molecules, termed VL9 peptides, that act as a negative regulator of NK cell lysis by interacting with CD94/NKG2A molecules on NK cells. Therefore, maintenance of HLA-E surface expression with simultaneous downregulation of HLA class Ia allows for viral escape from both HLA class Ia-restricted cytotoxic CD8+ T cells and HLA-E-recognizing NK cells. However, it has been reported that, in addition to VL9 peptides, HLA-E can also present virus-derived peptides which could instead be a target for protective HLA-E-restricted CD8+ T cells.
Here we utilized SARS-CoV-2 spike (S) protein transduced cell lines for a target agnostic approach to sort HLA-E-restricted CD8+ T cells from the peripheral blood of healthy donors. Isolated cells were cloned and specificity for SARS-CoV-2 S confirmed by proliferation, cytokine secretion, degranulation, and cytotoxicity. To refine target recognition, epitope mapping using overlapping peptides spanning the SARS-CoV-2 S sequence was performed. In addition, we sequenced the TCR of positive clones to ensure distinct clonotypes were isolated.
Our data show that HLA-E-restricted SARS-CoV-2 S-specific CD8+ T cells are present in the peripheral blood of healthy donors. These clones are multifunctional, secreting activating cytokines such as IFNγ, GM-CSF, and IL-13. In addition, these SARS-CoV-2 S-specific clones effectively lyse target cells transduced with the S protein. Overall, these results highlight a potential use for HLA-E-restricted CD8+ T cells in immunotherapy applications, such as adoptive cell transfer, or to synergize with vaccine-induced antibody responses by modifying vaccine design. However, these HLA-E-restricted CD8+ T cells are rare and a deeper understanding of how to better elicit HLA-E-restricted virus-specific CD8+ T cells is needed.

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WS06 – EPIDEMIOLOGY AND IMMUNOGENETICS

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2197 – WS06.1

Compromised T cell development in Down syndrome is driven by intrinsic defects in early thymocytes

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Purpose: Down Syndrome (DS), caused by trisomy 21, is clinically associated with immunological dysfunction. Infants with DS have a small thymus with structural and functional stromal abnormalities, suggestive of a compromised thymocyte development. However, a detailed analysis of T cell development in DS is still missing and the underlying mechanisms of immunological alterations are not yet understood.
Methods: We performed multiparameter flow cytometry analysis on paired thymic and peripheral blood immune cells from 32 DS and 40 children with non-syndromical congenital heart disease, all under one year of age. We used CITE-seq for the analysis of thymocytes in carefully selected age- and heart condition-matched donors. Finally, we generated artificial thymic organoids (ATOs) using thymic CD34+ progenitor cells to study the particularities of T cell development in DS *in vitro*.
Results: Analysis of DS thymocyte subpopulations revealed lower frequencies of immature single-positive CD4 cells and an overrepresented CD8 compartment. *In vitro* modelling showed accelerated differentiation of DS cells into CD8+ T cells, indicating that these changes are intrinsic to the hematopoietic cells. CITE-seq analysis of DS thymocytes revealed differential expression of genes relevant for T cell development and lineage commitment. Finally, multiparameter flow cytometry analysis of peripheral blood at the time of heart surgery revealed higher frequencies of γδ T cells in infants with DS.
Conclusions: Our data demonstrate significant changes in T cell development in infants with DS, which are likely predetermined in thymic progenitor cells. The identification of a disproportionate CD4/CD8 composition in the thymus of DS children, together with the characteristic transcriptomic signature, advances our understanding of the impaired immune fitness observed in individuals with DS.

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555 – WS06.2

Multi-omic immunophenotyping to identify predictors of mRNA vaccine responsiveness in immunocompromised individuals

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Purpose: Recent advances in -omics technologies enable detailed evaluation of the human immune system in the context of important clinical phenotypes. Here, we apply such technologies to investigate predictors of vaccine immune responsiveness in individuals with a range of immunosuppressive conditions who are vulnerable to COVID-19 vaccine failure.
Methods: Using bulk and cellular indexing of transcriptomes and epitopes (CITE)-sequencing, Olink proteomics and spectral flow cytometry we assessed the pre- and 21 days post-third dose COVID-19 mRNA vaccine immunophenotype of 133 individuals with various immunosuppressive diseases (inflammatory bowel disease, rheumatic conditions, solid organ and haematological stem cell transplant, cirrhosis, haematological malignancies and primary immunodeficiencies) and 22 healthy individuals. By integrating this data with vaccine-induced antibody (binding and neutralisation) and functional T-cell responses, we use machine learning enabled analyses to find associations between the baseline immunophenotype and vaccine immune responsiveness.
Results: We identified distinct pre-vaccination transcriptional signatures which predicted post-vaccine antibody responsiveness across immunosuppressive conditions. The signatures included increased B and plasma cell related gene modules and decreased inflammatory, monocyte and neutrophil-related gene modules. Spectral flow cytometry of peripheral blood samples confirmed that the changes in B-cell and monocyte-related gene signatures were partially explained by changes in the frequency of these cell-types. Cytometric analysis of B-cell subsets found that transitional and memory B-cell frequencies were significantly associated with vaccine-induced antibody responses.
The differences identified in pre-vaccine inflammatory gene signatures were validated at the protein level through analysis of 364 inflammatory proteins in pre-vaccine plasma samples. Further analysis identified a network of 16 inflammatory proteins which when measured together could predict post-vaccine immune responsiveness with an area under the receiver operating characteristic curve value of 0.82.
Persistence of the altered pre-vaccine immunophenotype was observed post-vaccine using CITE-sequencing. This gave insights into the cellular identity of transcriptomic and proteomic signatures related to vaccine immunogenicity and identified key regulators of vaccine immune responsiveness which were perturbed across immunosuppressive conditions.
Conclusion: Through comprehensive immune profiling of pre- and post-vaccine blood samples from immunocompromised individuals we identified novel cellular and molecular signatures that predict vaccine responsiveness and give insight into mechanisms of vaccine failure.

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186 – WS06.3

Celluloepidemiology - A novel paradigm for quantifying infectious disease dynamics on a population level

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Purpose: Although serology has been a powerful tool in infectious disease epidemiology supporting epidemiologists and mathematical modellers to parameterize their simulation models thereby allowing epidemic forecasting and public health decision-making (e.g. SARS-CoV-2 vaccination), serological analysis of infected individuals is complicated by the variability of pathogen-induced antibody responses and is limited due to its use of antibodies as the sole marker of immunity against pathogens. On the other hand, pathogen-induced cell-mediated immunity is considered more sustained but remains poorly characterized at an epidemiological level.
Methods: In this ERC-funded study, we would like to introduce the celluloepidemiology method which analyses systematically T-cell responses against pathogens from an epidemiological perspective. We have applied the proposed celluloepidemiology method to characterise SARS-CoV-2 specific T-cell responses in convalescent patients recovered from COVID-19 for over 3 months and compared them to controls, household members of the recovered COVID-19 patients, general practitioners, hospital healthcare workers, and pre-COVID era donors.
Results: Applying flow cytometry and machine learning approaches to data from more than 500 individuals, we showed that the number of activated and exhausted $CD4^+$ and $CD8^+$ T-cells (having positive expression of CD154, OX40, CD137, CD69, TIGIT, and LAG-3) could be used to distinguish COVID-19 patients from controls and pre-pandemic participants, identify asymptomatic patients from those with mild, moderate, and severe COVID-19, as well as differentiate healthcare worker groups with different SARS-CoV-2 exposure/infection status. In addition, in our T-cell receptor (TCR) repertoire analysis, we found that recovered COVID-19 patients do not only harbour TCRs specific to SARS-CoV-2 but also those reactive against other coronaviruses (inc. MERS-CoV). This highlights that the celluloepidemiology approach is useful for discerning between different infections and backward tracing of pathogen-infected patients thanks to the added layer of TCR specificity.
Conclusion: We believe that the proposed celluloepidemiology method is complementary to conventional seroepidemiology in offering high dimensionality, sensitivity, and deep insight into the heterogeneity of human immune response against pathogens. Although the celluloepidemiology method was introduced and evaluated using the SARS-CoV-2 model in this study, the same concept is highly transferable to different pathogens and infectious diseases in the future.

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1887 – WS06.4

The multi-omics landscape of systemic inflammation in people living with HIV

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Background: People living with HIV (PLHIV) with viral suppression because of HIV-treatment have a higher susceptibility to non-AIDS co-morbidities, such as cardiovascular diseases (CVD), due to systemic inflammation. Systemic inflammation is potentially caused by immune activation by viral glycoproteins, but its underlying mechanisms are largely unknown.
Methods: We analyzed 1342 virally suppressed PLHIV participating in the 2000HIV project. By integrating five different omics layers together with immune function profiling, we deciphered mechanisms underlying persistent inflammation.
Results: Integrating epigenomics, transcriptomics, proteomics, metabolomics, and cytokine responses resulted in a set of co-morbidity related factors capturing inter-individual variation across layers. Specifically, we identified latent factors associated with CVD and accelerated aging. Furthermore, we used genomics to understand the sources of inter-individual variation in the different omics layers and identified 4,766 expression-QTLs, 1,155 protein-QTLs, 22 metabolite-QTLs and 2 cytokine-QTLs at study-wide significance level. The identified QTLs were largely concordant with existing QTL studies performed in healthy cohorts. Mendelian randomization analysis revealed baseline determinants of immune responses, such as gene *TNFRSF8* and protein IL17D as regulators of immune responses to HIV. Lastly, by linking genetic variation to the latent factors we identified, we discovered that the NLRP12 locus regulates the inflammasome complex at different layers. This suggests a causal factor in determining systemic inflammation in PLHIV.
Conclusion: All in all, we discovered intricate molecular mechanisms underlying inter-individual variation in systemic inflammation in 1342 virally suppressed PLHIV, identified molecular QTLs in PLHIV, and pinpointed baseline regulators orchestrating the immune response to HIV stimulation.

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102 – WS06.5

Modeling gene-enviroment interactions in Parkinson's disease: Helicobacter pylori infection of PINK1 -/- mice induces autoreactive cd8 T/ regulatory T cell disbalance associated with motor and cognitive dysfunction

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Purpose: Parkinson's disease (PD) is a neurodegenerative disorder characterized by the progressive loss of dopaminergic neurons in the brain that manifests in motor dysfunction upon the loss of the larger portion of neurons in substantia nigra. Genetic risk factors, environmental triggers, and dysregulated immune response have been implicated in PD. Understanding PD pathophysiology in its pre-motor prodromal phase is needed for earlier diagnosis and intervention. Here, we aim to study the impact of the PD-associated gene - *PINK1* and pathogen-*Helicobacter* (*H.*) *pylori* on PD development and immune autoreactivity.
Methods: We established *H. pylori* infection in *Pink1* $^{-/-}$ mice and wild-type littermate controls, with unaltered or depleted CD8 T cells. At two months post-infection mice underwent Y-maze, beam crossing and Pole behavioural tests. The stomachs, brains and spleens were harvested for immunophenotyping, multiplex cytokine assay, *H.pylori* qPCR; and detection of mitochondrial antigen reactive (MitAg+) CD8 T cells. We developed additional *in vitro* assays to assess *Pink1*and *H.pylori* effects on MitAg+ CD8 T cell priming and suppression by regulatory T cells (Treg).
Results*: H.pylori* infected *Pink1-/-* mice developed a PD-like motor-behavioural dysfunction that was abrogated by CD8 cell depletion prior to infection. Motor-behavioural phenotype in infected *Pink1-/-* but not wild type mice strongly correlated with the yield of autoreactive MitAg+ CD8 cells and CD8 T cell brain infiltration. *H.pylori* infection *in vivo* and *in vitro* altered Treg FoxP3 expression, with a more robust FoxP3 loss in *Pink1 -/-* Tregs. The absence of PINK1 in dendritic cells exposed to *H.pylori* triggered robust priming of primary MitAg+ 2C T cells resulting in activation and proliferation. Addition of Pink +/+ Tregs to the co-culture effectively supressed 2C CD8 T cell proliferation, while PINK1-deficient Tregs lost their suppressor function.
Conclusion: Using a model that integrates genetic PD susceptibility and a PD-relevant pathogen we recapitulated the major features of complex PD pathophysiology including immune dysregulation that consisted of simultaneous Treg dysfunction associated with increased yield of autoreactive CD8 T cells

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291 – WS06.6

Comparison of immune variability between healthy African and European adults reveals broad similarities and specific differences

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Recent developments of immuno-modulating therapies and their application to a broad range of pathologies ranging from cancer to infectious diseases is a major advance in medical research. Nevertheless, responses to these treatments vary from one patient to another which highlights the need to better understand immune response variability. This is particularly striking regarding low and middle resources countries, as most in-depth immunological studies have so far focused on western populations.
To address this limitation we compared two cohorts, the Milieu Interieur cohort, which consists of 1000 healthy French donors and the HGGP Senegal cohort, which consists of 48 healthy Senegalese donors. These two populations are subjected to different environmental and pathogen exposures with different genetic backgrounds, as well as different lifestyles and diets. We characterized their baseline immune cells using multi-analyte flow cytometry to quantify circulating granulocytes, myeloid cells, NK cells and T and B cell subsets. To assess their functional immune responses, we stimulated their whole blood in TruCulture systems with LPS, Poly:IC, and SEB which covers bacterial, viral, and T cell immunity. 13 cytokines were measured in the stimulated supernatants by Luminex, and the stabilized cell pellet was assessed by Nanostring hybridization arrays. We also assessed microbiome populations by 16S sequencing in fecal samples and nasal swabs.
Our preliminary results show remarkably comparable cellular population and cytokine levels in both baseline phenotypes and after stimulation between these two very different healthy human populations. Notable exceptions were observed for certain phenotypes for example Senegalese donors had significantly lower naïve CD4 T cells, higher IL-8 in Null and LPS conditions, and lower IL-17 after LPS stimulation. In contrast to systemic immune responses the microbiome composition in both fecal and nasal samples were significantly different between the two cohorts, with much greater diversity observed in the Senegalese donors.
Ongoing analysis will focus on how such diverse microbiomes may impact immune homeostasis and responses.

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WS07 – PARASITE AND BACTERIAL IMMUNOLOGY

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484 – WS07.1

Ruxolitinib restores disease tolerance in malaria-infected glucocorticoid receptor knockout mice by preventing lethal hypoglycemia

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Malaria is a global disease with yearly around 600 000 deaths. Lethality is caused by various complications, including dysmetabolism. Previously, we have shown that adrenal hormones prevent excessive inflammation and severe hypoglycemia in malaria, and we recently detected decreased glucocorticoid (GC) sensitivity in both patients and mice with severe malaria. To further investigate the specific effects of endogenous GCs in malaria, we infected wildtype (WT) and tamoxifen-inducible global glucocorticoid receptor (GR) knockout (GRiKO) mice with *Plasmodium chabaudi* AS parasites. A 50% lethality rate was observed in infected GRiKO mice while all WT mice survived the infection, indicating that GR is critical for disease tolerance. Lethality in the GRiKO mice was coinciding with severe hypoglycemia and hyperlactatemia. With [18]F-fluorodeoxyglucose-PET/MRI imaging, drastic increases in glucose uptake and/or retention in liver and spleen of infected global GRiKO mice were observed. The hypoglycemic phenotype observed in the infected global GRiKO mice did not develop upon infection of hepatocyte, T cell or myeloid-specific GR knockout mice, suggesting that a combined dysregulation in both hepatic and immune cells might be required for the development of lethal hypoglycemia in GRiKO mice. Furthermore, RNAseq analysis of the liver revealed glycolytic reprogramming and an aggravated impairment of gluconeogenic gene expression in infected GRiKO mice compared to WT. A similar glycolytic trend was observed in the spleen, and highly significant correlations were found between hypoglycemia and the glycolytic transcriptome. Transcription factor enrichment analysis of the transcriptome suggested STAT3 involvement. Correspondingly, increased STAT3 activation, and elevated *il6* mRNA levels were found in liver and spleen of infected GRiKO. Importantly, Ruxolitinib, a JAK/STAT inhibitor, prevented the development of severe hypoglycemia, and improved the survival of GRiKO mice upon infection. Overall, this study provides proof of a protective role for GCs during severe malaria. Impairment of GC signaling during malaria infection leads to an exaggerated activity of the JAK/STAT pathway, which ultimately drives development of lethal hypoglycemia. Inhibition of the JAK/STAT pathway by administration of Ruxolitinib restored normoglycemia and prevented lethality upon infection of GRiKO mice. Therefore, this study uncovers a potential new therapeutic strategy for the treatment of dysmetabolism in severe malaria.

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1093 – WS07.2

Microbial infections trigger tissue mechano-responses to initiate innate immunity.

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Neutrophil recruitment from the bloodstream to tissues is a key early step in inflammation but how this happens remains unclear. This process occurs in response to both sterile and microbial injuries, suggesting the existence of a general strategy to minimize tissue damage while preparing for the containment of potential infections. This raises questions about the involvement of Pattern Recognition Receptors (PRRs) in early neutrophil recruitment during microbial infections and their role in microbial inflammation.
To investigate the mechanisms regulating neutrophil recruitment and function upon infection, we utilized a skin infection model wherein microbes are injected intradermally to minimize tissue damage while introducing PAMPs in large amounts. We employed different types of microorganisms, a fungus, *C. (Candida) albicans*, and Gram-positive or negative bacteria, *S. (Staphylococcus) aureus* and *P. (Pseudomonas) aeruginosa,* respectively.
To study the molecular mechanism leading to neutrophil recruitment during infection, we first characterized the immune infiltration through multi-parametric flow cytometry both in WT and mice KO for PRRs or interleukin receptors. We then characterized in depth the molecular cascade leading to neutrophil recruitment *in vivo* through qPCR and ELISA. Finally, we corroborated the results obtained in KO mice with pharmacological and genetic approaches.
We discovered that neutrophil recruitment is temporally defined by different upstream mediators, all converging to MyD88. Specifically, we identified two "waves" of neutrophil recruitment and were able to mechanistically define the first one. This initial wave occurs during the first 6 hours and is mediated by an LTB_4 (Leukotriene B4)- IL-1α axis, regardless of the type of infection. Neutrophils are recruited to the infected site via IL-1-CXCL1 axis, and conditional deletion of $CD11c^+$ immune cells abrogate neutrophil recruitment only at early stages. Surprisingly, this event is largely PRR-independent and instead relies on mechanoreceptor PIEZO1 and TRPV4, which directly regulate LTB_4 synthesis *in vivo*.
Thus, we propose a model of early neutrophil recruitment acting through a lipid-cytokine-chemokine axis that is pathogen-independent and relies on mechanosensing without major contribution from PRRs. This is a general mechanism deployed during various infection contexts.

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1973 – WS07.3

Spatial multi-omics identifies acyltransferase activity associated with granulomas

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Purpose: We sought to elucidate the metabolic state of immune cells within granulomas that form in response to intracellular pathogens such as mycobacteria and Leishmania. Despite progress in understanding the phenotypic characteristics of these cells, their metabolic profiles, particularly lipid metabolism, remain largely unexplored. We studied granuloma immuno-metabolism in the context of experimental *Leishmania donovani* infection, to understand lipid metabolism regulation within these complex structures and its implications for disease outcomes.
Methods: We integrated spatial lipidomic and transcriptomic data. Liver sections from both *L. donovani* infected and control mice were subjected to non-targeted lipidomic (MALDI MSI) and transcriptomic profiling (10x Visium). The data obtained were then co-registered and analyzed using graph-based clustering to discern the distinct lipid and transcriptomic signatures between the hepatic granulomas and the surrounding parenchyma. Matched single-cell RNA sequencing data (10x) was used to spatially resolve cell-type deconvolutions and enrichment. Additionally, immunohistochemistry was used to validate the upregulation of certain enzymes, Lysophosphatidylcholine acyltransferase 2 (LPCAT2), ApoE and parasite specific protein OpB within the granulomatous regions.
Results: Our analysis revealed significant compositional differences in lipid signatures between granulomas and the adjacent liver parenchyma. We found that certain lipid classes were preferentially localized to granulomatous tissue, indicating a unique lipidomic profile. Several lipid classes were upregulated in these granulomas, highlighting the potential for lipid-led heterogeneity within these structures. Specifically, Lands cycle acyltransferases were associated with granulomas, with their enzyme products and substrates detected as lipid components in the MALDI data and the corresponding gene expression in the transcriptomic data. Single-cell expression of LPCAT2 suggested a myeloid-specific signature. Finally at the protein level, LPCAT2 expression was found to be upregulated in granulomas.
Conclusion: This study provides novel insights into the lipid metabolic changes that occur within granulomas during L. donovani infection. The distinct lipidomic and transcriptomic profiles identified suggest a specialized regulation of lipid metabolism in granulomatous regions, which may be crucial for the pathophysiology of granulomatous diseases. Our data serves as a platform for further investigation into the role of lipid metabolism in disease progression and treatment.

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1448 – WS07.4

Differential antigen patterns during cyst degeneration in porcine neurocysticercosis

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Purpose: Neurocysticercosis (NCC), caused by *Taenia solium* larvae invasion in central nervous system of humans and pigs, remains as a public health concern as the leading cause of acquired epilepsy worldwide. Cyst degeneration process, linked to focal epilepsy, is accompanied by active release of parasite antigens causing neuroinflammation. However, antigen distribution across cyst stages (viable, degenerating, calcified) and their impact on neuronal structures remain poorly explored. We utilized anti-*T.solium* monoclonal antibodies (moabs), targeting antigens from total cysts, vesicular fluid, and excretory/secretory (E/S) products, aiming to identify antigen patterns within cerebral cysts and surrounding parenchyma from NCC-infected pigs during cyst degeneration.
Methods: To identify antigens targeted by our moabs, we performed western blots against recombinant *T.solium* antigens related to anchoring, scaffold formation, and secretion (rGP50, rT24H and sTsRS2-sTs14/18, respectively). Subsequently, we optimized moab-based immunohistochemistry (IHC) to evaluate antigen distribution in brain tissue samples containing viable, granulomas and calcified cysts from 17 pigs post-antiparasitic treatment (2-, 4-, 8-, and 12-month). Image J software analysed images assessing immunoreactivity percentages.
Results: Moabs against total cysts targeted rGP50, rT24H and sTsRS2, while moabs against vesicular fluid and E/S products principally recognized sTs14/18 and sTsRS2. Two moabs targeting anchoring antigens (TsW5/TsW8) and two targeting E/S products (TsV3/TsE1) were selected for IHC evaluation. Visual examination identified two different antigen patterns. TsW5/TsW8 recognized antigens in cyst wall, vesicular fluid, and in the spiral canal in viable cysts, whereas TsV3/TsE1 recognized antigens only in cyst wall and vesicular fluid. In degenerating cysts, all moabs recognized antigens in cyst wall, vesicular fluid and surrounding tissue. Surprisingly, in calcified cysts residual antigens were detected and distributed inside the cyst for TsW5/TsW8 and only in surrounding parenchyma (500µM) for TsV3/TsE1, with a significant and gradual decrease in immunoreactivity (4 months:30.8%, 8 months:7.5%, 12 months:1.8%, $p<0.0005$).
Conclusion: Our findings reveal the dynamic nature of cyst degeneration and antigen distribution, suggesting antigen diffusion from the parasite into surrounding tissue, potentially inducing structural alterations in brain cells. This study contributes to understanding NCC pathogenesis and lays the groundwork for future investigations into the relationship between brain cell antigens, neuronal structure changes, and clinical NCC features.

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1591 – WS07.5

A vaccination strategy utilizing exosomes induces protective immune responses in an experimental model of Leishmania infantum infection in BALB/c mice

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Purpose: Leishmania, a protozoan parasite of the trypanosomatid family, is the causative agent of leishmaniasis which has wide range of clinical manifestations including cutaneous, mucocutaneous and visceral leishmaniasis. Annually, 0.9–1.6 million new infections are reported and 20–50 thousand deaths occur due to Leishmania infection. Current chemotherapy for treating leishmaniasis exhibits numerous drawbacks while there are no effective human vaccines with protective immune responses which could help on elimination of the disease. To this end, exosomes, extracellular vesicles that contain protein, RNA and DNA constituents of the cells that secrete them, play a new role in the vaccination approach of a variety of diseases such as cancer, Toxoplasma gondii etc.
Methods: In the current study, exosomes derived from Leishmania infantum promastigotes were isolated by density gradient ultracentrifugation and were characterized. Subsequently, BALB/c mice were immunized with the isolated exosomes with two intramuscular immunizations with 15-day interval in the presence and absence of Addavax adjuvant. The mice were then infected with L. infantum parasites and the effect of the regimen was evaluated on the acute and chronic phase of the disease (1 and 3 months upon infection, respectively).
Results: Vaccination with exosomes in the presence of adjuvant resulted in a dramatic reduction of the parasitic burden in both the acute and the chronic phase of the experimental model of Leishmania infantum infection in BALB/c mice, accompanied by an immunomodulatory effect on the host. More specifically, the parasitic burden was reduced by 93% in both liver and spleen during the acute phase and 80% in spleen and 90% in liver during the chronic phase of the disease. The reduction in parasite load was accompanied by an increase in antigen-specific $CD4^{+}IFN\gamma^{+}$ T-lymphocytes in the host.
Conclusion: According to the above data, an exosome-based vaccine regimen emerges as a new potent vaccination approach and promising tool in the fight against the parasitic disease leishmaniasis.

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401 – WS07.6

Leishmania mexicana-infected Lutzomyia longipalpis sand flies induce inflammasome derived IL-1β in C57/BL6 mice

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Leishmaniasis is the second cause of mortality for a parasitic disease after malaria. Cutaneous leishmaniasis manifests in different forms, ranging from uncomplicated self-healing skin lesions (*Leishmania major*) to chronic diffuse lesions (*L. mexicana*). Here, we compare the early immune response to bites of *L. mexicana*-infected ($LmxSF_i$) and *L. major*-infected *Lutzomyia longipalpis* ($LmjSF_i$) sand fly bites in mice. We demonstrate that at 6 hours, $LmxSF_i$ and $LmjSF_i$ produced an acute inflammatory response with a 1.94-fold and a 1.77-fold higher recruitment of $Cd11b^+$ cells, respectively, compared to steady state earskin. Sand flies infected with either $LmxSF_i$ or $LmjSF_i$ produced a comparable influx of both neutrophils (median [M] of 33400, and 34750), and inflammatory monocytes (iMOs; M of 11750 and 10000), respectively, compared to steady state for neutrophils (M= 925) and iMOs (M=1250). A significant production of IL-1β was observed after infected sand fly bites, albeit at a lower magnitude in iMOs for both $LmxSF_i$ and $LmjSF_i$ (M= 2600, 3450) compared to neutrophils (M = 24280, 28400), respectively. Western blots of ear cell lysates from both *Leishmania spp*. 6 hours after sand fly bites show production of NLRP3 and cleavage of pro-IL-1β into its active form, indicative of pore formation and canonical inflammasome activation. Collectively, we show that a robust recruitment of innate immune cells after $LmxSF_i$, accompanied by a significant upregulation of inflammasome-derived IL-1β, reinforces the signature early host immune response to infected sand fly bites that regulates skin inflammation and modulates disease outcome.

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WS08 – RESPIRATORY IMMUNOLOGY

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2159 – WS08.1

Profiling maternal immune activation-induced alterations and training effects in offspring's immune cell compartments

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Background and objectives: The developmental origins of health and disease (DOHaD) concept has revealed the profound impact of the prenatal environment on postnatal health outcomes. We have previously shown that maternal chronic infection shapes the fetal immune system and response to antigenic challenges later in life. In the present study, we explore whether timely exposure to maternal immune activation (MIA) in utero alters stem cell development and modifies innate and adaptive immune cell compartments and their respective functionality.
Methods: We utilize a mouse model of MIA induced by the administration of Poly: IC during mid-gestation (E12.5) and analyze changes in 3-, 7- and 10-week-old offspring in regards to hematopoietic stem cell development and tissue-specific alterations of immune cell populations (DCs, B). Especially, we assess the MIA-induced alteration of FACS-sorted DC functionality.
Results: So far, we have observed a decrease of cDCs, especially cDC2, in lungs of MIA-offspring, including a decreased expression of CD80. This effect is more pronounced in females than in males, suggesting a sex-dependent maternal impact. Furthermore, we have observed alterations in the hematopoietic stem cells (HSCs) and other precursor cells, specifically the long-term (LT-HSC) and short-term (ST-HSC) HSCs, within the bone marrow. These changes indicate a shift towards activated hematopoiesis, where the cells are primed for differentiation rather than being maintained as stem cells. Moreover, we have also shown tissue-specific patterns in the immune alterations induced by MIA.
Conclusions: This research contributes to a better understanding of the developmental origins of health and disease theory and provides insights into the underlying mechanisms associated with immune alterations in offspring. Further work is ongoing to explore the extent of these changes and their influence on the development of allergic airway inflammation.

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938 – WS08.2

The Influenza Virus Hemagglutinin (HA): A Novel functional Ligand for the Immunosuppressive Receptor LAG-3

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In the realm of clinical research, there is growing interest in Lymphocyte Activation Gene 3 (LAG-3, CD223) as a promising inhibitory receptor, expressed on the surface of activated and exhausted CD4+ and CD8+ T cells, as well as regulatory T-cells. Its potential for enhancing immune responses against cancer and viral infections has sparked considerable attention. However, the identification of LAG-3 ligands remains elusive, impeding the development of LAG-3-targeted therapeutics. Concurrently, influenza viruses continue to pose significant global health challenges, with an observed inverse correlation between clinical symptoms and the frequency of influenza-specific T cells following infection. As such, there is a critical need for comprehensive research to unravel the role of LAG-3 during influenza virus infection and its potential implications.
The interaction between LAG-3 and the Influenza-virus infected cells, as well as the interaction with the influenza virus different proteins, was evaluated using Flow cytometry and ELISA and other immunological and virological assays. C57BL/6 mice model were used for the in vivo studies to understand the effect of LAG-3-Ig on the Influenza virus immune response.
We found that the Influenza virus hemagglutinin (HA) glycoprotein is a novel functional ligand for the immunosuppressive receptor LAG-3. Furthermore, blocking the interaction between LAG-3 and HA resulted in an improved antiviral $CD8^{+}$T cells response in vivo. Moreover, we showed that this binding is mediated by the glycans residues present on LAG-3. We suggest that blocking the LAG-3-HA interaction should serve as a new immune checkpoint target in Influenza virus infection.
We identified the Influenza virus HA protein as a new functional ligand for the inhibitory immune receptor LAG-3 and demonstrated that this interaction is glycan mediated. Thus, we uncovered a new immune checkpoint pathway that modulate the T-cell response during viral infection.

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1899 – WS08.3

Role of NK cell circadian clock in Influenza pathogenesis

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Purpose: Circadian rhythms provide an anticipatory system for the host to adapt to changes in its environment, including the threat of pathogens. Influenza infection causes extensive immunopathology; few consistently effective therapies exist. A hallmark of circadian control is that the response of the host varies by the time of day at which the insults are sustained. We have previously shown that mice infected at dawn had 3-fold better survival than those infected at dusk. Depletion of NK cells abrogated this time-of-day specific protection from IAV. This was mediated by exaggerated immunopathology but not by controlling early and peak viral titers. While the depletion experiment strongly supports the role of the NK cells in mediating the circadian regulation of lung injury, whether the NK cell-intrinsic clock is relevant to this process is not known.
Methods: To distinguish NK cell-intrinsic clock mechanisms from extrinsic circadian influences over this population, we generated a model wherein the clock is disrupted in the NK cells, *Ncr1cre*$^{+}$ *Bmal1*$^{fl/fl}$ (*Bmal1Δ*^{NK})**.** *Bmal1Δ*^{NK} and their WT littermates were infected with IAV (PR8) and lungs harvested for flowcytometry, viral titration, ELISA for cytokines and histological analyses, including Immunofluorescence staining. Further, we also sorted NK cells from the lungs of naive and flu-infected (day 4 post-infection) mice, and performed sc-RNA sequencing and analyses.
Results: *Bmal1Δ*^{NK} lungs had more leukocytes, but fewer NK cells in the uninfected state, and after infection, had 3-fold higher mortality, worse immunopathology, but fewer NK cells than WT littermates. Following IAV infection, the lungs of *Bmal1Δ*^{NK} mice had more Krt5^{+} areas and lesser SPC^{+} alveolar type 2 epithelial cells than WT controls, suggesting dysplastic repair in the mutant mice. Single-cell RNA sequencing of sorted pulmonary NK cells from both *Bmal1Δ*^{NK} and WT littermates revealed that the NK cell clock may be relevant to not only the regulation of host immune response but also lung repair processes during recovery from IAV.
Conclusion: In summary, NK cell- intrinsic circadian clock mediates regulation of host response to IAV through the regulation of acute inflammation and thereby affecting chronic repair.

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1031 – WS08.4

Deletion of Dipeptidyl peptidase 3 in mice unleashes protective antibacterial immunity against Klebsiella pneumoniae

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Klebsiella pneumoniae is a common cause of pneumonia, particularly in elderly and immunocompromised patients, often leading to sepsis. Over the past decades, the emergence of multi-drug resistant strains made the treatment of *K. pneumoniae* a big challenge. Recent studies have demonstrated that host defense plays a critical role in eradicating *K. pneumoniae*.
The dipeptidyl peptidase 3 (DPP3) is a ubiquitous cytosolic metallopeptidase acting in the degradation of various bioactive peptides. In addition, it has been involved in the Nrf2 antioxidant pathway. Its high blood concentrations, related to massive cell death and inflammation, predict high risk organ dysfunction and mortality in patients with septic shock. To address the relevance of DPP3 in *K. pneumoniae* infections, we applied a model of lung infection in the DPP3 knock-out mice. We evaluated the mortality and the pulmonary and systemic bacterial load at different time points post infection. The levels of pro- and anti-inflammatory cytokines were measured, histopathological damage and inflammation were assessed in the lung. Results showed that DPP3 deficiency was associated with markedly decreased lung colonization and systemic bacterial burden, resulting in a significant survival advantage. Upon bacterial infection, DPP3-/- mice exhibited reduced tissue damage and weakened pulmonary and systemic inflammation compared with WT mice. Adoptive transfer experiment indicated that deficiency of DPP3 in immune cells was sufficient to enhance the lung bacterial clearance. Thus, we further used transcriptomics and cellular assays to define the specific role of DPP3 in lung immune cell biology. We found that lack of DPP3 induced transcriptional and metabolic programs characteristic of effector cells, associated with enhanced inflammatory signaling pathways. Accordingly, both innate and adaptive DPP3-deficient immune cells exerted more potent antimicrobial responses, particularly ROS and Th1/Th17 cytokines production. These findings point to DPP3 as novel immune checkpoint that shapes immunity by controlling the threshold for activation and resistance pathways. These results provide a framework for new therapeutic strategy against K. pneumoniae through improving host immunity.

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411 – WS08.5

Mast cell granules serve as endogenous C-type lectin receptor ligands skewing dendritic cell function towards type II immunity

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Purpose: Mast cells (MCs) are best known as key effector cells of type I allergic reactions. Despite increasing evidence for an additional, critical impact of MCs on adaptive immunity, the underlying mechanisms are poorly understood. We recently reported that, upon skin inflammation, dermal dendritic cells (dDCs) engulf MC secretory granules (MCG), which boosts DC functionality and thereby adaptive immunity remotely (Dudeck et al. 2019). Based on this study, we intended to decipher underlying molecular mechanisms and consequences on DC functions.
Methods: Different transgenic mouse lines were used to clarify mechanistic details of the MCG uptake process. Mouse models of inflammatory disorders, basically hapten-induced skin inflammation and allergic airway inflammation, were used to determine the relevance of involved mechanisms for the respective immune response.
Results: We could show, that immature DCs sense and engulf MCG in a Card9-dependent manner. Consistently, Card9 deficiency resulted in a reduced T cell priming/differentiation upon hapten sensitization and diminished T cell driven adaptive ear swelling response upon elicitation. Questioning the MCG sensing DC surface receptors, we could identify that the c-type lectin receptors (CLRs) MCL and SIGNR3 bind to MCG and mediate their uptake. Most importantly, MCG uptake by DCs modulated their antigen-presenting capacity towards a type II immune response. Hence, mice that lack both, *Mcl* and *Signr3* expression, showed reduced allergic airway inflammation, in line with reduced cDC2 numbers and Th2 cytokine production.
Conclusions: Herein, we show that MCG are endogenous CLR ligands that translate (MC degranulation inducing) danger signals into adjuvant effects promoting lymph node-borne adaptive immunity. Understanding the uptake mechanism and DC modulating properties of MCG may give rise to therapeutic strategies to either intentionally boost adaptive immunity or dampen elevated immune responses.

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1286 – WS08.6

Molecular regulation of innate lymphoid cell type 2 in inflammatory lung disease

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Airway inflammatory diseases like asthma and chronic obstructive pulmonary disease (COPD) are common disorders of increasing prevalence, for which no curative therapies currently exist. The phosphoinositide-3 kinase (PI3K) is often hyperactivated in these chronic lung inflammatory disorders, thereby promoting disease. The inositol phosphatase SHIP1 is a negative regulator of the PI3K pathway in hematopoietic cells, and SHIP1-deficient mice (styx) spontaneously develop a chronic airway inflammatory disorder that exhibits characteristics of both asthma and COPD. Here, we found that genetic ablation of IL-33 signaling prevented lethal inflammatory lung disease in styx mice. Innate lymphoid cells type 2 (ILC2s) were the main cells expressing of IL-33 receptor in lung infiltrates of styx mice, suggesting these cells as disease initiators. Indeed, lymphocyte deletion entirely rescued airway disease in lymphocyte-deficient Rag2-/-/Il2rg-/-/styx mice. Furthermore, depletion of ILC2s prevented, while adoptive transfer of ILC2s or associated cytokines restored lung disease in our model. Compared to other immune subtypes in the mouse lung, SHIP1 expression is particularly high in ILC2. In vitro experiments and single-cell RNA sequencing analyses indicate an intrinsic role of SHIP1 in restraining both murine and human ILC2 function. Lastly, SHIP1 expression is altered in lung ILCs from COPD versus control patients, suggesting a contribution of SHIP1 to ILC regulation in humans with chronic lung inflammatory disorders.
These findings collectively emphasize the critical role of SHIP1 in restraining ILC2 activity during airway inflammation and provide compelling support for the exploration of small-molecule SHIP1 agonists as a therapeutic approach for individuals suffering from asthma or COPD.

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WS09 – NEUROINFLAMMATION I

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1688 – WS09.1

IgA production decrease is associated with B cell differentiation and proliferation defects in multiple sclerosis

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Objectives: Multiple sclerosis (MS) is an inflammatory disease of the central nervous system leading to major disabilities in young adult. Growing evidences show the important role of B cells through their antibody/proinflammatory cytokine production and antigen presentation in MS pathology. Opposite to IgG+ B cells exercising inflammatory functions, IgA secreting plasmablasts / plasma cells are able to downregulate neuroinflammation in experimental autoimmune encephalomyelitis. The failure of TACI-Ig as an MS therapy, a treatment that targets all B cells including plasma cells, confirmed the protective and regulatory role of plasma cells in MS. We aim here to studying plasma cells in MS.
Methods: As plasmablast/plasma cells are rare in blood, they were differentiated *in vitro* from sorted blood B cells of MS patients and healthy controls (HC). Cytokines and immunoglobulins from B cell culture supernatants were measured by Luminex. In blood and cerebrospinal fluid (CSF), immunoglobulins were measured by ELISA. A single cell RNA sequencing was performed on PBMC from MS patients and HC using 10X genomics technology.
Results: We reported a lower differentiation and a lower proliferation of B cell from MS patients associated to a decrease of IgA production. MS patients also exhibited a lower concentration of serum IgA and IgA represented a lower proportion of immunoglobulins in the CSF compared to HC. Moreover, we observed a lower frequency of IgA^+ memory B cells and their activated subset in the blood of RRMS patients during relapse compared to HC. Finally, memory IgA+ B cells exhibit a specific transcriptomic signature in MS patients compared to controls.
Conclusions: IgA^+ B cells, that have been described as a potential reservoir of regulatory B cells, exhibited defects of proliferation and survival in MS. With a specific transcriptome not observed in other Ig-secreting B cells, IgA^+ B cells appear as an important component of B cell response in MS, which could be a target of future therapeutic strategies.

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72 – WS09.2

6-Formylindolo(3,2-b)carbazole ameliorates Graft-versus-Host Disease of the central nervous system via arylhydrocarbon receptor/NF-κB pathway in microglia

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Purpose: 30-50% of patients receiving allogeneic hematopoietic cell transplantation (allo-HCT) develop acute Graft-versus-Host Disease (aGvHD), also affecting the central nervous system (CNS). The majority of allo-HCT patients need to undergo antibiotic therapy due to infectious complications which alter the microbiome, thereby promoting GvHD. Therefore, we investigated the mechanism through which microbiota modulates GvHD of the central nervous system to offer a potential new therapeutic target.
Methods: In a murine MHC II-mismatch transplantation model (C57BL/6 to Balb/c), we administered antibiotic treatment orally every day. We analyzed the brain using histological techniques including confocal imaging and investigated underlying cellular mechanisms *in vitro* using primary murine microglia and the arylhydrocarbon receptor (AhR) ligand 6-formylindolo(3,2-b)carbazole (FICZ) as *in vivo* experiments revealed reduced AhR signaling. We further confirmed our results *in vivo* by treating GvHD mice with FICZ.
Results: Antibiotic treatment of GvHD mice led to a significant increase in microglia and T-cell numbers in the brain, indicating increased inflammation. Immunofluorescence analysis showed a proinflammatory phenotype for microglia with increased NF-κB and Src activity. In addition, their morphology shifted to a highly branched phenotype compared to vehicle treated GvHD mice. Staining for the arylhydrocarbon receptor, a receptor for microbial metabolites, revealed a significant decrease in nuclear localization following antibiotic treatment. Alongside, treatment of primary murine microglia with the AhR ligand FICZ resulted in reduced expression of Syk and p38 MAPK as well as disrupted NF-κB signaling. FICZ treatment reduced the expression of NF-κB and significantly blocked its nuclear translocation upon lipopolysaccharide stimulation in primary microglia. Our observations on diminished NF-κB signaling were confirmed by the reduced secretion of the cytokines IL-6, MCP-1 and TNF-α. Microglia's functional analysis showed a decrease in phagocytosis and migration *in vitro*. When translating these findings to the *in vivo* model, we could demonstrate that FICZ treatment reduced T-cell infiltration into the brain, the number of microglia and the morphological changes of microglia in GvHD mice, indicating reduced inflammation.
Conclusion: Antibiotic treatment increases brain inflammation in GvHD. The AhR ligand FICZ rescues CNS inflammation by inhibiting NF-κB, thereby providing a potential target to treat Graft-versus-Host Disease of the CNS.

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434 – WS09.3

Long-term use of rituximab increases T cell count in MS patients

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Purpose: Rituximab has been used to treat MS patients in Iceland for over a decade. However, long-term effect of rituximab on the leukocyte population has not yet been elucidated. We investigated whether a long-term effect of rituximab on the leukocyte population in Icelandic MS patients was present and evaluated whether the effect is dose dependent (1000 mg vs 500 mg).
Methods: Retrospective analysis of flow cytometric data from 349 patients visiting the neurological ward at The National University Hospital of Iceland from 2012 to 2023 for rituximab treatment. Differential counts and classification of leukocytes were analysed.
Results: No difference in efficacy of B cell depletion was detected in patients treated with 500mg compared to 1000 mg of rituximab. Efficacy of the treatment was neither age nor sex dependent. The persistence of depletion was stable for both doses for 300 days. However, long-term use of rituximab led to an increase in T cell count (p=0.0015) in patients receiving 3-8 doses of rituximab (1.5-8 years of treatment). The increase occurred in both CD4+ (p=0.0028) and CD8+ T cells and led to a decrease in the CD4/CD8 ratio (p=0.004).
Conclusion: Since no difference in B cell depletion was detected between the two patient groups, 1000mg might be an excessive dose. However, the clinical implications of long-term treatment of rituximab and its effect on the T cell pool needs to be explored further. Based on this data a personalized dosing regimen might have therapeutic and financial advantages that should be explored further.

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538 – WS09.4

Targeting pyruvate kinase M2 to limit T cell pathogenicity in multiple sclerosis

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Multiple sclerosis (MS) is a chronic autoimmune inflammatory disease of the central nervous system (CNS) in which infiltration of leukocytes into the CNS leads to neuronal death, cognitive impairment, and disability. Among immune cells, T lymphocytes are key players in MS pathogenesis, controlling the development of CNS inflammation. Immunometabolism studies have shown that T cells regulate their activity by modulating their intracellular metabolic profile, and that targeting T cell metabolism represents a novel strategy for the treatment of autoimmunity. In particular, recent works suggested that the glycolytic enzyme pyruvate kinase M2 (PKM2) may be a potential therapeutic target in T cell-mediated autoimmune neuroinflammation. PKM2 can translocate into the nucleus in its monomeric/dimeric form, where it performs moonlighting functions different from its canonical enzymatic one, such as regulation of gene transcription. Several pre-clinical studies have demonstrated that PKM2 moonlighting activity controls T cell pathogenicity in the CNS and modulates neuroinflammatory responses in experimental autoimmune encephalomyelitis, a mouse model of MS. This project aimed to investigate the role of PKM2 in T cell inflammatory potential in human donors and MS patients. We first observed that PKM2 is differentially expressed in blood T cell subpopulations, with CD4+ T cells showing higher PKM2 levels compared to CD8+ T cells. Moreover, central memory, effector memory, and effector T cells display higher PKM2 expression, compared to naïve T cells. Interestingly, T cells from MS patients may display higher PKM2 levels than T cells from control individuals, depending on the disease form and activity. We then evaluated whether limiting PKM2 moonlighting activity with the PKM2 allosteric activator TEPP-46 may impact the inflammatory profile of T cells from healthy and MS individuals. We found that TEPP-46 inhibits T cell proliferation and decreases pro-inflammatory cytokine production by T cells in both control donors and MS patients, suggesting that PKM2 may control T cell pathogenicity. Overall, our data indicate that PKM2 may represent a novel biomarker for T cell activation and MS activity, and that targeting PKM2 moonlighting functions may be of relevance in MS. PKM2 allosteric activators thus represent promising pharmacological tools for MS treatment.

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1985 – WS09.5

Natalizumab Treatment Induces Proinflammatory CD4 T Cells Preferentially in the Integrin β7+ Compartment

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Natalizumab, a monoclonal humanized antibody targeting integrin α4, inhibits the transmigration of lymphocytes into the CNS by preventing the interaction of integrin α4β1 with V-CAM expressed on brain vascular endothelial cells. Although natalizumab treatment reduces the clinical relapse rate in patients with relapsing-remitting MS, its discontinuation after reactivation of the JC virus is associated with a rebound of the disease in 20% of patients. The mechanisms of this rebound are not elucidated, but natalizumab increases the frequencies of circulating CD4 T cells expressing proinflammatory cytokines as well as the proportion of circulating Th17/Th1 cells (Th1-like Th17 cells). Gut-derived memory CD4 T cells are a population of growing interest in the pathogenesis of MS, but whether and how their properties are affected by natalizumab is not known. Here, we studied the phenotype and cytokine expression profile of circulating gut-derived memory CD4 T cells in patients with relapsing-remitting MS under natalizumab. We identified gut-derived memory CD4 T cells by their expression of integrin β7 and compared their properties and those of integrin β7- memory CD4 T cells across healthy donors and patients with relapsing-remitting MS treated or not with natalizumab. We also compared the capacity of integrin β7- and integrin β7+ CD4 T-cell subsets to transmigrate in vitro across a model of blood-brain barrier. The proportions of proinflammatory Th17/Th1 cells as well as of IL-17A+IFNγ+ and IL-17A+GM-CSF+ cells were higher in memory CD4 T cells expressing integrin β7 in patients receiving natalizumab compared with healthy donors and patients with relapsing-remitting MS not receiving natalizumab. By contrast, integrin β7 negative memory CD4 T cells only presented a modest increased in their proportion of Th17/Th1 cells under natalizumab. We further observed that integrin β7+ Th17/Th1 cells migrated as efficiently as integrin β7- Th17/Th1 across a monolayer of brain microvascular endothelial cells. Our study shows that circulating integrin β7+ memory CD4 T cells of patients with relapsing-remitting MS under natalizumab are enriched in proinflammatory cells supporting the hypothesis that integrin β7+ memory CD4 T cells could play a pathogenic role in the disease rebound observed at natalizumab discontinuation.

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1283 – WS09.6

Primed monocytes in gamma-herpesvirus-induced EAE exacerbation

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Purpose: Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS). MS is a multifactorial disease caused by genetic, immunological and environmental factors. The most strongly associated environmental risk factor for MS is Epstein-Barr virus (EBV), for which it is still unclear how it contributes to disease development. While most studies on EBV and MS focus on lymphocytes, here the contribution of EBV-imprinted myeloid cells to MS development is investigated.
Methods: Experimental autoimmune encephalomyelitis (EAE) is induced in mice pre-infected with Murid Herpesvirus 4 (MuHV-4). Phenotypic changes of CNS infiltrating immune cells are determined by flow cytometry. To find human translation, the phenotype of monocytes of EBV seropositive and negative donors are compared.
Results: A significantly worse EAE outcome and an increase in CNS infiltrating immune cells were found in pre-infected mice. MuHV-4 infection led to a high activation status of monocytes in blood, shown by an upregulation of MHC-II, Sca-1, CD86, Saa3 and CXCL9 and this phenotype remained present after the lytic infection phase. After EAE induction, an increased infiltration of these monocytes is found in the CNS of MuHV-4 infected mice. Also, highly activated microglia are seen after MuHV-4 infection. EBV seropositivity in humans altered the phenotype of monocytes resulting in more CXCL9 and CCR2, while reducing the anti-inflammatory phagocytosis receptor CD93 and the myelin scavenger receptor CD36. MuHV-4 infected mice show a similar disease aggravation after adoptive transfer of MOG35-55 T cells, further indicating that the observed MuHV-4-mediated EAE aggravation is not directly mediated by T cells. Finally, monocyte depletion during EAE diminished clinical symptoms to the level observed in non-infected mice without affecting T cell phenotype, indicating a causative role of monocytes in MuHV-4-mediated EAE exacerbation.
Conclusion: EBV mediated priming of the myeloid cell compartment is seen in both humans and mice and leads to worse disease. Future research in this understudied field focuses on what myeloid changes worsen disease symptoms and thus provide support for EBV targeted interventions.

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WS10 – BACTERIAL AND VIRAL IMMUNITY

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361 – WS10.1

A Comprehensive 31-Months Longitudinal Study on SARS-CoV-2 Seroconversion, Reinfection, and Neutralization Spanning Several Variant Waves and Vaccination Campaigns

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Background: Longitudinal monitoring of SARS-CoV-2-specific antibody responses in the population has enriched our knowledge about the virus. In the face of ever-changing SARS-CoV-2 variants, regular sample collection irrespective of the presence of symptoms provides representative data on infection and seroconversion/seroreversion rates.
Methods: Our 31-months longitudinal seroepidemiological study (n=1446) in the community of the first German SARS-CoV-2 superspreading event includes analyses of acute infection, seroconversion, virus neutralization, T cells and cross-protective immunity against SARS-CoV-2 omicron variants.
Results: Spike(S)-specific IgAs decreased shortly after initial infection, while IgGs remained stable. Expectedly, both increased significantly after vaccination. Our longitudinal analysis predicts an 18-months half-life of S IgGs upon natural infection. N-specific responses declined over a period of 12 months after the initial infection, but showed an increase ($p < 0.0001$) during omicron. Frequencies of SARS-CoV-2-specific $CD4^+$ T cells expressing TNF-α and IFN-γ declined for 12 months after infection ($p < 0.01$; $p < 0.01$, respectively). S antibody levels and neutralization titers were highest in triple-vaccinated participants infected late in the pandemic compared to those infected early. Cross neutralization against omicron strains BQ.1.18 and XBB.1.5 was extremely low in all groups.
Conclusion: N-specific antibodies strongly increased during new sub-variants´ infection waves, but did not correspond to better neutralization against new variants.

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283 – WS10.2

SUV39H1 controls the differentiation of CD8+ tissue resident memory T cell precursors

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Introduction: Activation of CD8+ T cells leads to the differentiation of short-lived terminal effectors and long-lived memory precursors. Some of these memory precursors remain in lymphoid organs and become central memory cells (TCM), while others home to non-lymphoid peripheral tissues early after antigen recognition and give rise to tissue resident memory T cells (TRM). The early stages of memory precursor differentiation into TCM and TRM remain poorly understood.
Aim: Explore the role of the histone methyl-transferase SUV39H1 on the differentiation of CD8+ TRM cells
Methods: We characterized CD8+ T cells residing in different lymphoid and non-lymphoid tissues of SUV39H1-KO and conditional SUV39H1-flox-CD4-Cre mice and WT littermates at steady state and upon flu infection by flow cytometry. We also explored tissue homing and TRM differentiation of SUV39H1-KO or WT TCR transgenic OT-I CD8+ T cells upon adoptive transfer into lymphopenic mice and upon flu infection by flow cytometry and CITE-seq. Finally, we analysed the capacity of SUV39H-KO lung-homing cells to delay lung tumor metastases compared to WT OT-I cells.
Results: We show that at steady state, during homeostatic proliferation and upon flu infection, lack of SUV39H1 in CD8+ T cells leads to the accumulation of CD49d+ CD69+ CD103- TRM cells in lungs and other non-lymphoid tissues. CITEseq analysis showed that SUV39H1-defciency increased proportions of CD49d+ CD8+ T cells which can differentiate into CD69+ CD103- TRM cells, suggesting that SUV39H1 can restrain TRM precursor differentiation. Consistently, SUV39H1-deficient CD8+ T cells accumulating in lungs upon flu infection give rise to CD69+ CD103- TRM after adoptive transfer. Finally, SUV39H1-defective TCR transgenic OT-I TRM cells that accumulated in lungs delay tumor growth more effectively than SUV39H1-sufficient OT-I cells. These results show that SUV39H1 in CD8+ T cells restricts the differentiation of TRM precursors, reducing CD69+ TRM colonization and persistence in lungs and other peripheral non-lymphoid tissues. Together with previous studies showing that SUV39H1 restricts the differentiation of TCM precursors, the results presented here indicate that TCM and TRM share epigenetic mechanisms of precursor differentiation.

Authors' Disclosures: SA is Scientific co-founder and Chief Schientific Oficer of MNEMO therapeutics and owner of a patent on SUV39H1.

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1242 – WS10.3

Latent Cytomegalovirus upon Intranasal Infection provides immune protection against COVID in experimentally infected mice

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Introduction: Cytomegalovirus (CMV) is a ubiquitous herpesvirus that elicits a uniquely strong and lasting memory T cell response, which is a major driver for immune system variation. Limited clinical data have argued for a potential adverse effect of latent herpesvirus infections on the outcome of COVID-19 infections, but experimental and mechanistic validation have been lacking.
Methods: Using a mouse model of intranasal infection with mouse CMV (MCMV) and superinfection with a SARS-CoV-2 adapted variant (MA-10), we tested the effects of a latent CMV infection on COVID clinical outcomes and virological parameters. Mice were latently infected for at least 3 months, challenged with MA-10, followed by clinical, immunological and virological analysis and comparison to MCMV-naïve controls.
Results: We observed that both young and aged mice latently infected with MCMV display less weight loss and milder clinical symptoms than control mice upon MA-10 challenge. This was accompanied with lower SARS-CoV-2 RNA copy numbers and less plaque forming units in lungs, arguing for antiviral and protective effects of latent MCMV. In mice with latent MCMV infection, we also observed higher frequencies of CD8 T cells recognizing a SARS-CoV-2 antigenic peptide upon challenge. Interestingly, the same mice featured strong CD8+ tissue resident memory T cells (T_{RM}) in lungs and inflationary effector memory T cells in the blood (T_{EM}) before challenge. Latency upon intraperitoneal MCMV infection was not protective against COVID in a related model of challenge, and induced no T_{RM} responses in the lungs. To define the role of these cells on protection against COVID, we used mice that lack NFATc1 in T cells (N*FATc1*$^{fl/fl}$*Cd4cre*). These mice mount primary CD8+ T-cell responses to MCMV infection, but not the lasting CMV-specific T_{EM} or T_{RM} responses (Chaudhry et al. 2024). While naïve N*FATc1*$^{fl/fl}$*Cd4cre* mice were equally affected by SARS-CoV-2 infection as the parental C57BL/6 mice, we observed a difference in mice latently infected with MCMV: in NFATc1-deficient mice, the protective effect of MCMV against COVID was absent.
Conclusion: Our results strongly suggest that latent MCMV infection was protective against SARS-CoV-2 infection in mice due to an activation of T_{RM} CD8+ T cells, which facilitated antiviral defenses.

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531 – WS10.4

Bifidobacterium shapes antimicrobial T-cell responses during infancy and adulthood

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Bifidobacterium is the first microbial coloniser of the gut and is therefore involved in the establishment of tolerance to innocuous environmental antigens. Although *Bifidobacterium* is thought to have a beneficial effect on health, its mechanism of action is not well understood. Our study examines age-related differences in T-cell responses to different strains of the bacteria *Staphylococcus sp.* and *Bifidobacterium*.
To achieve this, we enriched naive $CD4^+$ T cells from children of different ages, as well as umbilical cord and peripheral blood from healthy donors, using magnetic cell sorting. We characterised these cells using flow cytometry and functional tests. To stimulate the enriched T cells, non-classically matured monocytes were used. This was achieved by pulsing $CD14^+$ monocytes with heat-inactivated extracts of *S. aureus*, *S. epidermidis* and *B. infantis*.
Analyses of T-cells from the different age groups show that $CD4^+$ T-cell responses are initiated from birth with bacterial-specific properties. T-cells activated by *Staphylococcus sp.* display an enormous proliferative capacity, increased plasticity and diversity, whereas *B. infantis* induced T-cell responses were significantly attenuated. Interestingly, there is an inverse correlation between the percentage of proliferating T-cells induced by *S. aureus* and the age of the children. Additionally, we observed by RNA sequencing that *B. infantis*-stimulated T-cells exhibited a regulatory phenotype. In line with these results, we found that *B. infantis* increased the frequency of CTLA-4-dependent $FOXP3^+$ T-cells, suggesting an induction of Treg cells. High levels of galectin-1 was detected in cell culture supernatants of *B. infantis*-stimulated T-cells, which may act as an autocrine negative growth factor regulating cell proliferation and activation. Strikingly, pre-stimulation of T-cells with *B. infantis* not only suppressed activation of *S. aureus* and *S. epidermidis*-specific T-helper cells but also dampened the "cytokine storm" of critically ill COVID-19 patients. These observations suggest that *B. infantis* could be used at any age as a tool to mitigate and resolve immune system overreactions.
Our study demonstrates, for the first time, how *B. infantis* specifically modifies the responsiveness of $CD4^+$ T-cells and the underlying regulatory mechanisms that play an important role in shaping antigen-specific responses of human T-cells.

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1179 – WS10.5

Cross reactive responses between filovirus species amongst a cohort of Ebolavirus Zaire survivors in Guinea, West Africa

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Purpose: New and emerging filoviruses such as Ebola and Marburgvirus pose a major risk to public health as demonstrated by recent outbreaks across West and Central Africa. Amongst a cohort of Ebolavirus disease survivors in Guinea, West Africa we examined the cross-reactive response to Ebolavirus Sudan and Marburgvirus glycoproteins.
Methods: We used an in-house anti-glycoprotein ELISA and pseudovirus neutralisation assays to investigate the cross-reactive serological response. Additionally, we used IFNγ ELISpot and memory B cell phenotyping to examine the cross-reactive cellular response augmented by T and B cells.
Results: We assayed 40 serum or peripheral blood mononuclear cell (PBMC) samples from Ebolavirus disease survivors, who contracted Ebolavirus Zaire in 2015. Samples were collected in 2024 and it was found that at the humoral level all survivors showed a response to Ebolavirus Zaire glycoprotein, whereas 75% (30/40) showed a cross-reactive response to Sudan Ebolavirus and 40% (16/40) showed a cross-reactive response to Marburgvirus, however the magnitude of these responses varied. At the cellular level we found that 70% (28/40) showed a T cell response to Ebolavirus Zaire glycoprotein peptides, whereas 10% (4/40) and 0% (0/40) showed a T cell response to Ebolavirus Sudan or Marburgvirus glycoprotein peptides respectively. Using Ebolavirus species specific fluorescently conjugated glycoprotein we were able to identify Marburgvirus or Ebolavirus Sudan cross-reactive memory B cells and show that these are primarily activated memory, memory or atypical memory B cells.
Conclusion: We conclude that the natural immune response to Ebolavirus Zaire leads to the generation of detectable immunity across the species. It is unknown what level of protection, if any, these cross-reactive responses will provide. However, these cross-reactive responses will provide the foundations for any future immune response across the filovirus family. The identification of cross-reactive memory B cells could have implications for the identification of broadly reactive B cell clones that could be used to design therapeutics.

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675 – WS10.6

Investigating the effects of variability of *Streptococcus agalactiae* isolates on macrophage immune response and pathogenesis

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Purpose: *Streptococcus agalactiae* (Group B *Streptococcus*, GBS) remains one of the main causes of invasive neonatal infections, usually manifesting as sepsis, meningitis, or pneumonia. As intrapartum antibiotic prophylaxis is only effective in preventing early-onset disease, but not in preventing late-onset disease and infections in the elderly and immunocompromised individuals, further research into GBS-associated pathogenesis is needed. To date, 10 different serotypes (Ia, Ib, II – IX) have been described, which differ in their virulence and the immune response they elicit.
Methods: Since the severity of the disease depends on both the GBS isolate and the immune status of the individual, we investigated how genotypic and phenotypic differences between GBS isolates influence the innate immune response of macrophages. The immune response was studied at multiple levels, from phagocytic uptake to inflammatory mediator production, inflammatory cell death and metabolic changes, both at the functional assay level and at the gene expression/protein production level using flow cytometry, Seahorse extracellular flux analyzer, quantitative real-time PCR, fluorescence microscopy, and multi-modal microplate readers.
Results: Significant isolate-specific differences in phagocytic uptake and expression of polarization markers characteristic of M1 or M2 macrophages were observed. By measuring inflammatory and anti-inflammatory cytokines and chemokines as well as the expression of genes involved in antimicrobial activity and inflammation at different time points, the data also revealed that different isolates have a different potential to become invasive or remain colonizing. By measuring LDH secretion, IL-1β and IL-18 production, as well as caspase-1 and caspase-3 expression and activity, we further demonstrated that individual isolates strongly induce the formation of an inflammatory cell death, pyroptosis. Furthermore, by measuring extracellular acidification, oxygen consumption, and glycolytic gene expression, differences in the metabolism of infected macrophages were also observed.
Conclusion: Our data suggest that the severity of the disease depends not only on the immune status of the individual but also on the isolate itself, as infection with different isolates leads to significant differences in the immune response of macrophages and to varying degrees of induction of inflammation and cell death.

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WS11 – INNATE AND ADAPTIVE IMMUNE CELLS IN INFLAMMATION

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1346 – WS11.1

CXCR4 minor pocket ligands inhibit immune response by selectively blocking TLR7/8 mediated phosphorylation of NFκB

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Purpose: Toll-like receptors (TLRs) are stimulated by pathogens. The activation by viral DNA/RNA in the endosome or by bacterial components on the cell surface can be mimicked by synthetic resiquimod (R848, TLR7/8) or lipopolysaccharides (LPS, TLR2/4). CXCR4, a chemokine receptor, has recently been published to regulate the production of type I interferons in virus treated monocytes. Treating cells with CXCR4 minor pocket ligands (MPL) leads to a reduced immune response, this effect can be abolished by addition of the CXCR4 antagonist AMD3100 or by treatment with siRNA targeting CXCR4. It is unclear how MPLs reverse the immune response of TLRs. Here, the MPLs IT1t, CB, and NP1411 were investigated in their impact on TLR activation and in their signalling at CXCR4.
Methods: HTRF kits based on an antibody sandwich technique were used to look at the phosphorylation of key signalling proteins (NFκB, and IKK-β) in THP-1 cells. The activation of NFκB and the IRF pathways were further monitored in a THP1-dual cell line stably transfected with reporters for both pathways in the presence and absence of various compounds including R848, IT1t and PKA blockers. G protein activation recruitment was measured at CXCR4 in HEK293T cells transfected with BRET sensors. (All:n≥3)
Results: IT1t reversed the R848-mediated but not the LPS-mediated phosphorylation of the transcription regulator NFκB. CXCR4 was previously suggested to increase PKA activity via an increase of cAMP. In agreement, IT1t showed an inhibition of basal G_i-protein activity at CXCR4, thereby increasing cAMP and PKA activity. When testing the initial hypothesis by adding cAMP or PKA blocker, they surprisingly reduced the response of R848 on their own opposite to the previously stated mechanism.
Conclusion: So far, CXCR4's role as a regulator in immune response has not been fully elucidated. While our studies show a clear and direct impact of the compounds on TLR and CXCR4 signalling, it is unclear from which step onwards the signalling of R848 is impacted. The selectively towards TLR7/8 over TLR2/4 opens new questions. Differences could be caused by the endosome vs cell surface environment but could also be based on target engagement interference.

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250 – WS11.2

Investigation into obesity related defects in MAIT cells - is glutamine the missing link?

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Mucosal Associated Invariant T cells or MAIT cells are important innate effectors that produce pro-inflammatory cytokines such as IFNg and IL17. MAIT cells are not only critical for fighting bacterial and viral infections. They are also emerging to be important in defense against cancer. TCR+cytokine induced activation of MAIT cells results in a change in MAIT cell metabolism whereby MAIT cells show a significant upregulation of glycolysis. This glycolytic reprogramming is essential for MAIT cell function and proliferation. We have also previously shown that mTORC1 and cMyc play an integral role in enabling this metabolic switch. However, little is known about amino acid uptake and requirement to support MAIT cell function and metabolism. Here we show by proteomic analysis that several amino acid transporters including the glutamine transporter SLc1A5 is upregulated in activated MAIT cells. Along with this, the enzymes involved in glutamine lysis such as GLUD1 and GLS are also upregulated. We explore the requirement of glutamine in further detail by utilizing the inhibitor of GLS -CB839 which led to MAIT cell dysfunction in vitro. Furthermore, we show that enzymes involved in glutamine metabolism are defective in obesity. These data identify an essential role for glutamine and its metabolism in optimal MAIT cell responses upon activation.

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70 – WS11.3

Eosinophil peroxidase induces a pro-resolving phenotype in macrophages

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Purpose: Macrophages play a pivotal role in the development of rheumatoid arthritis. While classical macrophages contribute to inflammation, alternatively activated macrophages (AAMs) counteract inflammation through the production of anti-inflammatory cytokines and the clearance of dying cells via efferocytosis. Eosinophils are recognized as crucial regulators of AAM polarization. Recent findings indicate that this regulatory mechanism extends to the synovial environment, where eosinophils govern the resolution of inflammatory arthritis by inducing AAMs. Given that eosinophils are significant producers of eosinophil peroxidase (EPX) and heme peroxidases have been associated with monocyte/macrophage polarization, our study aims to elucidate the role of EPX in macrophage polarization and the resolution of inflammatory arthritis.
Methods: Bone marrow derived macrophages (BMDMs) were stimulated *in vitro* with EPX without and with the following stimuli: IL-4 for the polarization of classical AAMs and Lipopolysaccharide (LPS) for the polarization of classical pro-inflammatory macrophages. To dissect the phenotypic characteristics of macrophages in response to EPX, we examined their gene expression signature by bulk RNA sequencing, their polarization status by flow cytometry and immunofluorescence microscopy, their secretion profile by ELISA and their metabolic switch by extracellular flux assays. Moreover, the effect of EPX on the morphology and efferocytosis capacity of macrophages was illustrated by Spinning Disc confocal microscopy using BMDMs from *Cx3cr1^{cre}Rosa26*(*R26*)*-tdTomato* mice.
Results: The stimulation of macrophages with EPX caused their polarization into AAMs. Our data revealed a distinct anti-inflammatory macrophage subtype characterized by the upregulation of the anti-inflammatory markers PD-L2, CD206, along with induction of the efferocytosis markers CD36, CD93, and Trem2. In contrast, pro-inflammatory genes such as NOS2, IL-1β, IL-6, and TNFα were downregulated. Strikingly, EPX-treated macrophages displayed enhanced mitochondrial respiration and glycolysis, suggesting that these cells generally have a higher energy consumption. Visually and mechanistically, EPX altered the morphology of macrophages and enhanced their efficiency to take up apoptotic neutrophils.
Conclusion: These findings indicate a hitherto undiscovered pro-resolving signature in macrophages upon EPX treatment that is independent of the classical IL-4-mediated pathway. EPX induces the expression of AAM-related surface proteins, influences the metabolic state, promotes the secretion of anti-inflammatory cytokines, and facilitates the efferocytosis of apoptotic neutrophils.

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1644 – WS11.4

Endogenous Secretory leukocyte protease inhibitor contributes to regulation of inflammatory responses in peritoneal macrophages by modulating Matrix metalloproteinase-9 production

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Purpose: Secretory leukocyte protease inhibitor (SLPI) is described as a potent regulator of inflammation and tissue homeostasis with pleiotropic functions. It has been shown to inhibit NF-κB and proinflammatory responses in macrophages. However, its expression patterns and specific functions in different macrophage populations remain poorly understood.
Methods: SLPI expression was analysed in various mouse macrophage populations using databases, RNA-Seq data, flow cytometry, and gene expression analysis. Additionally, the impact of endogenous SLPI on macrophage activation was investigated by proteome array and myeloid cells were compared during *in vivo* inflammatory responses.
Results: Among macrophage populations, peritoneal macrophages exhibited the highest endogenous SLPI expression. While SLPI deficiency did not affect proinflammatory cytokine production in activated macrophages, it regulated Matrix metalloproteinase-9 (MMP-9) expression. Similar results were observed in thioglycolate-elicited macrophages. Furthermore, *in vivo* administration of LPS induced changes in SLPI expression across myeloid populations.
Conclusion: SLPI expression by macrophages is modulated during both homeostasis and inflammation. Contrary to expectations, endogenous SLPI does not inhibit proinflammatory cytokine production but specifically upregulates MMP-9 expression in peritoneal macrophages. These findings highlight a specific role for SLPI in inflammation, rather than broad anti-inflammatory properties and suggests that SLPI might play a role in tissue remodelling orchestrated by macrophages.
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302 – WS11.5

The involvement of plasmacytoid dendritic cells and endoplasmic reticulum stress in systemic sclerosis-associated fibrosis

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Systemic sclerosis (SSc) is a rare autoimmune disease characterized by microvascular damage, chronic inflammation, and fibrosis of the skin and, in some cases, of internal organs. Recent studies suggest that plasmacytoid dendritic cells (pDC), immune cells specialized in type I interferon (IFN-I) production, are key in the fibrotic process, although the mechanism remains to be elucidated. Due to their high secretory capacity, these cells possess a large endoplasmic reticulum (ER) and basal activation of the unfolded protein response (UPR), which is triggered by ER stress. We have found that pDC activation is impacted by ER stress, and it has been recently suggested there is dysregulation of UPR-related genes in pDC of SSc patients. Therefore, we are exploring the contribution of pDC and ER stress for fibrosis development. We found that CAL-1, a pDC cell line, and IMR-90 lung fibroblasts physically interact when in direct co-culture. Crucially, exposure of the co-culture to the ER stress inducer subtilase cytotoxin (SubAB) resulted in a significant increased expression of both fibronectin, an extracellular matrix (ECM) protein, and α-smooth muscle actin (α-SMA), a marker for fibroblast activation. This effect was found to depend on the presence of the ER stress sensor protein kinase R-like ER kinase (PERK) on CAL-1 cells. Moreover, cell-to-cell contact between IMR-90 and CAL-1 was required, since the soluble factors produced by SubAB-exposed cells were insufficient to induce fibroblast activation. The mechanisms behind this ER stress and pDC-mediated fibrosis are being further dissected. With this work we expect to identify molecular targets for treatment of not only SSc-associated lung fibrosis, but also other fibrotic diseases.

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1185 – WS11.6

Cytokine synergy and increased T cell polyfunctionality drives the aggressive synovial fibroblast phenotype in Down Syndrome associated Arthritis.

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Purpose: Down syndrome associated arthritis (DA) is an aggressive, erosive form of arthritis that occurs 20-times more frequently in children with Down syndrome (DS) than juvenile idiopathic arthritis (JIA), however little is known of the underlying pathogenic immunological mechanisms of disease. The aim of this study was to characterize T cell polyfunctionality and examine the effect of T-cell derived cytokines on primary DA synovial fibroblast (DA-FLS) and EC function.
Method: PBMC were isolated from children with DS, DA, JIA, and HCs and T-cell polyfunctionality and chemokine receptor expression was analysed by flow cytometry. DA-FLS and EC were stimulated with TNF-α, IL-17a and IFN-γ alone and in combination, or pre-primed for 24 hrs before cytokine combinations. Gene/protein expression of inflammatory mediators were quantified by RT-PCR, flow cytometry, ELISA/MSD-Multiplex assays, and cellular function assessed by adhesion and migration assays. Real-time metabolic activity was assessed by Seahorse XFe96 Analyser and RT-PCR.
Results: Higher CD8+ T-cell frequency and reduced CD4+ T-cell frequency was observed in DA compared to other groups. A significant enrichment of polyfunctional T-cells which simultaneously produced TNF-α, IFN-γ and IL17A was also demonstrated in DA compared to all other groups. Differential expression of chemokine receptors (CCR4, CCR5, CXCR3, CXCR6) in both CD4+ and CD8+ T-cells (effector, memory and T_{emra}) was also demonstrated. IL-17A and IFN-γ potentiated the effects of TNF-α on IL-6, MCP-1 and RANTES secretion in DA-FLS and EC compared to cytokine stimulation alone. Additionally, IFN-γ potentiated the effects of TNF-α on CXCR3,-CXCR4 and CCR6 in DAFLS and EC, and on leukocyte-adhesion, however differentially regulated adhesion molecule expression with ICAM-1 significantly induced in DA-FLS and VCAM-1 in EC. Cytokine synergy shifted the metabolic profile of DA-FLS to a highly energetic glycolytic phenotype, in addition to significantly inducing gene expression of key glycolytic mediators (HIF1a,-Glut-1,-HK2,-PKM2), however had minimal effect on EC metabolism. IFNγ-priming for 24hr further potentiated DA-FLS activation/invasive function in response to TNF-α. Finally, blockade with Tofacitinib inhibited cytokine-induced pro-inflammatory mediator expression.
Conclusion: These data have implications for combination therapy or manipulation of metabolic pathways for the treatment of this aggressive form of Inflammatory arthritis in children with DS.

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WS12 – IMMUNE REGULATION IN DISEASE

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2173 – WS12.1

Halting type 1 diabetes progression: use of regulatory T cell-derived extracellular vesicles

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Purpose: Type 1 diabetes (T1D) is a childhood autoimmune disease in which immune cells invade the pancreatic islets causing β-cell destruction and insulin loss. Defective $CD4^+$ regulatory T (Treg) cells have been reported in T1D individuals and, while much effort is devoted to expand/activate them for slowing disease progression, these "living drugs" are prone to cell plasticity and loss of immune suppressive capacity *in vivo*. We here aim at analyzing whether the extracellular vesicles (EVs) released by Treg cells may stably mediate the immune protective effects of the originating cells.
Methods: Peripheral blood mononuclear cells were separated from whole blood of healthy donors (HD) or subjects with T1D using Ficoll-Paque, and isolated Treg cells were expanded for 14 days and characterized for their phenotype and suppressive function through flow cytometric analyses. EVs released by expanded Treg cells (expTreg-EVs) were analyzed for their microRNA and protein content by RT-qPCR and proteomics, respectively; in parallel, expTreg-EVs are tested for their suppressive function on both allogeneic $CD4^+$ and $CD8^+$ T cells *in vitro*, and, for the *in vivo* part, in non-obese diabetic (NOD) mice.
Results: Our results show that expTreg cells from both HD and T1D subjects maintain substantial expression of Foxp3 and significant suppressive function. In parallel, expTreg-EVs demonstrated the ability to dramatically hinder $CD4^+$ and $CD8^+$ T cell proliferation compared to mock controls, although the immune suppressive ability was found to be diminished in T1D compared to healthy conditions. This EV functional impairment has been functionally linked to specific differences in terms of microRNA and protein EV-cargo between HD and T1D expTreg-EVs. *In vivo* expTreg-EV testing is in progress in order to optimize time of treatment and EV dose in NOD mice for efficiently delaying pancreatic β-cell destruction and insulin loss and hamper diabetic progression.
Conclusion: Based on the above results, we believe expTreg-EVs have to potential to be developed as novel cell-free therapeutics in autoimmune diabetes. Moreover, the identification of specific expTreg-EV associated molecular determinants of immune suppression may lead to the design of artificial nanoparticles with potentially enhanced pharmacological activity.

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475 – WS12.2

Unveiling CD300e as a pivotal immune checkpoint in colorectal cancer

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In the landscape of colorectal cancer (CRC), tumor-associated macrophages (TAMs) represent the primary immune cell population, fostering the pro-tumor and immunosuppressive features of the tumor microenvironment. Our recent investigation uncovered a compelling link between the activation of the immune receptor CD300e and a compromised antigen presentation capacity in macrophages. Remarkably, the majority of TAMs in CRC patients exhibit a $CD300e^{high}/MHC\text{-}II^{low}$ expression profile, suggesting a potential role for CD300e in shaping the immunosuppressive traits of TAMs and prompting the investigation of its involvement in immune evasion mechanisms and CRC progression. Supporting our hypothesis, human macrophages exposed to patient-derived tumor colon organoids strongly upregulated the surface expression of CD300e, concomitant with the acquisition of immunosuppressive features, compared to macrophages exposed to patient-derived normal colon organoids. Taking our findings to a murine system, we revealed that CD300e knockout (KO) macrophages, when co-cultured with mouse-derived tumor colon organoids, exhibited a prominent proinflammatory/antitumor profile compared to their wild-type counterparts. Importantly, this shift in macrophage profile correlated with diminished expression of genes associated with epithelial-to-mesenchymal transition and a decreased epithelial permeability in the tumor epithelium interacting with CD300e-deficient macrophages. Our *in vitro* observations find validation in an *in vivo* murine colitis-associated CRC model, where CD300e KO mice displayed improved protection from colitis and lower mortality rates compared to wild type counterparts. Most promisingly, CD300e-deficient mice developed a reduced colon tumor burden and an enhanced anti-tumor immune response, the latter being notably characterized by an enrichment of interferon-γ-producing $CD8^+$ T cells in local lymph nodes and a favorable cytokine milieu. Intriguingly, preliminary insights suggest a potential influence of CD300e on gut microbiota composition, with CD300e KO mice harboring an enrichment in CRC protective bacterial taxa. In essence, our data unveil a multifaceted role for CD300e in CRC progression, positioning this understudied receptor as a potential novel immune checkpoint. These novel findings pave the way for a comprehensive exploration of CD300e mechanisms in tumor progression, leading to the perspective of targeting CD300e as a therapeutic strategy for CRC patients.

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587 – WS12.3

Elucidating the interaction of CD96 and its ligand CD155 in T cells

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Co-receptors are vital in modulating immune responses, particularly in effector and regulatory T cells and thus are potential targets for immunotherapy for cancer and autoimmunity. CD96, CD226, and TIGIT are an immunoglobulin superfamily of co-receptors that bind to a shared ligand, CD155. TIGIT is thought to be co-inhibitory and CD226 co-stimulatory, while the role of CD96 remains unclear.
In humans, two natural CD96 isoforms (variant 1 and variant 2) differ in their domain 2 structure. To test CD96 variant interactions and ligand-dependency, we utilised the luciferase-based NanoBiT® system in stably transduced cell lines and demonstrated that CD96 isoforms form dimers irrespective of variant specificity or ligand availability. Using co-culture models of T cell lines stably transduced with CD96 variant 1 or 2 and APCs transduced with CD155, we showed ligand interaction with both variants and a ratio-dependent uptake of CD155, but with a higher uptake by CD96 variant 2 compared to variant 1 with a significant AUC difference ($p=0.0039$).
We assessed *in vitro* the binding kinetics of CD96 to a recombinant CD155Fc at various concentration and timepoints and found that CD96 internalises the ligand in stably transduced cell lines and primary T cells. Furthermore, we developed a CRISPR/cas9 knockout model of total CD4+ and Tregs and showed that CD155 uptake and internalisation was highly dependent on CD96 alone regardless of TIGIT/CD226 expression. Interactions were confirmed utilising confocal microscopy. In addition, we showed that the ligand is undergoing degradation within cell lines and primary T cells, which was inhibited by Bafilomycin A1.
Understanding the mechanism of CD96 interaction with the other co-receptors, as well as with their shared ligand, shape the activity of effector and regulatory T cells. This will be important in the development of more potent immunotherapy in cancer and/or autoimmunity targeting this co-receptor family.

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1122 – WS12.4

Regulatory T-cells in multiple sclerosis are activated by Epstein-Barr virus and produce IL-10 in the central nervous system

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Regulatory T-cells (Tregs) maintain immune homeostasis, but the antigens that activate adaptive Tregs in human pathologies are ill-defined. FOXP3$^-$EOMES$^+$type-1 regulatory T(EOMES+Tr1-like)-cells had a dysregulated homeostasis in multiple sclerosis (MS), which was related to their migration and activation in the CNS. Selectively EOMES$^+$Tr1-like cells were enriched and clonally expanded in patient's cerebrospinal fluid (CSF), and cells of the same clonotypes were present among circulating EOMES$^+$Tr1-like cells. EOMES$^+$Tr1-like cells were the major IL-10-producing T-cell population in the CSF in MS. Importantly, EOMES+Tr1-like cells and FOXP3$^+$Tregs in MS responded poorly to myelin-derived self-antigens, but were activated by Epstein-Barr Virus (EBV) to produce IL-10 and IFN-g. EOMES$^+$Tr1-like cells responded selectively to the latent EBV antigen EBNA1, whereas FOXP3$^+$Tregs responded also to lytic antigens. Notably, EBNA1-specific EOMES$^+$Tr1-like cells were associated with major MS risk factors, namely anti-EBNA1 IgG and HLA-DRB1*15. Finally, IL-10$^+$EOMES$^+$Tr1-like cells were identified in MS brain lesions, and some were close to EBV-infected B-cells. The aberrant anti-viral specificities of Tregs in MS may explain their inability to prevent CNS immunopathology.

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1063 – WS12.5

Regulatory functions of redox processes during host-pathogen interactions in tuberculosis

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Infection with *Mycobacterium tuberculosis* results in increased necrosis among phagocytes, impairing the ability of macrophages and neutrophils to effectively combat the pathogen. Instead, macrophages become a proliferative niche for *M. tuberculosis*, leading to uncontrolled pathogen growth and increased tissue damage caused by neutrophil necrosis. The specific signaling pathways and the role of reactive oxygen species underlying these processes remain unclear.
By use of the mass spectrometric method OxRAC we explore the proteome of different cell types for oxidative changes. Preliminary investigations reveal a modified oxidation status in various proteins within macrophages and neutrophils following *M. tuberculosis* infection. As alterations in the oxidation status of enzymes can affect their function and signaling molecules or antigens may influence subsequent pathways, this study aims to identify essential thiol modifications in the proteome of *M. tuberculosis*-infected macrophages and neutrophils. These modifications can serve as targets for further analysis.
In addition to the OxRAC-based proteome investigation, we examine the influence of cellular antioxidant systems and reactive oxygen species production through inhibition of key molecules to uncover the underlying signaling pathways. The resulting insights could provide a better understanding of how *M. tuberculosis* modulates the host's immune system, offering potential targets for host-directed therapies.

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243 – WS12.6

Disentangling the heterogeneity and differentiation process of CD11c+ B cells

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An alternative subset of B cells characterized by the expression of markers such as CD11c, T-bet, and FcRL5, is present at low levels in healthy individuals but expands upon inflammatory responses, such as certain infections or autoimmune disease. We have previously shown a substantial heterogeneity among CD11c$^+$ B cells, but it remains unclear if this is due to different stimulation cues, different origins, or different stages of differentiation.
Here, we investigate the origin and heterogeneity of CD11c$^+$ B cells in humans. Employing paired single-cell transcriptomic and VDJ sequencing of CD11c$^+$ B cells isolated from a patient two weeks after acute malaria, and CITE-seq with targeted single-cell RNA sequencing of four patients at three-time points (acute, two weeks, 12 months) after acute malaria, we observed the presence of early, intermediate, and late clusters of CD11c$^+$ B cells. These clusters differed in their expression of CD27, CD24, and CD1c, among other genes. Clonal analysis of V(D)J sequences further revealed a divergent origin for the intermediate clusters, while they were mixed in the fully polarized cluster. This suggests a convergent differentiation program of naïve and memory B cells to a shared fully polarized subset. To further explore if this was a general mechanism, we analyzed publicly available datasets from blood and tissues of SARS-CoV-2 infected patients. We also performed our own flow cytometric confirmation of both the malaria and SARS-CoV-2 gene expression data using peripheral blood mononuclear cells and tissue samples. Finally, we employed an *in vitro* stimulation protocol to delineate how different B cell activation cues lead to the progressive development of an atypical phenotype. Altogether, this work has allowed us to have a better understanding of the origin, heterogeneity, and differentiation route of CD11c$^+$ B cells.

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WS13 – GASTROINTESTINAL IMMUNOLOGY

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1990 – WS13.1

HIV-1 exploits LBPA-dependent intraepithelial vesicular trafficking pathway for persistent infection of human intestinal mucosa

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Despite the advent of highly suppressive antiretroviral therapies, HIV-1 infection remains a major public health concern. The intestinal mucosa, which houses HIV-1 target cells including dendritic cells (DCs) and CD4+ T-cells, is a major anatomical site of HIV-1 persistence and replication during acute and chronic HIV-1 disease. Retention of replication-competent viruses and residual viral production in secluded tissue compartments, such as the intestinal tract, represent major obstacles to HIV-1 curative strategies. However, the key underpinning mechanisms involved in HIV-1 penetration and latency within intestinal mucosal barrier remain elusive. Here, we present human 2D intestinal immuno-organoid systems to model intestinal HIV-1 pathogenesis. These systems recapitulate tissue compartmentalization and epithelial-immune cellular interactions. Primary cell-derived intestinal epithelial monolayers on cell-culture inserts were co-cultured with either subepithelial DCs or tissue-derived CD4+ T-cells on the basolateral side of the insert membrane. These co-culture models permitted the concomitant assessment of intestinal barrier integrity, luminal sampling and immune phenotyping alongside virus trafficking and infection pathways. Our data demonstrate that apical exposure of intestinal epithelium to HIV-1 results in viral internalization, with subsequent basolateral shedding of replication-competent viruses, in a manner that is impervious to antiretroviral treatment. Markedly, intraepithelial viral capture ensued an altered distribution of specialized endocytic vesicular pathways alongside durable sequestration of infectious HIV-1 within lysobisphosphatidic acid (LPBA)-rich endocytic vesicles. The retention of replication-competent HIV-1 and subsequent viral transmission by intestinal epithelium did not compromise tight junction function or barrier permeability. Furthermore, HIV-1 targeting to basolateral CD4+ T-cells allowed us to monitor cellular reservoir formation and HIV-1 induced T-cell activation/exhaustion signatures within epithelial-T cell co-cultures systems. Incorporation of subepithelial dendritic cells resulted in HIV-1 luminal sampling and amplification of the *de novo* mucosal HIV-1 replication. Notably, therapeutic neutralization of LBPA-dependent endosomal trafficking suppressed HIV-1 replication across immuno-organoid systems, and thereby demonstrated the pivotal role of intraepithelial multivesicular endosomes as niches for virulent HIV-1 within the intestinal mucosa *in vitro*. Our study showcases the application of primary human 2D immune-competent organoid cultures in uncovering mechanisms of intestinal HIV-1 invasion and disease pathogenesis, as well as a platform for preclinical antiviral drug discovery.

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644 – WS13.2

Investigating the impact of Parkinson's Disease-associated genes on intestinal homeostasis.

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Purpose: Intestinal epithelial cells (IECs) provide an essential physical barrier between luminal contents and host tissue. Dysregulation of IECs results in the loss of barrier function, causing pathologies in both intestinal and extra-intestinal diseases. While Parkinson's Disease (PD) is primarily a neurodegenerative disorder, there is increasing evidence linking PD progression and gastrointestinal dysfunction. Our group recently developed a model to investigate the role of the gut in PD, demonstrating that mice with genetic ablation of the PD-associated gene *Pink1* exhibited motor phenotypes only when previously infected with Gram-negative *Citrobacter rodentium* intestinal bacteria. As *Pink1* is expressed in IECs, we hypothesize that PD-associated gene mutations directly affect the epithelium, impacting early PD pathophysiology.
Methods: Single-cell RNA sequencing (scRNAseq) of IECs from *Pink1* WT and KO mice was conducted at steady state and following *in vivo C. rodentium* infection. Colons were collected seven days post infection to elucidate transcriptional differences between epithelial lineages of each genotype. *Ex vivo* colonic organoids (colonoids) were derived from primary *Pink1* WT and KO epithelium. Colonoids were either grown in Matrigel (basal-out) or inverted in suspension (apical-out), then stimulated with inflammatory cytokines or infected with gram-negative pathogens to determine how PINK1 loss-of-function affects the inflammatory response of the epithelium.
Results: ScRNAseq analysis revealed significant upregulation of interferon (IFN)-signaling genes in *Pink1* KO enterocytes (ECs) compared to WT at steady state; whereby 70% of upregulated differentially expressed genes (DEGs) were linked to type I or II IFN signaling pathways. Current validation through *in vivo* enterocyte isolation, RNAscope staining, and *ex vivo* colonoids is ongoing. Under infection, *Pink1* KO stem cells demonstrated downregulation in Wnt signaling pathway genes, whereas *Pink1* KO ECs showed increased expression of MHC I pathway genes.
Conclusion: Using *in vivo* and *ex vivo* models that encompass PD-genetic susceptibility and environmental stimuli, we identified dysregulation in *Pink1* KO epithelium, suggesting altered inflammatory responses at baseline and infection. Investigating PD genes in the gastrointestinal tract offers insights into early PD initiation and intestinal epithelium dynamics.

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1608 -WS13.3

Exploring macrophage heterogeneity in Crohn's disease

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Crohn's Disease (CD), one of the two main forms of inflammatory bowel disease (IBD) is a disabling condition with a growing incidence worldwide. CD is associated with severe complications, frequently leading to surgical resection. Anti-TNF therapy has transformed CD outcome but efficacy is limited to a subset of patients. We previously described a cellular response enriched in inflamed ileums of future non-responders to TNF blockers (Martin et al., 2019), which suggested major roles for dysregulated actions of mononuclear phagocytes (MNP). In this study, we cell-sorted MNP from surgical resections of CD patients and characterized them by single-cell RNA sequencing (scRNA-seq).
Using Metacells (Baran et al. 2019; Ben-Kiki et al. 2022) and a gene module-based analysis, we captured 17 molecular programs of coregulated genes present in the monocytes/macrophages compartment, the combinatorial expression of which allowed to resolve 10 molecular states of monocytes and macrophages. While monocyte-like cells were constantly enriched in inflamed ileums, our approach enabled the identification of an inflammatory subset characterized by coexpression of *CD274* and *IL3RA* that was uniquely enriched in inflamed tissues from patients with severe forms of the disease, including anti-TNF resistance and surgical recurrence.
Using spectral flow cytometry, we validated the enrichment of PD-L1$^+$ CD123$^+$ monocyte-like cells in inflamed ileums. Transcription factor binding motif enrichment analyses of the 17 molecular programs suggested the generation of PD-L1$^+$ CD123$^+$ monocyte-like cells depended on a complex inflammatory milieu, which was validated in *in vitro* reductionist approaches. Ligand:receptor analyses suggested central roles for PD-L1$^+$ CD123$^+$ monocyte-like cells through their specific interactions with pathogenic states from other lineages, including T and stromal cells. Importantly, PD-L1$^+$CD123$^+$ monocyte-like cells exhibited a high JAK/STAT activity *in vivo* and *in vitro*. Accordingly, the JAK inhibitor upadacitinib, which was recently approved by the FDA to treat patients resistant to anti-TNF therapy, interfered with the generation and activity of PD-L1$^+$CD123$^+$ monocyte-like cells in both in vitro and ex vivo experiments.
In summary, our study identifies a druggable molecular state of inflammatory monocytes uniquely enriched severe in CD, as compared to anti-TNF primary responders, and provides a molecular rational to help guide immunotherapy prioritization in ileum CD.

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2074 – WS13.4

IL4 prevents the gluten-induced inflammation in the gut mucosa of children with potential celiac disease

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Purpose: Celiac disease (CeD) is a chronic intestinal inflammation caused by gluten proteins in genetically predisposed individuals. Childhood CeD is characterized by two main forms: acute disease (positive anti-tissue transglutaminase-tTG antibodies and intestinal villous atrophy) and potential disease (positive anti-tTG and normal mucosa architecture). The mechanisms preventing the intestinal villous atrophy in subjects with potential-CeD, and on regular diet, have not been completely elucidated. Recently, we have demonstrated a marked infiltration of IL4-secreting T cells in the intestinal mucosa of potential-CeD children that correlated with clinical outcomes, such as anti-tTG antibody titers and histological scores of mucosal damage (1-2), By contrast, these cells are almost undetectable in gut mucosa of acute patients. In this study, we investigated the protective role of IL4 in preventing the inflammatory response to gluten that leads to villous atrophy in celiac gut mucosa.
Methods: Gluten-reactive T-cell lines (TCLs) were established from gut biopsies of either potential- and acute-CeD children (N=10 patients for each group), in absence or presence of exogenous IL4. The effect of IL4 treatment on cytokine production, cell infiltrates (CD4+, CD8+, TCR γ/δ+, Tregs) and gluten peptides recognition pattern of TCLs was evaluated collected at different time-frames by ELISA/Luminex and multiparametric flow-cytometric analyses.
Results: IL4 treatment induced a statistically significant reduction of IFN-γ production associated with a mild increase of IL10 production in culture supernatants of TCLs in potential-CeD patients, collected over time up to 6 weeks. Furthermore, a significant decrease of densities of CD8+ and TCR γ/δ+ T cells was detected in IL4-TCLs compared to control cultures. In addition, IFN-γ production in response to whole gliadin/immunodominant peptides was significantly reduced in IL4-TCLs. In contrast, no significant changes were observed in TCLs grown with IL4 generated from acute-CeD patients, for any of the experimental read-outs.
Conclusions: Our study demonstrated a hitherto unexplored immunoregulatory function of IL4 on gluten-induced inflammation in gut mucosa of patients with potential disease, suggesting an important role of this cytokine in counteracting the transition mechanisms to villous atrophy.

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1282 – WS13.5

BAMBI, a regulator of intestinal epithelial cell permeability and apoptosis, controls colitis and colon cancer development.

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The BMP and Activin Membrane-Bound Inhibitor (BAMBI) is an inhibitor of TGFβ signaling involved in the differentiation of $CD4^+$ T cells into Tregs and Th17 cells. Due to the importance of TGFβ and of Treg and Th17 cells in the maintenance of intestinal homeostasis, we have evaluated here the effects of BAMBI deficiency in the composition of gut-associated lymphoid tissue (GALT) and in the development of colitis and inflammation-associated colon cancer. We show that in the gut epithelium of wild type (WT) mice, BAMBI expression is mostly restricted to colon epithelial cells and its absence promotes changes in GALT composition, characterized by an increase of Tregs, total B cells and germinal center B cells in different GALT locations. In contrast, lack of BAMBI causes a marked and general reduction of Th17 cells. Furthermore, BAMBI deficiency protects mice against Dextran Sodium Sulfate (DSS)-induced colitis by TGFβ-dependent, but immune suppression-independent, mechanisms, tightly associated to increased resistance of intestinal epithelial cells to DSS-induced apoptosis and a decreased intestinal permeability. Finally, the absence of BAMBI inhibits the development of colon cancer associated to chronic inflammation. In conclusion, our present findings point to BAMBI as an essential regulator of intestinal homeostasis and as a new promising therapeutic target in colorectal pathologies such as colitis and colon cancer associated to chronic inflammation.

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817 – WS13.6

T cell responses to dietary antigens

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Proper nutrient absorption requires the host to develop an immune response that tolerates food antigens. For this purpose, naive T cells, in response to dietary antigens, acquire a regulatory program (iTregs) known to suppress peripheral inflammatory responses to these antigens, even when introduced in an inflammatory setting—a process known as 'oral tolerance.' Type 1 conventional dendritic cells (cDC1s) are traditionally viewed as the primary inducers of the iTreg response to food antigens. Although they have been shown to present food peptides on MHC class II, support Treg differentiation in vitro, and interact with antigen-specific T cells, mice lacking cDC1s still maintain a Treg response to food antigens as well as oral tolerance. Recently, we identified RORγt+ antigen-presenting cells (APCs) as a dedicated subset required for the induction of iTreg cells in response to Helicobacter hepaticus (Hh) (Kedmi R. et al., Nature 2022). These findings prompt us to revisit the cellular requirements for the induction of an immune response to dietary antigens. Using genetic mouse models, we now show that antigen presentation by RORγt+ APCs is also required for the induction of the iTreg response to food antigens. While our data suggest that antigen presentation by cDC1 is incapable of eliciting the iTreg program, we found that it still plays a unique role in mediating immune responses to food antigens. Our studies offer significant insights into the basic mechanisms of immune responses to dietary antigens and propose novel mechanisms for the pathogenesis of celiac disease.

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427 – WS14.1

CXCR4 blockade reduces the severity of murine heart allograft rejection by plasmacytoid dendritic cell-mediated immune regulation

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Purpose: Allograft-specific regulatory T cells (Treg cells) are crucial for long-term graft acceptance after transplantation. Although adoptive Treg cell transfer has been proposed, major challenges include graft-specificity and stability. Thus, there is an unmet need for the direct induction of graft-specific Treg cells. We hypothesized a synergism of the immunotolerogenic effects of rapamycin (mTOR inhibition) and plerixafor (CXCR4 antagonist) for Treg cell induction.
Methods: BALB/c (allogeneic) or C57BL/6J (syngeneic) mice served as heart donors and C57BL/6J as transplant recipients. Animal experiments adhered to EU directive 2010/63/EU and were approved (#G1071/09). Heterotopic intra-abdominal heart transplantation (HTX) was performed as previously described (1). Allograft function was evaluated daily by palpation. C57BL/6J recipients received injections with plerixafor (1 or 5 mg/kg s.c.) and/or rapamycin (0.4 mg/kg i.p.) two days before, immediately after HTX and every other day for 14 days. The subclinical dosage of rapamycin allowed to early distinguish differences in allograft survival.
Results: The combined treatment consisting of Plerixafor and Rapamycin lead to a longer prolongation of allograft survival compared to rapamycin-only ($p < 0.001$). Median allograft survival time in recipients from the non-treatment, plerixafor (P1 and P5 mg/kg), rapamycin and combined treatment group P1R or P5R were 8, 10, 10, 44, 49 and 78 days, respectively. Hearts of the respective syngeneic controls survived the whole observation period of 100 days.
Moreover, fibrosis and myocyte lesions were significantly reduced in the combined treatment group when compared to sole Rapamycin-treatment. Although less CD3+ T cell infiltrated, higher Treg cell numbers were observed. These findings were accompanied by a plerixafor-dependent plasmacytoid dendritic cell-mobilization as in vivo pDC-depletion abrogated the plerixafor-mediated increase in Treg cell numbers and led to a reduced allograft survival.
Conclusion: Our pharmacological approach allowed to increase Treg cell numbers due to pDC-mediated immune regulation. Therefore, pDCs can be an attractive immunotherapeutic target in addition to plerixafor treatment.

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2253 – WS14.2

Translation of tacrolimus resistant antiviral T cell products: In depth-efficacy evaluation and human based safety assessment

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Immunocompromised patients e.g. in the case of solid organ transplantation (SOT) are commonly treated with immunosuppressive drugs to circumvent organ rejection. This entails patients being equipped with a strongly diminished endogenous T cell response against pathogens e.g. viruses. Seasonal and omnipresent pandemic viruses are major health concerns for these patients. In fact, long-term immunosuppression leads to insensitivity to vaccines and compromised viral defense results in organ rejection and mortality. In this case, common antiviral drugs cannot combat viral complications.
To fully regenerate a protective T cell response, we have focused on manufacturing tacrolimus resistant CMV, EBV, and IAV specific T cell products from limited amounts of peripheral blood to be co-administered with immunosuppressive treatment. Our next-generation manufacturing protocol represents a GMP compliant process allowing the production of up to 300 million antiviral T cells within 2 weeks using bioreactors. Knock-out of FKBP12, the functional adaptor protein for the immunosuppressant tacrolimus is achieved by a GMP compliant CRISPR-Cas9 ribonucleoprotein based approach. The efficacy and safety of these gene modified T cells is studied by characterizing effector cytokine production and artificial target specific killing assays as well as in depth by CITEseq, proteomics and epigenetic analyses. We recently gained proof-of-concept results with CITE seq showing functionality of gene edited T cell products in presence of immunosuppressive drugs in comparison to unedited controls. This included also verification of a safety switch using an alternative immunosuppressant, cyclosporine A. Since pre-clinical safety assessment is of high importance for the transition into early phase clinical trials, we focused on T cell product characterization in co-cultures with primary human cells, highlighting such models as efficient and reproducable. With this, we want to take a further step towards relevant human model based T cell product evaluation, which offers particular advantages in the complex environment of cell and gene therapies and immunological questions. All in all, we gathered preclinical data to pave the way for clinical translation of tacrolimus resistant antiviral T cell products.

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2104 – WS14.3

Donor HLA-DQ landscape predicts the likelihood of controlling BK polyoma virus replication after kidney transplantation

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Aim: BK polyomavirus (BKV) infection is a major concern after kidney transplantation (KT) increasing the risk of graft loss. Because of the lack of specific antiviral treatment, obtaining an optimal balance between maintaining antiviral immunity (i.e. reducing immunosuppression) while preventing alloreactive responses is a major clinical challenge. Therefore, early identification of patients at high risk of BKV replication is crucial to best tailor immunosuppression. The HLA evolutionary divergence (HED) metric, which quantifies the sequence divergence between the peptide binding domains of two alleles of a given locus, has shown relevance as a predictor of outcome in other pathophysiological contexts. Here, we aimed to investigate whether HLA diversity might impact the risk of BKV replication after KT.
Methods: We studied a large retrospective KT cohort (n=532 patients) with systematic screening of plasma BKV replication (DNAemia) during 2 years post-transplant. HLA class-I and -II typing (two-field resolution) was available for all donors and recipients. For each patient, the HED was measured (Grantham distance) for each HLA locus. Prediction of BKV peptides binding was performed in silico using NetMHCIIpan-4.2. Only stable HLA-DQαβ heterodimers were considered for peptide binding prediction to avoid counting peptides that cannot be presented in vivo.
Results: During the 2-year follow-up, 18% of patients experienced BKV DNAemia. HLA alleles and diversity from the recipients were not associated to BKV infection. However, high donor HLA-DQ HED was a significant and independent predictor of BKV-free outcome (adjusted HR 0.55, 95%CI 0.35-0.85; p=0.008), confirmed in G-computation analysis (time-varying causal inference). More generally, we highlighted a bimodal distribution of the HED-DQA, with higher values corresponding to HLA-DQA1*01/non-DQA1*01 allele combinations. Furthermore, HLA-DQ divergence was positively correlated to the size of the BKV-derived immunopeptidome bound by donor HLA-DQ supertypes (R=0.51; p<0.0001), with predominant contribution of the BKV-VP2 protein.
Conclusion: We provided evidence for a direct link between HLA-DQ divergence, the size of the BKV-derived DQ-bound immunopeptidome, and the control of BKV infection, which likely reflects a stronger anti-viral immune response. This easily accessible predictor could provide a unique opportunity for personalized monitoring of BKV DNAemia and initiation of prophylactic or prompt therapeutic intervention when warranted.

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528 – WS14.4

Immunosuppressive therapy modifies anti-Spike IgG subclasses distribution after 4 doses of mRNA vaccination in a cohort of kidney transplant recipients

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Background and hypothesis: Immunization with mRNA vaccines against SARS-CoV-2 prompts an increase in IgG4 against the Spike protein in healthy populations. However, the response of immunosuppressed individuals remains unclear. This study assesses the immune response, including IgG subclasses, to four mRNA vaccine doses in kidney transplant recipients (KTR).
Methods: A prospective cohort study involving 146 KTR and 23 dialysis patients (DP) who received three mRNA-1273 vaccine doses and a BNT162b2 booster. We evaluated anti-Spike IgG titers and subclasses, T-CD4+ and T-CD8+ cellular responses, and serum neutralizing activity (SNA).
Results: At the fourth dose, 75.8% of COVID-19 naïve KTR developed humoral and cellular responses (vs 95.7% in DP). A correlation existed between anti-Spike IgG titers/subclasses and SNA ($p<0.001$). IgG subclass kinetics after the third/fourth doses varied between COVID-19 naïve KTR and DP. Immunosuppressive therapy strongly influenced IgG subclasses: mTOR inhibitors (mTORi) positively impacted IgG1 and IgG3 ($p<0.05$), while mycophenolic acid negatively affected IgG1, IgG3, and IgG4 ($p<0.05$). A correlation emerged between SNA after four vaccine doses and breakthrough infections over the next six months in KTR; lower SNA (20.3% vs 45.8%, $p=0.012$). Multivariate analysis revealed mTORi as the only factor associated with SNA>65% in naïve KTR [4.29 (1.21-15.17), $p=0.024$].
Conclusions: KTR exhibit weaker cellular and humoral immune responses to mRNA vaccines. Immunosuppressive regimens strongly influence IgG subclasses. Elevated SNA after four vaccine doses is protective against infection. mTORi treatment positively impacts the humoral response and could benefit non-responding KTR.

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1678 – WS14.5

Effects of corticosteroids therapy on immune reconstitution and function upon hematopoietic stem cell transplantation in cancer patients

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Hematopoietic stem cell transplantation (HSCT) represents the only curative option for many hematological malignancies and an efficient immune reconstitution is necessary for a positive outcome in cancer patients. However, HSCT remains a high-risk procedure associated with many complications including graft-versus-host disease, therefore a percentage of HSCT recipients need to be treated with the anti-inflammatory glucocorticoids (GCs). Since GC therapy has been found to regulate innate lymphoid cells function and to inhibit their HSC-derived differentiation, a detailed characterization of the effect of GC on immune reconstitution would be beneficial to avoid HSCT low efficiency. It is known that GCs directly regulate gene expression through their nuclear receptor and that they can induce stable chromatin modifications. Our hypothesis is that GC therapy may generate transcriptional and long-term epigenetic modifications on HSCs, influencing their development towards the different immune cell subsets and hampering the correct immune reconstitution following HSCT. Here we characterized the effects of GC treatment on HSCs differentiation upon HSCT, both *in vitro*, on cultured HSCs and *in vivo*, on immunodeficient mice receiving human HSCs. Furthermore, we evaluated the impact of GC treatment on immune function against leukemia relapse following HSCT in an *in vivo* model of immunodeficient mice receiving human HSCs and infused with leukemic NALM cells. Finally, through RNA- and Assay for Transposase-Accessible Chromatin (ATAC)-sequencing experiments, we were able to identify the transcriptional signature and the chromatin landscape induced by GC treatment on *in vitro* cultured HSCs. These data shed light on the molecular mechanism by which GC therapy affects immune reconstitution and represent an important step towards the development of novel strategies to improve HSCT outcome in oncologic patients.

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324 – WS14.6

Tissue-resident lymphocyte subsets are associated with poor glucocorticoid response in acute gastrointestinal graft-versus-host disease

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Acute graft-versus-host disease (GVHD) of the gastrointestinal tract (GI) is a serious life-threatening complication of allogeneic hematopoietic stem cell transplantation. Only half of all patients respond to first-line intravenous methylprednisolone therapy and steroid-refractory (SR) GVHD is associated with a poor long-term prognosis and survival. Cellular specificities of SR-GVHD remain incompletely understood and there is still an unmet clinical need for biomarkers predicting response to glucocorticoid therapy.
In this study, we monitored 253 patients treated with HCT at the Medical University of Graz for development of GI GVHD, which occurred in 55 patients. We obtained GI-biopsies at the first onset of acute GI GVHD prior to start of steroid treatment. Formalin-fixed paraffin-embedded (FFPE) tissue from 50 patients, 24 steroid-sensitive patients (SS group), 26 steroid-refractory patients (SR group) and 13 healthy-appearing mucosa samples from bowel adenoma resections (control group) was labelled with T cell surface markers and intracellular staining of transcription factors, with subsequent software-based computational analysis.
Patients with SR GVHD experienced significantly higher GVHD scores and dramatically increased mortality from GVHD. Compared to the control group, patients with acute GI GVHD showed but a decrease in CD103+ tissue-resident memory T cells (TRM) and increased type-3 (RORgt+) innate lymphoid cells (ILC3) in tissue biopsies. No differences in total T cell numbers or RORgt+ T cells were observed between groups. Compared to SS-GVHD, we detected increased percentages of TRM in samples from SR-GVHD, as well as an increased proportion of CD4+ cells within the TRM subset. Regarding ILC3s, the SS group exhibited a trend towards higher ILC3 percentages compared to the SR group and significantly higher proportions of ILC3 compared to the reference group.
Our study shows that cellular infiltrates in intestinal biopsies differ regarding TRM and also ILC composition at onset of GI GVHD in patients who subsequently respond to glucocorticoid therapy compared to those developing steroid-refractory disease. This implicates pre-existing resident lymphocytes as potential pathomechanistic factor for steroid sensitivity. Intestinal tissue infiltration patterns of TRMs and ILC3s at GI GVHD onset may serve as useful clinical prognostic markers for response to glucocorticoid therapy.

*J. Strobl and L. Gaksch contributed equally.

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WS15 – T CELLS IN AUTOIMMUNITY

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2007 – WS15.1

Autoantigen-specific CD4 T cells acquire an exhausted phenotype and persist in patients with antigen-specific autoimmune disease

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Purpose: Autoreactive pro-inflammatory CD4+ T helper (Th) cells orchestrate the self-destructive immune response in chronic autoimmune diseases but remain difficult to track. The molecular characteristics of human autoreactive Th cells persisting in face of chronic autoantigen-stimulation and immunotherapy remain therefore poorly understood. The *ex vivo* characterization of autoreactive T cells is essential to understand their lifestyle, how they adapt to and maintain chronic autoimmunity and thereby enable their targeted elimination with novel immunotherapies. Neuromyelitis optica spectrum disorder (NMOSD) is a prototypic chronic autoimmune disease of the central nervous system targeting aquaporin-4 (AQP4). AQP4-specific CD4+ Th cells are centrally involved in disease development including the Th cell-dependent generation of AQP4-specific antibodies. However, their functional and molecular properties as well as peptide/HLA specificities are poorly characterized due to technical limitations for their *ex vivo* detection.
Methods: Combined HLA-tetramer- and activation-based antigen-reactive T cell enrichment (ARTE) characterized *ex vivo* AQP4-specific CD4 Th cells isolated from blood of patients and healthy controls. We performed multiparameter cytometric and functional characterization, single cell RNA-sequencing and re-expression of autoreactive T cell receptors (TCRs) to confirm specificity and identify peptide/HLA class II recognition.
Results: In aquaporin4-antibody-positive neuromyelitis optica spectrum disorder patients, autoreactive Th cells expressed CD154, but proliferative capacity and pro-inflammatory cytokines were strongly reduced. Instead, exhaustion-associated co-inhibitory receptors were co-expressed together with FOXP3, the canonical regulatory T cell (Treg) transcription factor. The proliferative blockade of autoreactive Th cells was reversed *in vitro* by checkpoint inhibition and patient clones expanded in this way provided potent B cell help. The same exhaustion-like phenotype was demonstrated in autoimmune hepatitis and bullous pemphigoid, antigen-specific autoimmune diseases of liver and skin, respectively.
Conclusion: We identified a new exhaustion-like Th cell phenotype and a potential role of FOXP3 for the regulation of chronic autoreactivity in human autoimmune disease. A better understanding of the lifestyle of autoreactive Th cells in patients defines novel strategies for their therapeutic targeting. Our data suggest CD4+ Th cell exhaustion as a common mechanism of adaptation to chronic (self-)stimulation across disease types.

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477 – WS15.2

The tissue-resident memory Th17 (TRM17) cells, the predominant source of interleukin (IL)-17 in inflammatory arthritis, is largely IL-23 independent in their effector function and epigenetically regulated by Bromodomain Containing 1 (BRD1)

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Purpose: The IL-17 pathway plays a key role in the immunopathogenesis of inflammatory arthritis including Ankylosing Spondylitis (AS). Produced by a variety of immune cells, whether a specific cell type dominates IL-17 production in AS is debated. Recent studies have implicated γδ T cells, iNKT cells, and MAIT cells as the main sources of IL-17 in AS joints. Increasing evidence, however, is highlighting a role for CD4+ tissue resident memory Th17 (TRM17) cells as key IL-17 producers in tissues such as the lung and brain. It is therefore possible that a role for TRM17 in AS may have been missed in previous studies focused on synovial fluid samples.
Methods: Digested synovial tissue from patients with AS (n=5) was profiled using single-cell RNA sequencing. To model TRM17 in vitro, isolated memory CD4+ T cells from the blood of AS patients were first cultured in Th17 expansion condition for 6 days (to model initial Th17 activation in tissue), followed by the exposure to TGF-β and IL-7 for 7 days (to model the resolution phase). A library of epigenetic inhibitors and CRISPR were used to identify epigenetic regulators for TRM17.
Results: IL-17A/F expression was restricted to CD4+CXCR6+ TRM17 cells. The in vitro generated TRM17 phenocopied the TRM17 found in joint tissue and produced IL-17A/F in response to TCR stimulation. IL-1β and IL-23 enhanced the IL-17A/F production in the presence of TCR stimulation but were incapable of inducing IL-17A/F production on their own. CRISPR KO of BRD1 inhibited the generation of TRM17 from memory CD4+ T cells from AS patients. Cell interaction analysis predicted dendritic cells to be the antigen presenting cells for TRM17.
Conclusions: TRM17 cells are the predominant source of IL-17 in AS and require TCR stimulation for their effector function. IL-1β and IL-23 enhance IL-17 production by TCR-stimulated Trm17 but do not induce IL-17 on their own, explaining the lack of clinical efficacy in their blocking antibodies in AS. Epigenetic regulator BRD1 contributes to the generation of TRM17. Targeting TRM17, the “factory” of IL-17, provides a new therapeutic strategy potentially superior to IL-17 blockage in inducing long-term remission.

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2098 – WS15.3

T cell mediated immune responses in primary intestinal tissue slices from IBD patients ex vivo

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Purpose: Inflammatory bowel diseases (IBD) are characterized by the dysregulation of innate and adaptive immune responses, in particular massive T cell infiltration, leading to aberrant inflammatory processes within the intestinal tissue. Our aim is to gain deeper insights into immunological signatures associated with IBD and to modulate tissue-resident T cell responses using ex vivo primary intestinal tissue slices (Precision Cut Intestinal Slices; PCIS). PCIS represent an immunocompetent human-based ex vivo tissue model that reflects the complex intestinal architecture with all relevant intestinal cells in their native microenvironment.
Methods: PCIS were prepared from ileum resections of IBD and non-IBD patients and stimulated ex vivo with mitogens or pro-inflammatory cytokines (e.g. bacterial lipopolysaccharide (LPS), IL-1β, Concanavalin A) and/or anti-inflammatory treatments, e.g. pimecrolimus for 24 h. We analysed tissue viability (ATP/LDH-assay), cytokine release (multiplex assay) and PCIS morphology (H&E, Immunofluorescence).
Results: Primary intestinal tissue slices of IBD and non-IBD patients remained viable for 24 h. The investigated substances showed no significant effect on LDH release. PCIS from IBD patients exhibited disease-specific morphological changes such as immune cell infiltrates and higher abundance of CD4+ and CD8+ T cells in the subepithelial layer of the inflamed mucosa. Further, PCIS from IBD patients showed increased secretion of clinically relevant biomarkers and disease-dependent cytokine signatures compared to PCIS from non-IBD patients. Overall, we characterized the secretion patterns of 30 different mediators in intestinal tissue ex vivo. Concanavalin A led to a significant increase of several T cell cytokines including IL-2 (~2-fold), IFN-γ (~3-fold), TNF-α (10-fold) and IL-17A (~7-fold) in PCIS from IBD patients compared to PCIS from non-IBD patients. Further, Concanavalin A-induced T cell cytokines were specifically reduced by treatment of PCIS with the calcineurin inhibitor pimecrolimus, while the release of proinflammatory mediators such as IL-8 was less affected.
Conclusion: In conclusion, primary tissue slices showed distinct and disease-specific immune responses ex vivo. PCIS from IBD patients exhibited increased and modulable Th1 and Th17 cell-associated responses. This study highlights the potential of PCIS for investigating immunological mechanisms and disease-specific biomarkers, providing a test platform for new drugs based on a complex human tissue system.

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406 – WS15.4

A novel humanized mouse model to study rheumatoid arthritis associated anti-citrulline T cell reactivity

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Purpose: Two thirds of RA patients display anti-citrulline autoimmunity, however no existing animal model mimics such specificity, as they rely on immune reactivities unrelated to the human disease or that poorly represent reactivities observed in patients. Moreover, the majority does not consider the strongest genetic association with risk of RA – HLA-DRB1*04:01 (DR4). Hence, we generated humanized mice that better resemble patients autoreactivity, allowing a more straightforward translation of data to (pre)clinical settings.
Methods: Citrulline-reactive T cells were identified by peptide-HLA tetramers in peripheral blood and synovial fluid samples of DR4+ RA patients. Tetramer-positive CD4+ T cells were single-cell sorted and sequenced for their T cell receptors (TCRs). Identified paired alpha-beta TCRs were re-expressed in vitro to confirm their antigen and HLA specificity. Positively identified sequences were engineered into suitable DNA vectors for injection into murine zygotes. Germline alpha-beta TCR+ offspring were phenotypically assessed by flow cytometry and cell culture assays for antigen-reactivity.
Results: To date, we have generated TCR transgenic mice specific to citrullinated tenascin C (citTNC) and citrullinated fibrinogen (citFib). Here, we present data from the citTNC TCR transgenic line. citTNC mice develop both CD4+ and CD8+ T cells in the context of murine MHC. Analysis of double negative thymocytes indicate a skewed developmental stage towards a completely re-arranged TCR, and expression of the transgenic TCR was confirmed in single positive thymocytes by RT-PCR. When cultured in vitro with bone marrow derived dendritic cells (BMDCs), peripheral citTNC CD4+ T cells showed specific reactivity to their cognate antigen only in the presence of DR4+ BMDCs, but not murine H2-A^b expressing BMDCs. Similarly, when citTNC CD4+ T cells were transferred to citTNC-immunized DR4+ or H2-A^b+ recipient mice, antigen recall responses where only observed in DR4+ recipients.
Conclusion: We successfully generated transgenic mouse lines expressing patient-derived autoreactive TCRs of relevance to RA. citTNC mice develop CD4+ T cells that are activated in a HLA- and antigen-specific manner, showing both in vitro and in vivo functionality. These constitute the initial steps to developing a complex humanized mouse model for studying anti-citrulline immunoreactivity and better understand such immune responses in human RA.

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618 – WS15.5

CCL5/RANTES in the Pathogenesis of Systemic Lupus Erythematosus (SLE)

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Purpose: SLE is a highly heterogeneous systemic autoimmune disease, the cause of its initiation and severity needs to be revealed. Increased levels of CCL5 (or RANTES), a C-C chemokine, in sera/plasma and urine from SLE patients have been observed. Hyper-released neutrophil extracellular traps (NETs) promote plasmacytoid dendritic cells (pDCs) to secrete type 1 interferon (IFN), which is considered a cardinal feature of SLE. Earlier, we demonstrated that CCL5 could be upregulated by NET and IFNα presence. We subsequently studied CCL5 as a biomarker for SLE by investigating the proliferative or anti-proliferative effects of NETs, IFNα and CCL5 on T lymphocytes, to clarify how hyper-secreted chemokines in SLE contribute to the pathogenesis of the disease, to possibly pursue a universal therapeutic strategy from what we discovered.
Methods: We isolated peripheral blood mononuclear cells (PBMCs) and plasma from SLE patients and age-and-sex-matched healthy donors; IFNα and in vitro generated NETs are used as stimuli in healthy monocytes and PBMC cultures. CCL5 secretion was measured by ELISA; CCL5 stimulation-induced activation makers of monocytes and T cells, as well as T-cell proliferation were gauged by flow cytometry; Transcriptional levels of CCL5, CCR5 and other signaling molecules on monocytes were quantified by real-time PCR.
Results: We confirm that SLE patients exhibit elevated plasma levels of CCL5 compared to healthy subjects. We show that monocytes from patients express more CCR5 than monocytes from healthy donors, most notably in the intermediate monocyte subclass. Exposure to IFNα and NETs did trigger increased CCR5 expression and CCL5 secretion of monocytes. When PBMCs were cultured in presence of CCL5 and NETs, we observed an expansion of T-cell proliferation.
Conclusion: Dysregulated NETs and IFNα in SLE could induce CCL5 secretion and CCR5 activation on monocytes, the released CCL5 could further serve as signal 3, together with NETs to contribute to T cells activation and proliferation, whereas IFNα exhibits an anti-proliferative effects on T lymphocytes when they are exposed to NETs at the same time. Besides the well-known role in chemotaxis, we provide new mechanistic roles of CCL5 in SLE pathophysiology.

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1978 – WS15.6

Unravelling pathological mechanisms of Addison's disease using a novel animal model

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Adrenal glands play a crucial role in regulating metabolic, immune, behavioral, and cardiovascular processes through steroid production. Autoimmune Addison's disease is an autoimmune disorder that occurs when adrenocortical cells are attacked by the immune cells, leading to functional failure. However, due to the lack of human samples and suitable mouse models, the immune mechanism behind adrenal destruction remains unknown. To address this, we developed the first defined mouse model of experimental autoimmune adrenalitis (EAA) by immunizing C57BL/6 mice with immunogenic peptides derived from CYP11A1, a rate-limiting enzyme in steroidogenesis. Our model resulted in $CD4^+$ and $CD8^+$ T cell infiltration into the adrenals, as well as the recruitment of inflammatory monocytes, and B cells. These immune cells were predominantly located in the adrenal cortex, the site of robust steroidogenesis and CYP11A1 production. The initial adrenal inflammation led to pathological changes followed by adrenal functional failure manifested by corticosterone deficiency. Adoptive transfers demonstrated the significance of $CD4^+$ T cells in disease pathogenesis. Using single-cell RNA sequencing of adrenal-recruited $CD4^+$ T cells during EAA, we identified a broad heterogeneity of T cell responses, including a novel pro-inflammatory subset. Simultaneous analysis of T-cell receptor recombination of individual cells showed intense clonal expansion of adrenal-infiltrated $CD4^+$ T cells. To study expanded T cell clones representing individual $CD4^+$ T cells subset in detail, we have generated two different monoclonal populations using retrogenic mice. Our data revealed that both $CD4^+$ T clones are specific to the same adrenal antigen but have different affinities. In conclusion, our models not only allow us to study the pathogenesis of Addison's disease but also provide a unique opportunity to investigate the fate decisions of self-reactive $CD4^+$ T cells and their clonal lineages in a well-defined autoimmune setting.

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WS16 – B CELLS: DEVELOPMENT AND FUNCTION

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1211 – WS16.1

Systems level analysis of B-cell development across aging identifies BDNF as a driver for B-cell generation in the bone marrow

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Purpose: B lymphopenia is associated with increased morbidity and mortality in various clinical settings: following chemo/immunotherapy, aging and infections. Here, by leveraging a systems immunology approach, we identified drivers for B lymphopoiesis in the Bone Marrow (BM).
Methods: To prioritize B lymphopoiesis drivers, we analyzed scRNAseq data of BM samples from patients aged 24-67 and compared B lymphopoiesis trajectories using our CellAlign algorithm. Additionally, we measured 378 plasma proteins in healthy individuals and elderly B-cell depleted patients to find B-cell regeneration related drivers. Furthermore, we validated the drivers in biological experiments.
Results: To identify B-cell developmental genes, we initially created a B- cell trajectory that orders BM cells based on their developmental state, allowing high resolution identification of genes and regulatory programs that vary along B-cell development. As B lymphopoiesis is regulated by Mesenchymal Stromal Cells (MSC), we mapped 54 MSC expressed ligands interactions with B-cell developmental receptors, possible candidates for drivers of B-cell development. Leveraging age-associated differences in B lymphopoiesis, we aligned B-cell developmental trajectories of individuals in different ages to a common trajectory, and revealed 18 genes with significant differences in their expression profile dynamics upon aging. Additionally, we leveraged the B-cell rejuvenation process induced by depletion of B-cells in old patients to prioritize drivers. The upregulation of 17 proteins in elderly B-cell depleted patients restored to youthfulness, pointing to their role in B lymphopoiesis. Next, the putative drivers were filtered after analyzing their short stimulation impact on CD34+ Hematopoietic Stem and Progenitor cells (HSPC) assessed by scRNAseq. Following scoring the candidates by their results in the different analyses, we focused on one high scored putative driver, Brain Derived Neurotrophic Factor (BDNF). To validate BDNF we established an in-vitro biological system that mimics BM B lymphopoiesis process. Adding BDNF to isolated CD34+ HSPC led to their earlier and increased differentiation into progenitor B-cells.
Conclusion: High resolution systems-level analysis of B lymphopoiesis highlights BDNF as a candidate driver for B lymphopoiesis. Its expression may be used to predict B lymphopoiesis potential and to enhance their recovery toward restoring B lymphocyte function.

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1672 – WS16.2

The RNA binding protein HuR is required for self-replenishment and preservation of the restricted BCR repertoire of innate B1 cells

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Purpose: Innate B1 cells are developed from restricted progenitors found in foetal and neonatal tissues and, in the adulthood, these progenitors drastically wane. Instead, B1 cells are self-maintained by signaling through their restricted BCR repertoire and clonal expansion. Our previous findings uncovered the RNA binding protein (RBP) HuR as an essential modulator of B-cell adaptive immunity. Thus, we hypothesized that HuR could also control innate B cell immunity.
Methods: To address this hypothesis, we used HuR conditional KO mice and tamoxifen inducible – Cre mice to assess the intrinsic role of HuR in innate B1 cell development, self-renewal and survival. Phenotypical characterization of the B1 cell subsets, protein:RNA interactomics and transcriptomics analyses revealed an important role for HuR in B1 B cell homeostatic maintenance examined further in-vivo and in-vitro.
Results: Deletion of HuR in B1 cells resulted in a three-fold decrease in B1 cell numbers associated to impaired self-renewal capacity and increased apoptosis. Molecular characterization of HuR targets and function using iCLIP and RNAseq revealed that this RBP controls a specific genetic program for tonic BCR signalling and homeostatic maintenance. Indeed, mechanistic validation revealed decreased BCR signalling, impaired clonal expansion and lack of preservation of the BCR repertoire in the absence of HuR in B1 cells. Additionally, we found HuR to be required for the expression of TACI and BAFFR and B1 cell survival.
Conclusion: In summary, our data show that HuR-dependent post-transcriptional regulation is required for the homeostatic maintenance and expansion of innate B1 cells.

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1982 – WS16.3

Intracellular trafficking receptor SorLA is required for BCR uptake and trafficking, regulating B cell-mediated immune responses in vivo

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Antigen presentation by B cells is crucial for efficient humoral immune responses. B cells internalise specific antigens through the B cell receptor (BCR). Internalised antigens are trafficked to and processed within degradative lysosomal compartments where antigenic peptides are loaded onto major histocompatibility complex-II (MHC-II) for presentation to cognate T follicular helper cells. Subsequent B cell activation leads to the generation of a high-affinity antibody repertoire for efficient antigen clearance. Improved understanding of antigen uptake and trafficking mechanisms will offer insights into B cell immune responses to infection, malignancy, and autoimmune disorders associated with dysregulated B cell antigen presentation.

A genome-wide CRISPR-Cas9 screen has highlighted a novel role for intracellular trafficking receptor, SorLA (encoded by the *SORL1* gene), in B cell antigen uptake. We aim to elucidate the mechanisms behind this novel trafficking during B cell activation.

Our findings reveal SorLA interacts with the BCR in murine splenic and Ramos B cells, immunoprecipitating with the BCR and localising to internalised antigen clusters. Using CRISPR-Cas9-mediated knockout of *SORL1* in Ramos B cells, we illustrate that SorLA is required for efficient uptake of both soluble and membrane-bound antigens. Moreover, *SORL1* gene disruption alters downstream antigen-BCR trafficking and processing, leading to dysregulated MHC-II antigen presentation and humoral immune responses *in vivo.*

In conclusion, we report a novel role for SorLA in BCR trafficking and B cell-mediated immune responses, providing evidence of a novel mechanism of antigen-BCR trafficking during B cell activation.

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1218 – WS16.4

Active Inhibition of Chemokine-Mediated Migration Impacts Tumour-Lymph Node Crosstalk in TNBC

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In triple negative breast cancer (TNBC), draining lymph nodes (LNs) serve as both the first site of metastasis and the orchestrator of antitumour immune responses. We have demonstrated that TNBC patients with high tumour-infiltrating lymphocytes (TILs) and the presence of tertiary lymphoid structures (TLS) have more LN germinal centers (GC). These features associate with longer distant metastasis-free survival and imply crosstalk between the tumour and the LN. Despite these findings, only up to 50% of TNBC patients exhibit TLS, suggesting impairment in the tumour-draining LN axis in the rest. Understanding the factors governing this impairment is crucial for establishing novel therapies including for the induction of TLS.

We immunophenotyped the primary tumours and draining LNs in two orthotopic mouse models of TNBC at various time points post-implantation. Remarkably, we consistently observed minimal B cell tumour infiltration despite robust induction of tumour-draining LN GC responses. These observations underscore the utility of these models for elucidating the impairment of the primary tumour-draining LN axis.

We validated the LN as the primary source of the limited tumour-infiltrating B cells (TIL-B) by employing FTY720, a LN egress inhibitor. To investigate the chemotactic ability of the tumour secretome, we established a tumour slice culture method. Using a high-throughput approach, we assessed the murine tumour secretome for the presence of TLS-associated chemokines identified using microarray data from 124 TNBC patients. Tumour slice supernatants lacked TLS-inducing factors and did not induce the migration of B and T cells in transwell assays. Next, we tested the ability of key chemokines to induce chemokine-mediated immune cell migration. Intriguingly, we observed that chemokine-supplemented tumour slice supernatants prompted significantly less B cell migration than chemokine-supplemented culture medium, suggesting that the tumour secretome contains chemokine-mediated migration inhibitor/s.

Our findings suggest that the absence of TIL-B and TLS in TNBC may result from a combination of mechanisms: namely the failure by the tumour microenvironment to produce necessary chemokines to induce immune infiltration from the LN; and the active secretion of factors which hinder chemokine function. Insights into the active inhibition of chemokine-mediated immune cell migration may provide new treatment strategies in TNBC.

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609 – WS16.5

Human B-cell development at the single-cell level

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Purpose: B cells constitute an integral part of the immune system, actively participating in immune responses and forming immunological memory. Their development occurs in the bone marrow through a highly regulated process involving the generation of a diverse repertoire of B-cell antigen receptors. Although most studies of B-cell development have been carried out in mice, the advent of high-throughput single-cell sequencing methods enables us to unravel the complexity of human B-cell development in great detail. Understanding differences in B-cell development between mice and humans is essential, as it can uncover the mechanisms behind B cells in disease and lead to novel therapeutic strategies.
Methods: Here, we have analysed human bone marrow 'early' ($CD10^{+}CD24^{hi}CD38^{hi}$) B-lineage cells by 5´ single-cell RNA- and V(D)J-sequencing and flow cytometry.
Results: Based on distinct gene expression profiles, trajectory inference, and gene set enrichment analyses, we delineated a developmental pathway from progenitor (pro) B via large precursor B1 (L-preB1) and L-preB2 to small preB and subsequently to immature B1 (iB1) and iB2. The latter expressed higher levels of, for example, *MS4A1*, the gene encoding CD20. Flow cytometric analyses corroborated that the 'early' B-lineage cells contained two subsets of iB cells, $IgM^{+}IgD^{-}$ and $IgM^{+}IgD^{+}$, of which the latter expressed higher levels of CD20, indicating that they were more mature. Further confirmation that iB2 cells were indeed iB cells and not circulating transitional B cells came from integrative analyses of peripheral blood transitional B cells, which showed that the transcriptomes of iB2 and transitional B cells were distinctly different. Examination of differentially expressed genes between clusters revealed changes in the expression of transcription and survival factors, as well as signalling molecules. Gene set enrichment analyses highlighted differences in the involvement of different biological processes.
Conclusion: Our findings highlight the intricate journey of developing B cells in the human immune system, revealing a complex landscape. By using cutting-edge sequencing technologies, we provide a comprehensive perspective that not only enhances our understanding of lymphocyte development, but also opens new avenues for innovative strategies to study B-cell related diseases such as autoimmunity and B-cell driven leukaemia.

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598 – WS16.6

New mode of B-cell activation through extracellular release of native antigen by dendritic cells

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Dendritic cells (DCs) are antigen-presenting cells (APCs), which sample antigen (Ag) in the periphery and migrate to the lymph node (LN) where they activate T cells. Previously, we showed that DCs are able to store and to release from late endosomes native Ag into the extracellular medium. Alternative modes of B-cell activation by APCs, beyond cell-to-cell contact, such as extracellular release of Ag by DCs remain however not investigated. Using a subcutaneous delivery of Ag-loaded DCs in vivo and an in vitro co-culture system, we aimed: 1)- to visualize Ag trafficking by DCs to the LN; 2)- to investigate the modalities of Ag transfer and B-cell activation by the distinct DC subsets; and 3)- to probe the role of exosomes (Exo) in Ag release and its regulation by glucocorticoids (GCs). Here, we show that peripheral DCs are transporters of native Ag to the LN-B cell zone and potent B-cell activators both in vivo and in vitro. We highlight a novel extracellular mode of B-cell activation by showing that Ag release by DCs is sufficient to efficiently induce early B-cell activation through the transcription factor NF-κB/cRel. Furthermore, LN-resident DC subsets including conventional DCs (cDCs) and plasmacytoid DCs (pDCs) are also able to release native Ag with an unexpected superiority for pDCs. Strikingly, this new mechanism consists of an Exo-free release of native Ag, contrasting with the Exo-dependent extracellular T-cell activation by DCs. Interestingly, glucocorticoids inhibit Ag release and the subsequent induced B-cell activation by DCs. Thus, our study provides new mechanistic insights into the modes of Ag delivery for B-cell activation by DCs and a promising approach of drug modulation of the DC-elicited Ag-dependent B-cell responses.

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WS17 – NEUROINFLAMMATION II

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2195 – WS17.1

Meningeal γδ17 T cells and blood-brain barrier disruption: implications for neonatal Group B *Streptococcus* meningitis

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Neonatal Group B *Streptococcus* (GBS) meningitis remains a devastating disease associated with significant morbidity. Urgent demand exists for novel therapeutic and neuroprotective interventions. Here, we hypothesise that meningeal inflammation contributes to the neurologic sequelae observed in humans surviving GBS meningitis. Using a clinically relevant mouse model that mimics GBS vertical transmission from pregnant females to their progeny, we found altered γδ T cells in the meninges of infected pups. Flow cytometry analysis showed an increased frequency of γδ T cells at postnatal day (P) 3, independently of the brain bacterial burden. We also observed a significant decrease of IL-17A (IL-17 herein onwards) transcription in this subset while detecting an increase of IL-17 in the supernatant of cultured meningeal cells isolated from infected P3 pups after 24h of PMA/ionomycin stimulation. Neonatal thymic analysis showed no differences in γδ T cells or their commitment to IL-17 or IFN-γ production. Notwithstanding, two- and six-week-old infected mice presented increased numbers of meningeal γδ17 T cells, suggesting an important role of this subset in this disease. To investigate the contribution of γδ T cells to GBS meningitis pathogenesis, we performed infectious studies in TCRδ$^{-/-}$ mice. Interestingly, the absence of γδ T cells resulted in decreased brain bacterial load at P1 and P3 while showing no differences in the lungs and meninges. TCRδ$^{-/-}$ mice surviving infection presented decreased long-term sequelae. To understand how γδ T cells contribute to bacterial parenchymal invasion, we next assessed the blood-brain barrier (BBB) permeability both *in vitro* and *in vivo*, in TCRδ$^{-/-}$ versus TCRδ$^{+/+}$ pups. Western Blot quantification of the BBB tight junction protein occludin showed a significant decrease in TCRδ$^{+/+}$ infected pups compared to the TCRδ$^{-/-}$ uninfected ones. No differences were observed between TCRδ$^{-/-}$ infected and uninfected pups. Moreover, *in vivo* administration of FITC-dextran proved that BBB permeability is increased in both infected groups, with the TCRδ$^{+/+}$ group being most affected. Mice lacking IL-17 have increased bacterial control. Our study demonstrates that during GBS meningitis, the meningeal γδ17 T cell population contributes to BBB disruption and GBS invasion, being associated with behavioural abnormalities later in life.
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422 – WS17.2

The interplay between inflammation and oxidative stress supports autistic-related behaviors in two mouse models of Autism Spectrum Disorders

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Autism Spectrum Disorders (ASD) are highly prevalent neurodevelopmental conditions characterized by social communication deficits and repetitive and restricted behaviors. Several studies showed that inflammation may contribute to ASD, as high levels of pro-inflammatory molecules were described in the peripheral blood (PB) of individuals with ASD. Here we used RT-qPCR, RNA sequencing, metabolomic analysis and flow cytometry to show that molecules related to inflammation were increased in the cerebellum, PB, bone marrow and spleen of mice lacking Cntnap2, robust model of ASD. In parallel, pro-inflammatory molecules were additionally increased in the brain and PB of Shank3b mutant mice, another mouse model of ASD. The frequency and branching of microglia cells were impaired in Cntnap2$^{-/-}$ mice. In parallel oxidative stress was increased in the cerebellum of mutant animals compared to controls. Systemic treatment with the antioxidant N-acetyl-cysteine (NAC) rescued ASD-related behaviours in Cntnap2 and Shank3b mutant mice. Oxidative stress and inflammation in the cerebellum, as well as pro-inflammatory conditions in the PB, bone marrow and spleen were counteracted by NAC treatment. In addition, the phenotype of microglia cells as well as the branching were improved in Cntnap2$^{-/-}$ mice injected with NAC. Taken together, our findings suggest that the interplay between oxidative stress and inflammation may support the pathogenesis of ASD-related behaviors in mice.

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130 – WS17.3

Mapping midbrain immune response and potential gateway function of the choroid plexus in chronic gut inflammation

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Purpose: Chronic gut inflammation, as in inflammatory bowel disease (IBD), is associated with neuropsychiatric and neurodegenerative disorders, particularly Parkinson disease (PD). This link might be mediated by a gut-immune-brain axis suggested as a pivotal route of communication between the gut and the central nervous system. Specifically, gut-derived inflammatory factors are hypothesized to reach the systemic circulation and modulate brain function via distinct interaction sites, including the choroid plexus. Here, we used a mouse model of IBD to examine effects of chronic gut inflammation on the immune compartments of the choroid plexus and the midbrain. We thereby aim to better understand PD development in IBD.

Methods: Male and female adult C57BL6/J or *Hexb*$^{tdT/tdT}$ mice were fed with 2 % (w/v) dextran sulfate sodium (DSS) in drinking water for 5 days followed by normal drinking water for 10 days. The cycle was repeated thrice to induce chronic colitis that was confirmed by colonoscopy. Control mice received normal drinking water for the whole study period. Brain tissue was analyzed by multicolor flow cytometry and immunofluorescence. Bulk RNA-sequencing of three different brain regions was performed and complemented by proteomic analysis of the respective contralateral tissue. CD45^{+} immune cells were sorted from the midbrain and choroid plexus and subjected to single-cell RNA-sequencing.

Results: Mice treated with DSS developed colitis and showed signs of systemic, lung and liver inflammation. Transcriptomic and proteomic analyses revealed substantial reorganization of the immune cell compartment in the midbrain compared to other brain regions. By using a microglia-specific reporter gene mouse model, brain-resident macrophages (microglia) and microglia-derived molecules were identified as key elements in the inflammatory response. In addition, transcriptomic changes in the choroid plexus indicated endothelial dysfunction and increased blood-brain barrier permeability.

Conclusion: Our findings provide evidence for an innate and adaptive immune cell response in the midbrain to chronic gut inflammation, and point towards a key role of the choroid plexus in preventing immune cell entry into the brain parenchyma. These results significantly enhance our understanding of the gut-immune-brain axis that links peripheral inflammation to neurodegenerative processes, such as in PD.

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861 – WS17.4

Effects of microbial infection on the blood-brain barrier – high-dimensional tissue profiling using imaging mass cytometry

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Purpose: Blood-brain barrier (BBB) functions as an interface between the brain parenchyma and peripheral blood, regulating the exchange of metabolites and nutrients. Due to its critical role in maintaining health of the central nervous system (CNS), its breakdown may have long-term ramifications, including cognitive decline. In fact, it has been postulated that this vascular damage might be one of the early markers of Alzheimer's disease, the most frequent type of dementia. Therefore, in this study we postulate that one of the initiating factors of the BBB-breakdown might be the keystone periodontitis pathogen, *Porphyromonas gingivalis*, and that its translocation from the gingival tissue to the vicinity of BBB could cause pro-inflammatory response and vascular dysfunction, in the end resulting in the cognitive decline.
Methods: Tissues (brain, liver) of wild-type C57BL/6J and the 5xFAD murine strains orally infected with *P. gingivalis* for 22 weeks were collected. A novel panel of 40 metal-tagged antibodies suitable for imaging mass cytometry was designed, tested, and optimized using the harvested tissues to visualize tissue-specific protein expression and protein level changes resulting from *P. gingivalis* infection. Obtained data was analyzed *via* Steinbock pipeline.
Results: Of 40 markers, 9 were used to distinguish unique cell types (endothelium, pericytes, astrocytes, microglia, neurons, oligodendrocytes etc.). The rest allowed for characterization of the CNS' extracellular matrix, inflammatory responses, and BBB-specific markers (including GLUT1, AQP4, claudin 5). An antibody against *P. gingivalis*-derived virulence factor (rgpB) was included. Preliminary results indicate that rgpB can be reliably detected in tissues distant to the site of primary infection. Its presence induces local upregulation of pro-inflammatory markers (such as TLR4, IL17RA).
Conclusions: Oral infection with *P. gingivalis* caused pro-inflammatory response in distant tissues (liver and brain), which could contribute to the BBB breakdown and AD pathogenesis. Imaging mass cytometry can be successfully applied to characterize phenotypic changes of the BBB. Overall, the designed panel offers a unique opportunity to evaluate the blood-brain barrier breakdown and neuroinflammation in murine models.

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900 – WS17.5

Opposite innate immune signatures in Varicella Zoster Virus and Sendai Virus infected human induced pluripotent stem cell derived neurospheroids

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Innate immune signalling, and more specifically Type-I/II interferon (IFN) signalling, is a first line cellular defence mechanism against viral pathogens. With a specific focus on studying anti-viral neuro-immune responses in a human central nervous system (hCNS)-like environment, we first established and characterised a 5-month matured hiPSC-derived neurospheroid (NSPH) model containing TuJ1+ MAP2+ NeuN+ neurons and GFAP+ S100b+ AQP4+ Sox9+ CD49f+ astrocytes. Immune competence of these NSPHs was demonstrated by significant secretion of CXCL10 following stimulation with IL1b. Functional maturation of the NSPHs was confirmed by spontaneous and synchronized electrophysiological activity measured by calcium imaging.
Subsequently, NSPHs were infected with genetically engineered strains of either Varicella Zoster Virus (VZV-ORF23/GFP) or Sendai virus (SeV-eGFP). Live cell and immunocytochemical analysis here demonstrated that VZV spreads uninterrupted throughout the entire NSPH, while SeV spread was limited to the outer NSPH border. Next, using NanoString technology, broad transcript-level immune profiling was performed to explore the innate immune signatures of infected NSPHs. While SeV-infected NSPHs displayed a clear Type I/II IFN response, in VZV-infected NSPHs no Type I/II IFN response was activated. Even more, in the latter a strong suppression of genes related to the MHC Class I & II antigen presentation pathways was noted. Functionally validating these opposite innate immune signatures in VZV- and SeV-infected NSPHs, cytokine profiling of NSPH supernatant revealed increased secretion of IL6 and CXCL10 by SeV-infected NSPHs, but not by VZV-infected NSPHs. Similarly, immunocytochemical analysis demonstrated upregulation of Type I IFN activated anti-viral proteins Mx1, IFIT2 and ISG15 in SeV-infected NSPHs. Furthermore, CD74, a key part of the MHC class I antigen presentation pathway was found to be suppressed in VZV-infected NSPHs. We here demonstrate that our matured multicellular NSPH model is immune reactive, susceptible to viral infection, and most importantly, able to recapitulate VZV- and SeV-specific immune signatures. Therefore, this NSPH model will be suited to investigate viral neuro-immune responses and evasion strategies, as well as other neuro-inflammatory conditions following e.g., ischemic stroke and Parkinson's disease.

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1017 – WS17.6

Neuroimmune responses to intranasal poly(I:C) are primed by time of day

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Purpose: Neuroimmune responses are critical for survival. This is particularly evident with neurotropic (brain-targeting) virus infections, in which impaired immune signaling results in severe neuropathology and death. We previously found that the severity of neurotropic virus infection is impacted by time of day of infection. However, the mechanism by which time of day modulates this survival outcome remains unknown. To investigate potential mechanisms, our previous work shows that the olfactory bulb (OB), a site of neurotropic virus entry into the brain, rhythmically expresses neuroinflammation-related transcripts. These rhythmically expressed transcripts are enriched in genes associated with functional aspects of microglia. We also found that antiviral-related transcripts are upregulated in the OB at active phase onset, a time of enhanced survival following neurotropic virus infection. Here, we tested the hypothesis that time of day primes the OB to differentially respond to an intranasal virus-like challenge.
Methods: For Experiment 1, we intranasally challenged mice at resting phase onset (ZT0) or active phase onset (ZT12) with vehicle or poly(I:C) and collected tissues at 0-, 3-, 12-, and 24-hours post-inoculation. OB transcriptional responses were measured using NanoString technology. For Experiment 2, we intranasally challenged mice with vehicle or poly(I:C) at ZT0 or ZT12. We then isolated OB microglia at 24 hours post-inoculation and used imaging flow cytometry to analyze a population of cells characteristic of microglia.
Results: For Experiment 1, we found that intranasal poly(I:C) induced antiviral responses in the OB and that these responses unfolded more rapidly in mice challenged at ZT12 compared to ZT0. For Experiment 2, we found that time of day altered the number of OB microglia independent of treatment, with more OB microglia at ZT12 than ZT0. Surprisingly, we also observed a high proportion of microglia that contained intrinsically fluorescent puncta. The proportion of intrinsically fluorescent microglia was reduced following intranasal poly(I:C) at ZT12 but not affected following intranasal poly(I:C) at ZT0.
Conclusions: Time of day primes the OB to mount differential antiviral and microglial responses to intranasal virus-like stimuli, which may provide an antiviral gating mechanism underlying differential susceptibility to neurotropic virus exposure via the nasal route.

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WS18 – IMMUNE DEFICIENCIES AND IMMUNE DYSFUNCTION

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591 – WS18.1

Autoinflammatory patients with Golgi-trapped CDC42 exhibit intracellular trafficking defects leading to STING hyperactivation

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Purpose: Most autoinflammatory diseases are caused by mutations in innate immunity genes. Recently, four variants in the RHO GTPase CDC42 were discovered in patients affected by syndromes generally characterized by neonatal-onset of cytopenia and auto-inflammation, including hemophagocytic lymphohistiocytosis and rash (NOCARH syndrome). However, the mechanisms responsible for these phenotypes remain largely elusive.
Methods: Using microscopy and transcriptomics approaches, we analyzed protein trafficking, type I IFN response, STING activation and ER stress in both CDC42 patients' cells and THP-1 -transfected cells.
Results: We show that the recurrent p.R186C CDC42 variant, which is trapped in the Golgi apparatus, elicits a block in both anterograde and retrograde transports, and endoplasmic reticulum stress. Consequently, it favours STING accumulation in the Golgi in a COPI-dependent manner. This is also observed for the other Golgi-trapped p.*192C*24 CDC42 variant, but not for the p.Y64C and p.C188Y variants that do not accumulate in the Golgi. We demonstrate that the two Golgi-trapped CDC42 variants are the only ones that exhibit overactivation of the STING pathway. Consistent with these results, patients carrying Golgi-trapped CDC42 mutants present very high levels of circulating IFNalpha at the onset of their disease.
Conclusion: We report new mechanistic insights on the impact of the Golgi-trapped CDC42 variants. This increase in STING activation provides a rationale for combination treatments for these severe cases.

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1952 – WS18.2

NLRC4 mosaicism as the cause of a severe, early-onset autoinflammatory disease

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Purpose: To investigate the cause of an early-onset uncharacterized autoinflammatory disease (AID).
Methods: Genetics studies were performed using two different next-generation sequencing (NGS)-based methods: a targeted gene panel (TGP) for detection of germline variants and the amplicon-based deep sequencing (ADS) for postzygotic variants.
Results: The patient is an 18-month-old girl, who was born from a non-consanguineous couple of Egyptian ancestry, with no relatives affected by immune-related diseases. At the age of 6 months, she experienced for the first time recurrent febrile episodes (7-10 days), which recurred less than one week later, accompanied by extreme irritability. During her first year of life, she was hospitalized 6 times for suspected meningitis. Cerebrospinal fluid analysis always revealed the absence of pathogens, supporting for the presence of recurrent aseptic meningitis. Physical examination showed failure to thrive, bilateral arthritis in knees and urticarial-like rash. Laboratory tests revealed increased acute phase reactants (CRP and ESR), leukocytosis, neutrophilia, thrombocytosis and anaemia. A poor response to oral colchicine was observed. In contrast, the disease was relatively well controlled with the anti-interleukin-1 drug Anakinra, which was started on the suspicion of cryopyrin-associated periodic syndrome (CAPS). TGP did not detect any pathogenic germline variant in any of the genes causing monogenic AID. However, a specific analysis for post-zygotic variants identified the unreported c.1329C>A/p.His443Gln variant at the *NLRC4* gene, with an allele fraction (9.3%) compatible with that expected for variants causing gene mosaicism. Subsequent analyses in the patient's healthy first-degree relatives confirmed its de novo nature. This variant was absent in different databases (gnomAD v4.0, Kaviar) and bioinformatics analyses predicted an impairment in the protein function. Furthermore, a recent article reported this amino acid substitution as pathogenic, albeit as a consequence of a different nucleotide exchange[1]. Altogether, these evidences strongly support the pathogenic classification for the detected *NLRC4* variant.
Conclusions: Our findings demonstrate the novel, postzygotic c.1329C>A/p.His443Gln *NLRC4* variant as the plausible cause of the disease observed in the patient. These novel findings should be considered in the diagnostic evaluation of patients with early-onset AID mimicking CAPS.

[1]Wang J, et al. Ann Rheum Dis 2022; 81: 1173-8

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1586 – WS18.3

Functional analysis of inflammasome mutations in the inflammatory skin disease hidradenitis suppurativa

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Purpose: Hidradenitis suppurativa (HS) is a common and debilitating inflammatory skin disease with a prevalence between 1 and 4%. The aetiology of HS is poorly understood, and treatment options are limited. Further research into the pathophysiology of HS is required to create better optimised, personalised treatment options and investigating the role of inflammasomes in HS is a promising avenue. Inflammasomes are multiprotein complexes that play a central role in innate immunity. Inflammasome activation is crucial for host defence to pathogens, but also contribute autoinflammatory diseases. Inflammasomes have also been implicated in HS disease pathogenesis as they control the potent inflammatory cytokines IL-1β, which is uniquely overexpressed in HS compared to other autoinflammatory skin disorders. Recent studies provide evidence that HS patients may bear mutations in inflammasome proteins which lead to excessive inflammation.
Methods: We have generated a plasmid library to perform time-of-flight inflammasome evaluation (TOFIE) with an Apoptosis-associated speck-like protein containing a CARD (ASC) reporter cell line system (HEK293T-hASC-GFP) to assess alterations in inflammasome formation.
Results: We identified 25 mutations in 5 inflammasome genes (*NLRP1*, *NLRP3*, *NLRC4*, *MEFV* [Pyrin], *PSTPIP1*) using exome sequencing from 219 HS patients that are predicted to be damaging and/or consequential in HS.
Of these, we have identified a number of mutations which appear to alter inflammasome activation status.
Conclusion: By combining the disciplines of human genetics, immunology and dermatology offers promise in the pursuit of this goal to understand HS pathophysiology. Our study takes genetic insights and probes them with sophisticated molecular biological methods with a view to discovering druggable targets and providing evidence for the need for genomics screening in inflammatory skin disease.

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602 – WS18.4

Constitutive phosphorylation of STAT3 hallmarks different subsets of atypical T-bet-expressing B cells in immune mediated disorders.

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Purpose: Expansion of atypical CD21^{low}CD11c^{pos}CD19^{hi}T-bet^{pos} B-cells hallmarks several immunological conditions. Recent studies reported that JAK/STAT-signalling, activated by IL-21 plus IFN-γ, drives *in vitro* and *in vivo* the differentiation of naïve B-cells into CD21^{low}CD11c^{pos}T-bet^{pos} B-cells. *In vivo*, gain of function mutations of STAT3 cause uncontrolled accumulation of them in mice and humans. Our study explored the constitutive and inducible activation of STAT3 in T-bet^{pos} B-cells of normal subjects and of patients with immunological disorders. In vitro generation of T-bet^{pos}/phosphorylated STAT3-positive (pSTAT3^{pos}) B-cells was also investigated.
Methods: Freshly isolated peripheral blood mononuclear cells (PBMCs) of patients and healthy donors were fixed and permeabilized using BD-Phosphoflow Protocol III and intracellularly stained with fluorochrome-conjugated antibodies to T-bet and to STAT3 phosphorylated at Tyr705; this protocol permitted additional staining only of CD19 among relevant markers. CD11c and CD21 were investigated by staining viable cells followed by eBioscience-FoxP3 transcription factor kit treatment and T-bet staining. For *in vitro* experiments, PBMCs of healthy subjects were stimulated for five days with different combinations of CpG, anti-IgM, IL-21 and IFN-γ and stained with the two methods.
Results B-cells could be dissected into T-bet^{neg}, T-bet^{dim} and T-bet^{hi} populations with different levels of CD19, CD11c and CD21 expression. Constitutive STAT3 activation was significantly higher in CD19^{hi}T-bet^{hi} B cells than in T-bet^{neg} or T-bet^{dim} B-cells in patients with common variable immunodeficiency (n=9) or mixed cryoglobulinemia (n=6) and in healthy donors (n=12). Stimulation with IL-21 (15 min) further increased pSTAT3 in CD19^{hi}T-bet^{hi} B-cells whereas IFN-α and IL-6 did not. Five-day stimulation of normal PBMCs with CpG and anti-IgM induced the generation of CD19^{hi}T-bet^{pos} B-cells not expressing pSTAT3. The addition of IL-21 induced the generation of significant proportions of CD19^{hi}T-bet^{hi}pSTAT3^{hi} cells, which was not modified by co-stimulation with IFN-γ.
Conclusions Increasing levels of T-bet and pSTAT3 might identify distinct stages of differentiation of T-bet^{pos}-B-cells, and high constitutive activation of STAT3 hallmarks T-bet^{hi} B-cells in disease and health. IL-21, together with adaptative and innate B-cell stimuli, appears as a major player in the generation of T-bet^{hi}pSTAT3^{hi} B-cells. Further studies will need to address whether targeting JAK/STAT3-signalling could control the accumulation of these cells in autoimmunity.

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1075 – WS18.5

Deep immune cell profiling of patients with defects in the leptin-melanocortin signaling pathway

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The leptin-melanocortin signaling pathway plays an important role in body weight regulation and defects in this pathway can lead to rare monogenic forms of obesity. The most common defects include missense or nonsense mutations in the leptin receptor (*LEPR*), proopiomelanocortin (*POMC*), and the melanocortin-4 receptor (*MC4R*). While the effects of these mutations on the metabolism have been extensively studied, their role in immune regulation remains elusive.
To characterize the impact of the leptin-melanocortin signaling pathway on immune cell composition and function we collected a cohort of three patients with heterozygous mutations in *MC4R*, four patients with homozygous or compound-heterozygous mutations in *LEPR*, and six patients with homozygous mutations in *POMC*, as well as control groups of age- and sex-matched lean and obese individuals. Of note, most patients with *LEPR*, *POMC* and *MC4R* mutations included in this study were receiving the MC4R antagonist setmelanotide for treatment of monogenic obesity. Peripheral blood mononuclear cells (PBMCs) were isolated from all subjects, stimulated *ex vivo* for 4 h with ionomycin/PMA or lipopolysaccharide (LPS) and subsequently stained with a panel of 35 markers and analyzed by mass cytometry. By this, we performed an in-depth immune cell profiling of patients with monogenic obesity and compared the frequency and function of T cells, B cells, NK cells and myeloid cells to the control groups.
In patients with *LEPR* mutations, we detected a reduced frequency of pro-inflammatory IL-8$^+$ IL-6$^+$ TNFα$^+$ monocytes, whereas in patients with *MC4R* mutations we found an increased abundance of B cells. Patients with *POMC* mutations displayed increased frequencies of TNFα$^+$ IFNγ$^+$ CD4$^+$ and CD8$^+$ T cells as well as a significantly increased expression of TNFα and IFNγ in T cells.
Our study provides the first in-depth characterization of the immune cell composition of patients with monogenic obesity and indicate that LEPR, POMC and MC4R are not only important for appetite regulation but also have specific functions in the immune system. Functional assays are currently being performed to validate our findings and investigate underlying mechanisms leading to the observed differences.

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368 – WS18.6

Xpr1 deficiency dysregulates CD4+ T-cell responses by impaired calcium signalling and increased mTOR activation

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Purpose: Transmembrane receptors control signal transduction during T-cell stimulation. Here, we investigated the function of xenotropic and polytropic retrovirus receptor 1 (Xpr1) for stimulation, proliferation and differentiation of CD4+ T cells.
Methods: We compared thymocyte development and activation marker expression profiles in splenocytes from mice with or without conditional knockout of Xpr1 in CD4+ T cells using flow cytometry, gene expression analysis and enzyme-linked immunosorbent assays. Differential T-cell stimulation pathways in the absence of Xpr1 expression were assessed by phospho-specific immunoblotting of signal molecules. The impact of Xpr1 on proliferation and differentiation of isolated CD4+ T cells under Treg-, Th1-, Th2- and Th17-skewing conditions was analysed by flow cytometry and validated by siRNA-mediated XPR1 knockdown experiments in Jurkat cells. Calcium signalling and nuclear NFAT translocation as well as TCR internalisation was measured by live cell imaging and ImageStream analysis, respectively.
Results: We found that Xpr1 deficiency leads to hyperstimulation of T cells which increases positive and negative selection of thymocytes and enhances the expression of activation markers in the periphery. Moreover, gene ontology analysis revealed that Xpr1 deficient naive T cells readily express a stimulated phenotype upon TCR stimulation and release increased amounts of IL-2. We showed that CD4+ T cells with Xpr1 deficiency are hyperproliferative and display increased mTOR signalling, while ERK phosphorylation is non-sustained in comparison to controls. Consistently, expression of TNF-α and IFN-γ in Th1 cell- and IL-22 in Th17 cell-differentiation conditions increased in Xpr1 deficient CD4+ T cells and XPR1 siRNA-treated Jurkat cells. Mechanistically, we found that Xpr1 deletion promotes TCR internalisation but reduces calcium signalling and nuclear NFAT translocation.
Conclusion: In summary, Xpr1 deficiency promotes T-cell stimulation, proliferation and cytokine expression by altered T-cell stimulation pathways and may represent a potential target to regulate T-cell responses.

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WS19 – PATTERN RECOGNITION RECEPTORS AND INNATE IMMUNITY

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342 – WS19.1

AHR spying on bacterial communication and quorum during infection

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Purpose: The interaction between a bacterial pathogen and its host can be viewed as an "*arms race*" in which each participant continuously responds to the evolving strategies of the other partner. A mechanism allowing bacteria to rapidly adapt to such changing circumstances is provided by density-dependent cell-to-cell communication known as *Quorum Sensing* (QS). QS involves a hierarchy of signaling molecules, which in pathogenic bacteria is associated with biofilm formation and virulence regulation. We hypothesized that if a host sensor can detect and differentiate between bacterial QS molecules and their expression patterns, it will allow hosts to customize their immune responses according to the stage and state of infection.
Methods and Results: Taking advantage of different *in vitro* and *in vivo* (e.g., zebrafish and mouse) model systems, we demonstrate that infected hosts show differential modulation of the Aryl Hydrocarbon Receptor (AHR) signaling throughout a bacterial infection. AHR modulation depends on the relative abundances of different QS molecules, whereby their quantitative assessment enables the host to sense bacterial community densities that may have distinct gene expression programs and infection dynamics. The AHR is able to sense and bind to diverse microbial-derived ligands and regulate different host defence mechanisms, including ligand degradation, expression of pro-inflammatory mediators, immune cell recruitment and bacterial clearance. By sensing infection dynamics, the AHR regulates diverse host defense mechanisms and impacts bacterial clearance. Furthermore, AHR modulated by antibiotics and infection impacts therapeutic efficacy.
Conclusions: We propose that by spying on bacterial *quorum*, the AHR acts as a major sensor of infection dynamics, capable of orchestrating host defense according to the *status quo* of infection. Importantly, AHR modulation and activation status impact antibiotic therapeutic efficacy, potentially driving antimicrobial resistance and microbial adaptation strategies.

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1605 – WS19.2

PYHIN proteins modulate dendritic cell responsiveness to immune-stimulatory DNA via negative regulation of type I interferon

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The mouse PYHIN proteins AIM2 and IFI204, as well as their human ortholog and functional homolog - AIM2 and IFI16 respectively, contribute to innate immunity by sensing pathogen-derived nucleic acids to drive inflammasome activation and type I interferon (IFN-I) production. In addition to their role as innate sensors, PYHIN proteins modulate several cellular processes including differentiation, proliferation, apoptosis and senescence, whilst some also serve as viral restriction factors and direct regulators of cytokine gene induction. To date, the majority of work on PYHINs has been performed in cell lines, monocytes and macrophages, while there has been little investigation of PYHIN function in dendritic cells (DCs). As the crucial bridge between innate and adaptive immunity via their ability to engage in antigen presentation to lymphocytes, a better understanding of the role of PYHIN proteins in DCs may uncover avenues to target these proteins therapeutically to modulate downstream adaptive immune responses.
Using *in vivo* and *ex vivo* systems, we identify PYHIN proteins as negative regulators of DNA-induced responses in DCs, a somewhat surprising finding given their known roles as nucleic acid sensors and regulators of IFN-I transcription. In response to transfected DNA, but not RNA or TLR ligands, DCs from mice lacking the entire PYHIN locus ($ALR^{-/-}$ mice) produce increased IFN-I and pro-inflammatory cytokines, exhibit an enhanced ability to process antigen and also display increased expression of DC maturation markers, including MHC and CD86, on their cell surface. These phenotypes are recapitulated in $Aim2^{-/-}$ cells and mechanistically are due to enhanced activation of the cGAS-STING-TBK1-IRF3 axis. Using co-culture assays we demonstrate that this dysregulated innate immune response influences T cell proliferative and effector immune responses in an IFN-I-dependent manner. Furthermore, in a therapeutic MC38 *in vivo* tumour challenge model, intra-tumoral administration of chitin-derived polymers known to induce cGAS-STING-dependent IFN-I production exhibit markedly enhanced protective efficacy in $ALR^{-/-}$ mice, including improved survival and increased tumour eradication compared to wildtype animals undergoing the same treatment.
Overall, this work identifies PYHIN proteins as negative regulators of DNA-induced responses, particularly IFN-I, and suggests that they may represent promising therapeutic targets to modulate anti-viral and/or anti-tumour immunity.

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1461 – WS19.3

NLRP3 is a thermosensor that is negatively regulated by high temperature

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Inflammation is an essential response to infection and injury, but unregulated inflammation is damaging and must be limited by negative feedback signalling. Inflammasome activation is highly inflammatory, driving both local inflammation and systemic responses like fever. However, our understanding of how inflammasome signalling is negatively regulated is limited. Mutations in the inflammasome sensor NLRP3 cause Cryopyrin Associated Periodic Syndromes (CAPS) that are characterised by recurrent fevers. A subgroup of NLRP3 mutations cause Familial Cold Autoinflammatory Syndrome (FCAS) where, remarkably, NLRP3 activation is triggered by cold temperature. In healthy individuals NLRP3 is activated by a vast number of stimuli and senses perturbations of cytoplasmic homeostasis. As temperature is a fundamental environmental stressor, we hypothesised that NLRP3 inflammasome signalling would be sensitive to increased temperatures.
We investigated the effects of high temperatures on NLRP3 in mouse and human macrophages. Short-term incubation at high fever range temperatures significantly inhibits NLRP3 activation, while secretion of the inflammasome-independent cytokines TNF and IL-6 are much less affected. High temperature blocks NLRP3 inflammasome formation in a transcription-independent manner, and NLRP3 is highly sensitive to temperature-mediated inhibition relative to the NLRC4, AIM2, and NLRP1 inflammasomes. Using cellular and *in silico* assays we show that the effect of high temperature on NLRP3 is protein intrinsic. The activation of NLRP3 is associated with a decrease in the thermal stability of the protein and molecular dynamics simulations identify a peptide in the C-terminal of the FISNA domain (COFI) that undergoes a significant conformational shift at high temperature. The COFI peptide is specific to NLRP3 and is not present in closely related proteins such as NLRP12 or NLRP6. Cellular assays demonstrate that the COFI regulates NLRP3 stability and is required for activation.
Finally, *in vivo* experiments demonstrate that elevation of mouse body temperature negatively regulates LPS-induced inflammatory cytokine production. Our studies reveal that high temperatures associated with fever limit NLRP3 activity in a classical negative feedback mechanism and identify a novel role for NLRP3 as a protein thermosensor.

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2189 – WS19.4

Toll-like receptor 3 (TLR3) Leu412Phe (L412F): a candidate polymorphism in lung microbiome dysregulation and acute exacerbation in idiopathic pulmonary fibrosis patients

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Purpose: We previously established that the toll-like receptor 3 Leu412Phe (*TLR3* L412F; rs3775291) polymorphism attenuates anti-viral responses and is associated with accelerated disease progression and increased mortality risk in idiopathic pulmonary fibrosis (IPF) patients (*AJRCCM* 2013, 188: 1442). The role of *TLR3* L412F in bacterial infection, lung microbiome dysregulation and associated acute exacerbations (AE) in IPF patients is less well established.
Methods: Here, we investigated the effect of *TLR3* L412F on the IPF lung microbiome using 16S rRNA qPCR and pyrosequencing. The effect of *TLR3* L412F on anti-bacterial TLR-responses of primary IPF lung fibroblasts was quantitated. Hierarchical heatmap analysis was employed to establish bacterial and viral clustering in nasopharyngeal lavage samples from AE-IPF patients.
Results: We observed a significant increase in AE-related death in 412F-variant IPF patients. We demonstrated that 412F-heterozygous IPF lung fibroblasts have reduced anti-bacterial TLR responses to LPS (TLR4), Pam3CYSK4 (TLR1/2), flagellin (TLR5) and FSL-1 (TLR6/1) and have reduced responses to live *Pseudomonas aeruginosa* infection. Furthermore, 412F-heterozygous IPF patients had a dysregulated lung microbiome with increased frequencies of *Streptococcus* and *Staphylococcus spp*.
Conclusion: This study reveals that *TLR3* L412F dysregulates the IPF lung microbiome and reduces the responses of IPF lung fibroblasts to bacterial TLR-agonists and live bacterial infection. These findings identify a candidate role for *TLR3* L412F in viral- and bacterial-mediated AE-death.

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7 – WS19.5

SARM1 regulates pro-IL-1β expression in monocytes by an NADase-dependent mechanism

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Sterile alpha and HEAT/Armadillo motif-containing protein 1 (SARM1) is a member of the Toll/IL-1R (TIR) domain protein family and is highly conserved in evolution with a role in innate immunity and neuronal cell death in diverse organisms. We recently showed that SARM1 is a regulator of inflammasome responses in murine macrophages, although the detailed role of SARM1 in innate immunity remains unclear. A major recent discovery was that the TIR domain of SARM1 actually has NADase enzymatic activity, and this activity is required for SARM1-dependent axon degeneration in neurons. Furthermore, a wide range of TIR domain protein orthologues in lower organisms, but not mammals, also have NADase activity and this activity has been reported to be essential for innate immune regulation. SARM1 is identified as a unique NADase enzyme among TIR proteins and intracellular proteins. Therefore, this study aimed to determine the role of SARM1 and its NADase enzymatic activity, in innate immune responses in monocytes. Here we show that SARM1 regulates proinflammatory cytokine expression in both an NADase-dependent and -independent manner in monocytes. Using both loss-of-function and gain-of-function cell models, whereby enzymatically active or inactive SARM1 is expressed, we find that SARM1 negatively regulates TLR4-dependent TNF mRNA induction independently of its NADase activity. In contrast, SARM1 negatively regulates IL-1b secretion through both NADase-dependent inhibition of pro-IL-1b protein expression and NADase-independent suppression of the NLRP3 inflammasome and hence of pro-IL-1b processing to the mature secreted form. Interestingly, proteomics analysis revealed that SARM1 NADase-dependent inhibition specifically regulates pro-IL-1b protein expression levels, and other detected pro-inflammatory cytokines and chemokines are not affected. Further, we identified numerous proteins that participate in regulating metabolic pathways, which are decreased by SARM1 NADase dependently, suggesting those proteins require NAD and/or are regulated by SARM1. Thus, our data, reveal multiple mechanisms whereby SARM1 regulates pro-inflammatory cytokines in myeloid cells and shows a distinct NADase-dependent role for SARM1 in innate immunity compared to other TIR proteins.

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1460 – WS19.6

MicroRNAs released by UV-treated keratinocytes activate pDCs viaTLR7: a model mechanism of type I interferon triggering in psoriasis

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Purpose: Excessive production of type I interferons (IFNs) by plasmacytoid dendritic cells (pDCs) is the crucial event responsible for autoreactive T cell activation and tissue damage in a subgroup of autoimmune conditions known as "type I IFN-mediated diseases", including systemic lupus erythematosus (SLE) and psoriasis. We previously showed that circulating small extracellular vesicles (sEVs) from SLE patients activate pDCs via TLR7 triggering, suggesting a role for GU-rich microRNAs (miRNAs) as TLR7 endogenous ligands. Here, we used psoriasis as a model condition to investigate if deregulated miRNA secretion may represent a new pathogenic mechanism activating pDCs in type I IFN-mediated diseases.
Methods: Inflamed and UV-treated keratinocytes were used as in-vitro models of psoriatic skin to collect sEVs. Small RNAs contained in sEVs were sequenced and analyzed in terms of upregulated and TLR7-binding miRNAs. sEVs were used to stimulate primary human pDC activation, which was assessed in terms of type I IFN secretion and allogeneic $CD8^+$ T cell activation. The involvement of TLR7 was assessed by using specific inhibitors. An anti-BDCA-2 antibody and inhibitors of sEV production were also used. Psoriatic skin biopsies were collected and used to extract and quantify miRNAs by RT-PCR.
Results: TLR7-activating GU-miRNAs were selectively upregulated in sEVs derived from UV-treated keratinocytes as well as in psoriatic skin lesions. sEVs from UV-treated keratinocytes stimulated TLR7-dependent production of type I IFN and activation of cytotoxic $CD8^+$ T cells by pDCs. This activation was blocked upon triggering of the pDC-inhibitory receptor BDCA-2.
Conclusion: Our results identify miRNAs released by damaged keratinocytes as novel pathogenic mediators of pDC activation in the onset of psoriasis, setting the bases for the identification of new therapeutic targets and options in psoriasis and potentially in other type I IFN-mediated diseases.

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WS20 – MECHANISMS OF ANTIGEN PRESENTATION

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841 – WS20.1

Non-canonical proteome derived from transposable elements is a source of functional protein isoforms and cancer neoantigens

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Recent advances in ribosome profiling and mass spectrometry-based proteomics have expanded our understanding of the proteome by uncovering non-canonical proteins. Among these, transposable elements (TEs), which constitute 45% of the human genome and are repetitive sequences dispersed throughout it, play a significant role. TEs can serve as hosts for open reading frames in non-genic regions or, when transcribed, undergo non-canonical splicing events with protein-coding exons. In this study, we explore the role of splicing between exons and TEs as a source of unannotated functional isoforms and tumor-specific neoantigens.
Using transcriptome assembly, ribosome profiling, and mass spectrometry we showed that exonized TEs can be efficiently translated, resulting in a population of low-abundance isoforms that are generally shorter but stable. We characterize their subcellular localization and demonstrate that their functions can diverge from those of canonical isoforms. While many TE-derived isoforms are specific to individual samples, some are shared across different individuals. Moreover, a subgroup of these isoforms is recurrent in lung cancer patients but absent in healthy tissues.
Importantly, we provide evidence using immunopeptidomics that these unannotated isoforms encode HLA-I presented peptides, which are immunogenic and can be recognized by infiltrating CD8 T cells in lung tumors and tumor-invaded draining lymph nodes. Different TE families display varying capacities to encode stable protein isoforms or, on the contrary, short-lived polypeptides that enter the HLA-I processing pathway.
Our findings underscore the evolving nature of TE-derived proteins, which, while exploring new functions, may produce unstable and rapidly degraded products, giving rise to HLA-presented, recurrent, immunogenic, and tumor-specific antigens in cancer patients. Overall, our study emphasizes the clinical relevance of TE-derived antigens as promising targets for cancer immunotherapy and offers insights into the role of non-canonical splicing in TEs during protein evolution.

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595 – WS20.2

Ribosome profiling and immunopeptidomics reveal tens of novel conserved HIV-1 open reading frames encoding T cell antigens.

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In human cells, recent advances in genomics and peptidomics challenged the definition of Open reading frames (ORFs) demonstrating that thousands of small ORFs (sORFs) encode polypeptides or microproteins. It has been estimated that 85% of the translation products originate from non-annotated regions of the human genome and mostly from out-of-frame sequences of ORF, so-called alternative ORF (ARF). The latest studies revealed that a significant fraction of peptides presented by MHC molecules is derived from sORFs encoded polypeptides that are specific or overrepresented in tumour cells.
In the context of HIV-1, T cells are also required to maintain the viral set-point and in some donors to naturally control viral replication to undetectable levels, without treatment. Others and our team have previously shown that HIV-specific T cells target peptides presented by MHC molecules derived from HIV ARFs. However, to date, the existence of ARFs in the HIV genome has been only highlighted using indirect approaches such as T cell-based assays and HLA footprint analyses.
In the present study, we defined the translatome of HIV-1 in infected CD4+ T cells. Using ribosome profiling, we provide an unbiased assessment of actively translated viral mRNA sequences in HIV-infected cells and highlight that HIV-1 genomes harbours 98 ARFs corresponding to sORFs and located in the 5' UTR region or overlapping ORF encoding canonical HIV-1 proteins. Using a database of HIV genomes, we show that most ARF amino-acid sequences are highly conserved among clade B and C of HIV-1, with 8 ARF-encoded amino-acid sequences being more conserved than the overlapping ORFs. In addition, using two complementary and independent approaches, detection of ARF-derived peptide-specific T cells in the PBMCs of people living with HIV (PLWH) and direct isolation of MHC-bound ARF-derived peptides using mass spectrometry-based immunopeptidomics, we readily demonstrated that the HIV-1 ARFs encode viral polypeptides capable of inducing broad and potent T cell responses. Remarkably, ARF-derived peptides are recognized by CD8+ and CD4+ T cells from PLWH.
Our findings broaden the spectrum of HIV-1 immunogenic antigens that will help designing efficacious vaccines. It might also reveal the existence of HIV-1 microproteins or pseudogenes.

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721 – WS20.3

Engagement of TNF superfamily regulates the formation of the dendritic cell immunological synapse.

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Dendritic cells (DC) activate and prime T cells through the formation of a highly specialised interface called the immunological synapse. Expression of co-stimulatory or inhibitory molecules from the tumour necrosis factor (TNF) superfamily at the DC surface induces crucial signals for T cell differentiation, survival, proliferation, and cytokine production. Reciprocally, TNF superfamily members on T cells regulate DC maturation and survival. Interaction of CD40L, on CD4 T cells, with CD40, on DCs, leads to a new DC functional state, which increases their capacity to prime CD8 T cells. This phenomenon, known as "DC licensing", remains to be completely understood.
TNF superfamily receptors and ligands require a particular spatial organisation to induce signalling. Similarly, the spatial organisation of surface receptors and signalling proteins at the T cell side of the immunological synapse plays a crucial role in T cell activation. However, the spatial distribution of the TNF superfamily at the DC side of the immunological synapse has never been studied.
Here, we investigated the role of TNF superfamily members on the morphology and organisation of DC immunological synapses in the context of interactions with CD4 and CD8 T cells. We showed that interactions of CD40 on DCs with CD40L on CD4 T cells lead to larger DC synapses, where CD40 is concentrated at the centre. This process requires Myosin IIA activity and is necessary for proper DC licensing. We further showed that engagement of CD70 with its receptor CD27 in the context of interaction with CD8 T cells induces drastic changes in the DC synapse morphology and composition, which are even more pronounced in DCs that have been licensed through CD40. Altogether, our data indicate that engagement of TNF superfamily receptors and ligands regulate the DC immunological synapse and by doing so the very nature of their interactions with T cells.

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526 – WS20.4

Interferon-α promotes neo-antigen formation and preferential HLA-B-restricted antigen presentation in pancreatic β-cells

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Background: Interferon (IFN)-α is the earliest cytokine signature observed in individuals at risk for type 1 diabetes (T1D), but its effect on the repertoire of HLA Class I (HLA-I)-bound peptides presented by pancreatic β-cells is unknown.
Methods: Using immunopeptidomics, we characterized the peptide/HLA-I presentation in in-vitro resting and IFN-α-exposed β-cells.
Results: IFN-α increased HLA-I expression and peptide presentation, including neo-sequences derived from alternative mRNA splicing, post-translational modifications - notably glutathionylation - and protein cis-splicing. This antigenic landscape relied on processing by both the constitutive and immune proteasome. The resting β-cell immunopeptidome was dominated by HLA-A-restricted ligands. However, IFN-α only marginally upregulated HLA-A and largely favored HLA-B, translating into a major increase in HLA-B-restricted peptides and into an increased activation of HLA-B-restricted vs. HLA-A-restricted CD8+ T-cells. A preferential HLA-B hyper-expression was also observed in the islets of T1D vs. non-diabetic donors, and we identified islet-infiltrating CD8+ T-cells from T1D donors reactive to HLA-B-restricted granule peptides.
Conclusion: The inflammatory milieu of insulitis may skew the autoimmune response toward epitopes presented by HLA-B, hence recruiting a distinct T-cell repertoire that may be relevant to T1D pathogenesis.

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1896 – WS20.5

scATAC-seq in epithelial cells of human and mouse thymic medulla reveals a conserved HIVEP3-dependent program sustaining their maturation and the control of self-antigen expression

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Promiscuous expression of tissue-restricted self-antigen (TRA) genes in the thymus is crucial to the control of central T cell tolerance and autoimmunity. TRAs are expressed by mature medullary epithelial cells (mTECs) and presented to developing thymocytes, leading to depletion of those that recognize their cognate antigens and to Treg fate direction. Although the autoimmune regulator (AIRE) controls a wide array of TRAs, the induction of a large fraction of them is independent of AIRE and is specific to distinct sets of mTECs according to their level of maturation/differentiation. Here, to uncover the conserved missing transcription factors (TFs) that are implicated in TRA expression in regard to mTEC maturation, we carried out chromatin-accessibility analysis on individual human and mouse mTECs.
The diversity of mTEC chromatin landscapes led to the delineation of clusters that match the known maturation and differentiation states of these cells. TF motif and feature analyses revealed a striking enrichment of HIVEP recognition sites in pre-mature and mature AIRE-positive mTECs. HIVEP motif enrichment showed a similar pattern to the non-canonical nuclear factor NF-kB that was reported to control mTEC development, therefore highlighting a role for HIVEP TFs in mTEC maturation. Remarkably, we found a strong enrichment of HIVEP recognition sites in AIRE-dependent and independent TRA genes, and a conserved expression of the HIVEP3 gene in human and mouse mature mTECs. Thus, these findings point to a role of HIVEP3 in the control of mTEC maturation and TRA expression. In addition, motif enrichment comparison between AIRE-positive mTECs and terminal differentiated "mimetic" mTEC subtypes, revealed a dramatic reduction of HIVEP accessibility in favor of open chromatin for specialized TFs corresponding to extra-thymic cell types, such as HNF4G for microfold mTECs. Importantly, comparative analysis of lineage-specific master regulators revealed the involvement of conserved TFs in human and mouse mimetic mTECs while a few are species-specific.
Together, our data unveils a role for HIVEP3 on mTEC development and TRA expression in pre-mature and mature AIRE+ mTECs, in a mirror image with lineage-specific TFs that are implicated in mimetic mTEC subtypes and specific TRA expression programs.

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2016 – WS20.6

Mapping the RA synovial immune-peptidome

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Autoreactive CD4+ T-cells play a critical role in the pathogenesis of rheumatoid arthritis (RA), based on strong genetic associations at the *HLA-DRB1* locus. Identification of the autoantigens and responding CD4+ T-cells involved in the initiation and progression of RA is essential for a comprehensive understanding the disease process and for developing tolerising immunotherapies. Here, we determined the synovial immune-peptidome from synovial tissue (ST) of ACPA+ patients and assessed CD4+ T cell reactivity to these autoantigens.
Naturally presented HLA-DR-bound peptides were identified in seven ST samples from *HLA-DRB1* shared epitope-positive (SE+) ACPA+ patients using immunoaffinity capture and nUPLC-MS/MS. Expression of genes corresponding to parental source proteins was analysed across 18 synovial cell clusters using AMP1 phase 1 datasets[1]. Responses to ST-peptides in peripheral blood mononuclear cells from ST donor or genotyped-matched patients were analysed by IFN-γ fluorospot or 7-day CFSE proliferation assays. Peptide binding affinities to HLA-DRB1*01:01/*04:01/*10:01 allomorphs were determined by fluorescence polarisation.
274 HLA-DR bound peptides (79 source proteins) were identified in ST, with 39% of peptides identified in all biopsies across all patients, regardless of *HLA-DRB1* alleles. Genes corresponding to source proteins were expressed across distinct ST cell clusters, including fibroblasts, monocytes, and B cells. The number of antigen-specific responses observed in the periphery was variable across autologous/genotyped-matched patients with positive responses ranging from 6% to 56% out of 138 ST peptides tested. The highest frequency responses to a subset of ST-peptides (STAB1, GPNMB, DSG2, IGHG1) were observed in up to 80% of patients. Interestingly one of these GPNMB is enriched in monocytes and negatively correlates with risk of RA. Strong binding to HLA-DRB1*01:01 and HLA-DRB1*10:01 was confirmed for all lead peptides (IC50 range = 0.07-1.13mM). More variable binding was observed for DRB1*04:01 (0.31-13.43mM).
RA T-cells respond to a diverse repertoire of naturally processed peptides in the context of a range of *HLA-DRB1* alleles with parental proteins from a range of synovial resident cell types . The unique characteristics of peptide binding, TCR responses and the state of host immunity appear to dictate CD4+ T cell responses to these novel synovial-derived peptides (see accompanying abstract by Pook et al).

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WS21 – CANCER IMMUNOGENETICS

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1757 – WS21.1

Harnessing NK cell-mediated killing of prostate cancer cells through epigenetic modulation

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Prostate cancer (PCa) is one of the most common malignancies worldwide. Although available therapeutic options for early-stage PCa are generally efficient, treatment of advanced disease remains a clinical challenge. Epigenetic mechanisms, such as methylation or histone modifications play an important role, not only in in PCa development and progression, but also in tumor immune-evasion. Enhancer of zeste homolog 2 (EZH2) is the catalytic subunit of the polycomb repressive complex 2 (PRC2), involved in transcriptional repression by histone 3 trimethylation (H3K27me3). EZH2 is known to be involved in PCa progression. Moreover, EZH2 was shown to support tumor immune escape through MHC downregulation and PDL1 induction. Natural killer (NK) cells are main innate anti-tumor effectors, targeting tumor cells through an array of activating and inhibitory receptors that interact with cell surface ligands on target cells. A recent study showed that inhibition of EZH2 can result in upregulation of NK ligands on hepatocellular carcinoma. Here, we propose to use CPI-1205, a epi-drug targeting EZH2, to increase PCa sensitivity to NK-mediated killing.
PCa patient tissues immunohistochemistry showed an increase in EZH2 and H3K27me3 expression through disease progression. Inhibition of EZH2 with CPI-1205 in PCa cells resulted in the upregulation of NK cell activating ligands, particularly NKG2D ligands. CUT&RUN revealed H3K27me3 binding at the promoter region of those genes, which was reduced upon CPI-1205 treatment. Importantly, epi-drug treatment of PCa tumor cells co-cultured with NK cells led to an increased NK cell-mediated killing. Moreover, we established a PCa patient-derived explant (PDE) organotypic model accounting for tumor heterogeneity and architecture. Remarkably, CPI-1205 treatment of PCa-derived PDEs also induced NK ligand upregulation, supporting the results in a patient-derived model.
Our findings suggest that EZH2 inhibition with CPI-1205 can modulate PCa tumor cells rendering them more sensitive to NK cell-mediated killing, in both cell lines and a microtumor model, supporting the design of novel epigenetic-based therapeutic approaches for PCa patients.

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603 – WS21.2

Exploring the interplay between the RNA editing enzyme ADAR1 and innate immune responses in cervical cancer

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Purpose: The main function of ADAR1 enzyme is editing double-stranded (ds) RNAs by deamination of adenosines into inosines. This prevents aberrant activation of innate immune pathways (e.g. IFN-I production) upon sensing of endogenous dsRNAs, which may resemble viral structures. Several tumors exploit ADAR1 to evade immune detection and, indeed, ADAR1 deletion causes a decrease in tumor viability and a global reshaping of infiltrating immune cells. This study aims to investigate the role of ADAR1 in immune evasion mechanisms of cervical cancer (CC), the fourth most common tumor causing death in women. To this end, we are defining the innate lymphoid cells (ILCs) within CC biopsies, and dissecting *in vitro* the impact that ADAR1 manipulation in tumor cells has on the activation of innate lymphocytes, mainly NK cells, via IFN-I signaling and pro-inflammatory cytokines.
Methods and Results: We characterized ADAR1 expression in CC biopsies via immunohistochemistry (IHC), observing higher ADAR1 expression in pre-malignant lesions and in CC tissues compared to controls. In fresh CC biopsies, FACS analysis showed a reduced percentage of $CD7^+$ innate cells in tumors compared to healthy samples. Among these, $CD56^+$ NK cells showed signs of a repressed activity, with a decrease in cytotoxicity markers and an increase in the inhibitory signature. Silencing of ADAR1 in CC cell lines (SiHa, CaSki) reduced cell proliferation and sensitized cells to exogenous IFN-β treatment, as shown by live cell imaging. Additionally, RNAseq analysis of ADAR1-silenced cells demonstrated increased expression of pro-inflammatory cytokines and chemokines (e.g., *CXCL9, CXCL10, CXCL11, IL-12α, IL-15, IL-18, IL-23*), and conditioned supernatants collected from these cells potentiated NK cell responses in terms of proliferation, migration and cytotoxicity. NK cell activation upon ADAR1 silencing in SiHa cells was confirmed in organotypic tissue models.
Conclusion: We provided evidences that ADAR1 expression increases with CC progression and it is accompanied by an inhibited NK cell phenotype in the tumor microenvironment. *In vitro,* ADAR1 silencing caused an enrichment of inflammatory factors able to potentiate anti-tumor NK cell activities. These findings suggest that inhibition of ADAR1 expression and/or editing might represent a therapeutic perspective for cervical and other cancers as well.

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1419 – WS21.3

Clonal evolution analysis of paediatric lymphoma reveals a highly diversified and continuous cellular status and potential target for precision medicine

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Lymphoma is the third most common paediatric cancer (10-15%) worldwide. Although with high survival rate, current treatment for paediatric lymphoma is associated with severe long-term side-effects, including infertility, neurological defects, heart failure and secondary malignancies. Moreover, relapsed lymphoma is associated with drug resistance and poor survival. These issues post an urgent need for precision medicine that specifically target lymphomagenesis and spare normal cells to the largest extend. To reveal mechanisms underlying lymphomagenesis, we combined single cell RNA sequencing (scRNAseq) and immune receptor sequencing (VDJ-seq) on paediatric samples covering non-cancerous reactive lymph nodes (n=7) and the 3 major types of paediatric lymphoma; Burkitt (BL, n=3), Hodgkin (HL, n=4), and T lymphoblastic lymphoma (TLL, n=3). VDJ clonal expansion was used for cancer cell annotation for BL and TLL. To investigate the tumour microenvironment in HL, we integrated patient-paired scRNAseq, spatial transcriptomics (Visium), together with immunohistochemistry (IHC) using fresh frozen tissues. We identified a cycling cluster that mainly consisted of dark-zone germinal centre B cells (DZGCBs) in reactive lymph nodes but harbours cancer cells from the BL and TLL samples, suggesting that the highly proliferative programme normally used by DZGCB cells is important for lymphomagenesis. Based on VDJ-seq, we identified single- hyperexpanded lymphoma clones in each BL and TLL. To our surprise, these lymphoma clones had a diverse transcriptomics profile, with BL sharing programme with germinal centre B cells, memory B cells and plasma cells. TLL underwent a unique transcriptomic shift bridging the T helper cell cluster and germinal center-like cycling cluster, suggesting lymphomagenesis is continuous and organised. Moreover, trajectory analysis showed increased expression of cell cycle genes and cytoskeletal remodelling towards the cycling cluster, indicating the mechanism of this transition. Using a predictive treatment score, we observed inter- and intra-tumoral heterogeneity that explained the efficacy and short-coming of current treatment against BL and TLL. Finally, cell type decomposition with spatial resolved transcriptomics revealed exhausted T cells proximal to Reed-Sternberg cells in HL. More investigation is underway. Taken together, our data reveals that paediatric lymphomagenesis is characterized by its diversified transcriptome which is valuable information to guide novel therapeutics development.

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753 – WS21.4

Light induced expression of gRNA allows for optogenetic gene editing of T lymphocytes in vivo

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Purpose: Spatial transcriptomics is revolutionizing our knowledge of cellular states in complex tissues. However, to understand the function of individual genes, we need more precise methods for spatiotemporal gene editing. Recently, optogenetic CRISPR has emerged as a promising method for this, but due to its current shortcomings it has not been broadly implemented. To solve this, we have developed a conceptually new optogenetic platform (BLU-VIPR) allowing for efficient optogenetic CRISPR in vivo based on blue-light induced expression of gRNA.
Methods: The BLU-VIPR optogenetic platform is based on combining a new potent light-responsive transcription factor (VPR-EL222) with ribozyme-flanked gRNAs. The transcription factor VPR-EL222 ensures robust transcription induced by blue light, while the ribozyme-flanked gRNA design ensures precise excision of multiple gRNAs from the resulting mRNA transcript. We demonstrate that this design allows for multiplexed and orthogonal optogenetic gene editing, and simultaneous light-induced expression of gRNAs and proteins (e.g. fluorescent reporters), making the system very versatile. Since the light-induced gRNAs can be combined with different Cas proteins off-the shelf, the BLU-VIPR platform allows for optogenetic control of different CRISPR functionalities.
Results: Using BLU-VIPR in cells *in vitro* we achieved optogenetic Cas9 mediated knockouts, optogenetic CRISPR activation and optogenetic base editing. Since BLU-VIPR is compact and genetically encoded, we could deliver it to primary T cells using viral vectors. Indeed, after transduction of $Cas9^{+}$ T cells with BLU-VIPR retrovirus, followed by adoptive transfer into $TCRb^{-/-}$ mice, we were able to achieve site-specific optogenetic knockouts in T cells *in vivo*.
Conclusion: We have for the first time achieved optogenetic gene editing of T cells in vivo, thus paving the way for spatiotemporal dissection of immune responses in vivo with high precision.

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504 – WS21.5

A combined multi-omics approach reveals the impact of epigenetic therapies on the immunopeptidome of acute myeloid leukemia

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Hypomethylating agents (HMA) and histone deacetylase (HDAC) inhibitors, also referred to as epigenetic modifying therapies, have shown promising results in the treatment of acute myeloid leukemia (AML). Recent data point to an immunological mode of action suggesting that HMA-induced gene expression of endogenous retroviral element (ERV)-encoded promoters and various cancer/testis antigens (CTA) might result in the presentation of novel peptides on human leukocyte antigen (HLA) molecules. Thus, we investigated the impact of the DNA methyltransferase inhibitor decitabine (DAC) and the HDAC inhibitor vorinostat (VOR) on the immunopeptidome of AML cell lines and primary AML cells to evaluate novel treatment-induced antigens as targets for combinatorial T-cell based immunotherapies.
Implementing label-free quantitation mass spectrometry, we assessed HLA class I and II peptide presentation of (i) AML blasts of patients treated *in vivo* with DAC (n=3), or (ii) primary AML samples (n=7), and (iii) AML cell lines (n=3), after *in vitro* treatment with DAC, VOR, or a combination of both. In total, 612,763 HLA class I binders and 398,435 class II presented peptides were identified with 69,467 and 68,519 unique identifications, respectively. A comparative analysis of treated samples with respective untreated controls revealed 13,951 treatment-exclusive HLA class I binders and 17,330 treatment exclusive HLA class II peptides. Overlap analysis of these HLA class I binders with published benign samples (n=332) and untreated AML samples (n=47) revealed that 57% (7,950) of treatment-exclusive peptides were not priorly identified on benign or untreated samples, suggesting that these HLA ligands were induced upon treatment. Moreover, HLA ligands from several CTA (ATAD2, KI20B, PRAME) were found to be exclusively presented after epigenetic modifying therapy. Integrating mass spectrometry-based immunopeptidomics with genome-wide CpG methylation screening, and deep short- and long-read RNA sequencing technologies followed by reference-guided transcriptome assembly, further revealed treatment-induced HLA-ligands from canonical proteins as well as novel open reading frames (ORF).
Our results demonstrate that epigenetic therapies modify the immunopeptidome of primary AML cells by induction of treatment-exclusive novel HLA ligands, that will be further evaluated for their eligibility as targets for immunotherapeutic approaches.

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2261 – WS21.6

Lurbinectedin-induced transcriptional reprogramming: a pathway to sensitise SCLC to immunotherapy

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Background: For decades, platinum-based chemotherapy plus etoposide (ChT+E) has remained unchallenged at the first-line treatment for extensive-stage-SCLC (ES-SCLC), that is, until the introduction of immune-checkpoint blockade (ICB) anti-PD-L1. ChT+E susceptibility of SCLC relies on the high genomic instability observed in SCLC, further increased by ChT+E and surpassing DNA Damage Repair (DDR) capabilities. In the context of DNA damage and cell-intrinsic immunity, the addition of ICB improves the response of SCLC to ChT+E through tumour immunogenicity. Although initially responding to treatment, almost all patients relapse within the first 6 months, leading to limited options at second-line treatment, Lurbinectedin (LUR) among them. While LUR acts by degrading RNA polymerase II and, therefore, inhibiting transcription, it also causes DNA damage synergising with ICB. 2SMALL is a phase 1/2 study assessing the safety, tolerability and efficacy of LUR plus atezolizumab (ATZ) as second-line treatment for ES-SCLC.

Methods: 2SMALL Phase-I was a dose exploration trial combining increasing doses of LUR plus a fixed dose of ATZ every 3 weeks. The study endpoints included the safety profile and recommended dose for LUR, as well as, Objective Response Rate (ORR), Progression-Free Survival (PFS) and Overall Survival (OS). Pre-clinically, efficacy of LUR plus anti-PD-L1 mAb was evaluated in vivo in a syngeneic immunocompetent mice model for SCLC (Rb1-/-Trp53-/-), and several SCLC cell lines and PDX-derived-spheroids.

Results: LUR+ATZ was well tolerated, without unexpected toxicities. ORR were observed in 16/24 patients, including: 3/24 Complete Responses; 13/24 Partial Responses; 5/24 Stable Disease; and 3/24 Progressive Disease. Median PFS was 4.7 months (3.37-7.4) and median OS 14.5 months (9.5-23.4). In pre-clinical in vivo experiments, LUR+anti-PD-L1 achieved tumour rejection in 16/28 mice, from which 13/16 mice were protected against tumour-rechallenge. We observed that LUR increases expression of the Antigen Processing and Presentation (APP) machinery, activates IFN type-I signalling and promotes immune infiltration when combined with anti-PD-L1. Furthermore, anti-tumour effect of LUR+anti-PD-L1 was mainly mediated by CD8+ T lymphocytes.

Conclusions: LUR+ATZ anti-tumour activity is remarkable in both patients and pre-clinical in vivo experiments. Our results show effective tumour-activating IFN type-I, which increases immunity against tumours and provides the rationale to combine LUR with ICBs.

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WS22 – CANCER VACCINES AND IMMUNOTHERAPIES

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462 – WS22.1

Bacterial nanoparticles as a novel platform for personalized cancer vaccines

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Contemporary vaccine formulations face challenges related to production time, costs, stability and storage, requiring stringent conditions. Bacterial derived nanoparticles called protein bodies (PBs) exhibit these properties and offer other, improvements such as high antigenic multiple epitope content, low toxicity and relative resistance to proteases. Therefore PBs are optimally suited as an antigenic vaccine formulation for T cell responses to linear epitopes, enhancing immune responses in cancer immunotherapy. We have demonstrated that *in vitro* PBs enhance DC maturation and drive strong CD8+ and CD4+ T cell responses *((Schetters et al. (2020))*. To eliminate bacterial derived endotoxin that would trigger TLR4, we generated the PBs in a *ClearColi BL21*, a LPS free bacterial strain of *E.Coli* to investigates it's *in vivo* efficacy. We generated PBs expressing the ovalbumin (OVA)-derived OT-I and OT-III epitopes and injected in a prophylactic and therapeutic vaccination setting using the B16OVA mouse melanoma model. Interestingly, the PB vaccinated mice showed no tumor outgrowth, compared to full tumor outgrowth observed in unvaccinated counterparts. Even in mice injected with PB vaccines after 7 days when tumor growth was present complete tumor eradication was observed, further emphasizing their efficacy. Although depletion of CD8 T cells restores tumor growth implicating an important role for the induction CD8+ T cells by PB vaccine, the percentage of antigen-specific CD4+ and CD8+ T cells remained relatively low compared to the soluble equivalent that did not eradicate the tumor as efficiently as PBs. These findings hint to additional immune players that orchestrate the efficacy of the anti-tumor response induced by PBs. Taken together, PBs show promising results for the use of effective therapeutic cancer vaccine formulation that triggers a full repertoire of immune players.

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636 – WS22.2

In-situ vaccination with Flt3L elicits immune responses against hepatocellular carcinoma and improves checkpoint blockade therapy

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Purpose: Hepatocellular carcinoma (HCC) is a highly aggressive tumor developing in chronic inflammatory liver diseases. Current systemic therapies, including checkpoint blockade, insufficiently inhibit tumor progression. In a pre-clinical mouse model, we developed an *in-situ* vaccine (ISV) to recruit and activate classical dendritic cells type 1 (cDC1) and enhance cross-presentation of tumor-associated antigens to cytotoxic T lymphocytes.
Methods: Male C57BL/6J mice received a single injection of N-nitrosodiethylamine (DEN), followed by either repeated intraperitoneal carbon tetrachloride (CCl_4) injections or Western Diet feeding, inducing orthotopic HCC in liver fibrosis. The *ISV* combined systemic injections of the DC growth factor Fms-like tyrosine kinase 3 ligand (Flt3L), a Fas agonist, and two adjuvants (agonistic anti-CD40 or polyIC). ISV treatment was also combined with checkpoint blockade. Immune responses were evaluated using spectral flow cytometry, multiplex immunofluorescence and bulk RNA. Tumor growth was monitored with longitudinal magnetic resonance imaging. Signatures of cDC1 were explored in the human TCGA-HCC patient dataset.
Results: Injections of Flt3L significantly expanded intratumoral and systemic cDC1 numbers. Injection of both adjuvants induced upregulated markers of antigen presentation and co-stimulation on Flt3L-recruited cDC1s, indicating maturation of cDC1. Mice bearing fibrosis-related HCC and treated with ISV displayed significantly delayed tumor growth and prolonged survival compared to non-vaccinated animals. While depletion of CD4+ T cells and NK cells did not impair vaccine efficacy, CD8+ T cell depletion abrogated the tumor-controlling effect of the ISV, resulting in similar survival and largest tumor diameters as non-vaccinated mice. Due to high checkpoint expression in the immune microenvironment (mainly PD-1 and PD-L1), ISV was combined with anti-PD1 and anti-PD-L1 treatment. While HCC tumors did not respond to checkpoint monotherapy, the combination of ISV with anti-PD1 significantly prolonged median survival. This effect was synergistic as survival was increased compared to ISV alone. In the human TCGA-HCC dataset, a higher expression of cDC1 signatures were associated with adaptive immune response signatures and higher progression-free 24-month survival, independent of tumor stage, grading, and resection status.
Conclusion: Flt3L-based in situ vaccination elicits anti-tumor immunity and induces response to checkpoint blockade in previously unresponsive HCC tumors.

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534 – WS22.3

Polymeric nanoparticles targeting conventional type 1 dendritic cells enhance antigen cross-presentation and CD8+ T cells by activation of the non-canonical inflammasome

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Vaccination is considered one of the major milestones in modern medicine, facilitating the control of life-threatening diseases, even reaching eradication in case of smallpox. Subunit vaccine antigens require the addition of adjuvants to enhance immunogenicity and influence the nature of the adaptive immune response induced. In this context, there is a need for adjuvants that more effectively promote cell-mediated immune responses, particularly $CD8^+$ T cells. Additionally, resolving the mechanisms underlying adjuvant and vaccine mode of action is critical to provide a basis for rational adjuvanted vaccine design and allow tailoring of adjuvanted vaccines for specific diseases and target groups.
The size of particulate adjuvants has emerged as a key parameter influencing adjuvanticity. Previous work from the Lavelle lab with polymeric particles demonstrated that particle size was instructive in the induction of $CD8^+$ T cell responses by polystyrene and poly D,L-lactic-co-glycolic acid particles. We explored the mechanism of action of these adjuvants and identified a critical role of components of the non-canonical inflammasome- ROS, caspase 11 and gasdermin D (GSDMD), in the mediation of particle-induced cell-mediated immunity. Furthermore, we demonstrated that antigen-specific $CD8^+$ T cells and IFN-y responses were dependent on type 1 conventional dendritic cells (cDC1) *in vivo* by using Batf3 deficient mice and results were confirmed using a cDC1-$CD8^+$ *in vitro* co-culture model. In addition, 50nm polymeric nanoparticles with ovalbumin (OVA) were used as a therapeutic vaccine in mice bearing either MC38 colon adenocarcinoma (hot tumour) or B16F10 melanoma (cold tumour) expressing OVA. Vaccination led to a significant reduction in tumour growth and increased probability of survival. Furthermore, this polymer-based particulate vaccine adjuvant outperformed the effect of anti-PD1 monotherapy in both tumour models, and demonstrated a synergistic effect when both therapies were used together in B16F10 tumours. Therefore, this work enhances our understanding of the mechanism of action of 50nm polymeric particulate adjuvants having a direct application in prophylactic vaccines against intracellular pathogens, as well as therapeutic cancer vaccines.

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1106 – WS22.4

MV130 as a new innate memory-based mucosae vaccine against cancer

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Trained immunity (TI), known as innate immune memory, is described as the ability of innate immune cells of responding to a homologous or heterologous secondary challenge with enhanced proinflammatory response upon the exposure to certain TI-inducing stimuli. The most studied TI-inducers are the BCG vaccine and the fungal β-Glucan. However, BCG and β-Glucan have limitation for their clinical application. MV130 is a mucosal immunotherapy based on heat-inactivated bacteria indicated for recurrent respiratory infections. MV130 was demonstrated to induce TI in pre-clinical models against respiratory viruses included Sars-Cov2 or *C. albicans*. However, the role of MV130 in conferring cancer protection is unexplored. We observed that C57BL/6J mice subjected to the prophylactic regimen of six doses of intranasally administered MV130 exhibited delayed tumor development and improved tumor control against subcutaneously injected LLCs (Lewis Lung Carcinoma) cells, administered seven days after the last immunization. The protection is maintained with a higher tumor burden or when tumor cells are injected 60 days after the last MV130 administration, indicating that MV130 induces a long-lasting memory, which is a main feature of TI. Furthermore, MV130-treated mice, present protection against orthotopically lung tumors and melanoma spontaneous lung metastasis as well as a pro-inflammatory lung microenvironment. Upon *ex vivo* heterologous restimulation of lung innate populations, alveolar macrophages and neutrophils presented an enhanced proinflammatory phenotype (TNF production), revealing a potentiated response due to TI. Moreover, chromatin remodelling by ATAC-seq shows the training effect on an epigenetic level. In conclusion, our data indicate that MV130 induces TI in innate immune cells by potentiating a proinflammatory antitumor response, paving the way for the induction of innate memory through mucosae as a novel cancer immnunotherapy strategy.

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494 – WS22.5

Can immunotherapies engaging the CD3/γδ TCR complex help reactivate exhausted γδ T cells in a tumoral microenvironment?

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Recent successes of cancer immunotherapies have generated a renewed interest in understanding the contribution of gamma delta T cells (γδ T cells) in the immune response against cancer. γδ T cells have been initially described to play a broad role in responses against infections and cancer, making them good candidates for specific redirection against tumor cells. Human γδ T cells and conventional alpha beta T cells (αβ T cells) share some common features such as the expression of a TCR linked to the CD3 signaling complex, composed of three dimers (CD3δε, CD3γε, ζζ). However, they differ regarding the molecular mechanisms involved in CD3 engagement and activation, paving the way for potential strategies to activate specific subpopulations of T cells. Targeting CD3-based activation processes could represent a solution to activate γδ T cells more specifically and avoid high toxicity and lack of specificity that are triggered in many immunotherapeutic strategies (eg. bispecific antibodies). However, as other types of immune cells, γδ T cells undergo a state of exhaustion while in the tumoral microenvironment. This exhaustion can be caused by several factors, such as TCR overstimulation, immunosuppressive cytokines (TGF-β) or hypoxia for example. Here, we show that human γδ T cells exhibit a higher sensitivity for CD3ε-mediated activation, as compared to αβ T cells. We compare three different types of exhaustion protocols, either by TCR over-stimulation, TGF-β cytokine treatment or co-culture with Zoledronate-activated SKOV3 tumor cells. In each case, we assess the exhausted phenotype and the reactivation potential of γδ T cells in presence of anti-CD3 mAbs and we show that CD3 stimulation can be a trigger to overcome exhaustion and regain anti-tumor effector functions. Mastering the CD3-based activation processes of γδ T cells could help us design new immunotherapies. This project has been funded by Sanofi Aventis R&D.

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1276 – WS22.6

Enhancing oncolytic virus-infected cellular vaccines with tumor-rejecting lymphocyte transfer for the immunotherapy of triple-negative breast cancer

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Triple-negative breast cancer (TNBC) is a highly heterogeneous disease characterized, in part, by its relative immunogenicity and propensity for recurrences. This can be targeted therapeutically through personalized immunotherapies capable of eliciting a durable antitumor immune response. To this end, we are developing an oncolytic virus (OV)-infected cell vaccine (ICV) comprised of irradiated cancer cells infected by the oncolytic vesicular stomatitis virus (VSVd51). We have previously shown that the ICV can stimulate antitumoral immune responses and improve survival in a preclinical murine model of TNBC. We have also highlighted the critical importance of CD8+T cells for the survival of vaccinated mice. However, despite the ICV inducing a systemic antitumor immune response, complete responses (CR) are still limited to 50%. Therefore, we hypothesized that enhancing antitumor T cell immunity through optimal amplification *ex vivo*, away from the immunosuppressive tumor microenvironment, followed by adoptive transfer can augment rates of CR.
Thus, we explored the immunostimulatory capacity of the ICV *ex vivo* and sought to convert peripheral blood immune cells into tumor-rejecting lymphocytes (TRL) to provide a more convenient and powerful alternative to tumor-infiltrating lymphocytes. To accomplish this objective, we established a protocol for TRL activation and expansion relying on the co-culture of peripheral blood T cells with the ICV.
Preliminary data reveal the generation of a mixed population of T cells, including central memory T cells, which are associated with improved *in vivo* persistence. Additionally, CD8+T cells and the expression of granzyme B were increased suggesting enhanced cytotoxicity, as validated by tumor-killing assays. Tumor-reactivity assays demonstrated that TRL cultures can be activated to express CD137, IFNg and PD1 upon stimulation with TNBC cells. These findings suggest that TRL cultures are enriched for tumor-reactive T cells. Future studies will evaluate their efficacy in xenograft mouse models.
TRL and therapeutic cancer vaccines, together, have the potential to induce a strong and curative antitumor immune response that deter disease recurrence. Our research aims to translate such personalized immunotherapies into future clinical applications for patients with TNBC.

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WS23 – MACROPHAGE FUNCTION AND REGULATION

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1314 – WS23.1

MafB regulates monocyte-to-macrophage differentiation and imprints tissue resident macrophage identity and function

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Purpose: The transcription factor MafB is specifically expressed by monocytes/macrophages. It has been shown that MafB restricts Csf1-dependent proliferation in myeloid progenitor cells, as well as the self-renewal ability of differentiated macrophages. Recently we discovered that monocytes were able to first proliferate in a vacant tissue niche in a Mafb-restricted way before undergoing differentiation into one or the other subset of lung interstitial macrophages. To further understand resident tissue macrophage (RTM) development and homeostasis, it is fundamental to investigate the role of MafB in RTM differentiation, identity and function.
Methods: By applying intracellular flow cytometry, we examined the expression of MafB in blood monocytes and RTM across different tissues. We created *Lyz2*^{Cre}*Mafb*$^{fl/fl}$ mice with myeloid-restricted *Mafb* deficiency and used a combination of bone marrow competitive chimeras, high-dimensional flow cytometry, bulk RNA-sequencing (RNA-seq) and single-cell RNA-seq to study the effect of Mafb knock-out on macrophage differentiation and function, *in vitro* and *in vivo*. To identify transcriptional programs directly regulated by MafB, we performed CUT&RUN on bone marrow monocyte-derived macrophages (BMDM) and blood monocyte-derived macrophages (MDM) from mouse and human, respectively.
Results: MafB was found to be highly expressed in RTM across different tissues as compared to blood monocytes and MafB expression was found to be upregulated along the monocyte-to-macrophage differentiation axis. The *in vitro* and *in vivo* macrophage differentiation was heavily affected in *Lyz2*^{Cre}*Mafb*$^{fl/fl}$ mice as compared to littermate controls. In *Mafb* knock-out macrophages, the expression of monocyte signature genes increased, while the expression of macrophage signature genes decreased. In addition, the expression of tissue specific identity genes was reduced in *Mafb* deficient RTM. We identified MafB binding sites in promoters and close enhancers of many genes in a gene set that has been shown to be upregulated during macrophage differentiation. Moreover, MafB was also found to directly regulate core macrophage identity genes.
Conclusion: MafB plays a key role in macrophage differentiation by regulating conserved core transcriptional programs that imprint macrophage identity and function.

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1338 – WS23.2

Pentose Phosphate Pathway derived NO orchestrates macrophage immunometabolic responses through TCA cycle crosstalk; a target for Mycobacterium tuberculosis immune evasion

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Background: Glycolytic metabolism has emerged as a key feature in macrophage antimicrobial responses against Mtb infection. Recent work within our group demonstrates a Mtb mediated deceleration of macrophage bioenergetic metabolism, a process dependent on upregulation of anti-inflammatory microRNA-21 and subsequent targeting of glycolysis. Here, we further characterise this immuno-evasive phenomenon as means to regulate PPP-derived NO and subsequently target TCA cycle metabolism. NO is a soluble endogenous gas produced by iNOS and proposed to have direct bactericidal activity. However, this model is poorly understood and moreover dismissed in the context of Mtb infection of macrophages, with traditional dogma suggesting an inability of human macrophages to generate substantial NO in response to infection when compared to murine models. Recent evidence challenging this perspective in addition to the mycobactericidal capacity of NO necessitates a more comprehensive view of processes influenced by NO, including the orchestration of metabolic reprogramming.
Results/Methods: Isotope tracing models demonstrate that upon infection of both human and murine macrophages, Mtb specifically represses host-cell glycolysis and reroutes central carbon metabolism towards induction of a cyclic-PPP. This in turn promotes iNOS derived NO production through PPP mediated accumulation of enzyme co-factor, NADPH. Further models working to supplement and sequester cellular NO reveals this Mtb mediated manipulation as means to target TCA cycle metabolism in addition to further promoting PPP activity through positive feedback. Here, we find through inhibition of TCA cycle ACO2 and generation of isocitrate, Mtb derived NO results in compensatory upregulation of IRG1 and generation of itaconate, a metabolite facilitating reduced secretion of anti-mycobacterial cytokine IL-1β, thus enhancing Mtb survival. Exogenous supplementation of itaconate, in addition to use of *irg1*$^{-/-}$ models further highlight the ability of this metabolite to regulate cellular NO levels and consequently influence bacterial survival by means of regulating IL-1β.
Conclusion: Mtb demonstrates a unique capacity to evade innate responses, particularly that of its primary target cell of infection – the macrophage. Here, we describe a novel mechanism of metabolic crosstalk between PPP and TCA cycle activity, coordinated through cellular NO. Furthermore, we describe how Mtb employs this pathway as a means to elude host immunometabolic responses.

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1047 – WS23.3

CXCR3 mediates CD49a+ NK cell differentiation in colorectal cancer liver metastasis by promoting tissue residency in supportive macrophage niches

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Purpose: NK cell phenotype and activity in tumors is highly context-specific and both dysfunctional CD49a+ ILC1-like and preserved anti-tumor phenotypes have been described so far. This study aims at dissecting the mechanisms governing type 1 innate lymphoid cells (ILCs) function in metastasis engraftment and progression by investigating their phenotypic plasticity and behaviour in a metastatic model of colorectal cancer (CRC).
Methods and Results: By taking advantage of a MC38-induced CRC mouse model of liver metastasis, we show that NK cell/ILC1 are involved in controlling hepatic metastasis formation. We report poor infiltration of ILC1 and expansion of a CD49a^{+} NK cell population among metastasis-infiltrating (MI) lymphocytes. CD49a+ NK cells displayed preserved killing capacity against CRC cells, while other MI NK cell subsets were functionally impaired. By bulk RNA-seq of sorted type 1 ILCs and by multiparametric FACS analysis, we show that CD49a+ NK cells have increased expression of several molecules associated to immunoregulation and tissue retention, including PD-L1, CD73 and CD69, CXCR3.
In addition, we found that a population of CXCL9-producing F4/80^{hi} macrophages expands in metastatic nodules and promotes CD49a+ NK cell expansion through TGF-β. Anti-CSF1R antibody-mediated depletion of metastasis-associated macrophages (MAM) resulted in a marked reduction of CD49a+ NK cell metastasis infiltration, supporting an *in vivo* role for MAM in shaping NK cell phenotype. Along with this, an alternative SL4-induced liver metastasis model showed failed accumulation of CXCL9+ macrophages mirrored by a reduced generation of CD49a+ NK cells.
Finally, conditional deletion of Cxcr3 in NKp46+ cells markedly affected CD49a+ NK cell differentiation in MC38-induced metastasis, indicating that CXCR3 is required for correct NK cell localization in macrophage-supporting niches. Correspondingly, Ncr1ΔCxcr3 mice showed accelerated metastasis formation, indicating that CXCR3 expression by NKp46+ cells has a protective role.
Conclusion: Our data indicates that MI NK cells acquire ILC1-like features under the effect of metastasis environmental factors. Deficiency in accumulation of CD49a+ NK cells in Ncr1ΔCxcr3 mice suggests that CXCR3 plays a major role in promoting NK cell metastasis infiltration and/or in their crosstalk with MAM, thus influencing NK cell conversion and impacting liver metastasis development.

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2216 – WS23.4

Myeloid type i IFN sensing facilitates stress erythropoiesis

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Infections and inflammation can impair steady-state erythropoiesis and induce alternative extramedullary stress erythropoiesis in organs like liver or spleen. To date, there is limited understanding of how the erythroblastic islands' microenvironment facilitates stress erythropoiesis.
Here, we studied erythropoiesis in the murine spleen upon exposure of mice to the synthetic TLR9 agonist CpG ODN. We sorted myeloid and progenitor cells for single cell transcriptome sequencing (scRNA-seq) and modelled erythroid development by combining RNA-velocity and trajectory inference methods. To functionally determine the role of myeloid type I interferon (IFN) signaling, we challenged $CD169^{cre/+}$ and $LysM^{cre/+}$ $IFNAR^{fl/fl}$ (interferon alpha/beta receptor) mice and analyzed the impact on stress erythropoiesis at different time points.
Exposure to CpG strongly enhanced the transition of pro-erythroblasts towards the erythroblast stage at single cell level. Interestingly, dysregulation of Gata1/2 and altered IFN signaling in pre-erythroblasts explained enhanced erythropoiesis under stress condition. Further, we confirmed that effective stress erythropoiesis required type I IFN mediated activation of pre-red pulp macrophages (pre-RPM) by challenging $CD169^{cre/+}$ $IFNAR^{fl/fl}$ mice with CpG. Strikingly, IFNAR activated pre-RPM expressed the chemokine ligand 2 (CCL2), an agonist of the atypical chemokine receptor 1 (ACKR1) expressed on erythroblasts, which had been shown to be an essential regulator of hematopoiesis. Consequently, we challenged $LysM^{cre/+}$ $IFNAR^{fl/fl}$ mice and confirmed that loss of IFNAR signaling in monocytes strongly reduced CCL2 expression and prevented induction of stress erythropoiesis at day 1 and day 3.
Our results indicate that stress erythropoiesis depends on myeloid IFNAR signaling and uses alternative transcriptional programs compared to steady state erythropoiesis. Early monocyte recruitment enhances erythroblastic island formation and pro-erythroblast expansion while later scavenging of CCL2 by erythroblast ACKR1 might serve as a negative feedback loop to terminate stress erythropoiesis. To further dissect the temporal dynamics of the erythroblast microenvironment, we are currently analyzing a bulk-Seq time course experiment of challenged $IFNAR^{fl/fl}$ and $LysM^{cre/+}$ $IFNAR^{fl/fl}$ mouse with sorted cell type populations (monocyte, pre-RPM, PRM, erythroblasts). We anticipate that our results provide fundamental insights to improve our understanding of anemia of inflammation in the clinical setting.

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33 – WS23.5

Mutual immunomodulatory effect of a high-sodium diet and antihypertensive drugs on mouse macrophages

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Purpose: The development and maintenance of hypertension are associated with chronic systemic inflammation, and both processes drive each other. Additionally, excessive sodium intake triggers both elevated blood pressure and inflammatory processes. Since mechanisms of interaction of hypotensive drugs with the immune system are still not fully understood,
we focused on the investigation of impact of hypertension-associated high-sodium diet and six antihypertensive drugs on the modulation of peritoneal exudate macrophages (PEMs) function in mice.
Methods: Mice, fed since weaning with either standard (STD) or high-sodium diet (HSD), were intraperitoneally administered, for 8 consecutive days, with one of the drugs: captopril (5mg/kg), olmesartan (1mg/kg), propranolol (10mg/kg), carvedilol (5mg/kg), amlodipine (3mg/kg), or verapamil (5mg/kg). Mineral oil-induced PEMs were assessed for secretion
of ROIs (chemiluminescence), NO (colorimetric method) and cytokines (ELISA), surface marker's expression by flow cytometry as well as for phagocytic activity followed by induction of the humoral immune response against corpuscular antigen (SRBC) in vivo. Moreover, diet- and drug-induced modulation of contact hypersensitivity (CHS) response (PEM-induced CHS, adoptively transferred CHS, and actively induced CHS) was determined. Results were statistically analyzed using two-way ANOVA.
Results: HSD elevated both systolic blood pressure and heart rate, however, the effects were modulated by drugs. The assayed medications increased the generation of ROIs and NO secreted by macrophages from STD-fed donors, but they reversed both HSD-induced oxidative burst and enhanced secretion of pro-inflammatory cytokines. Some drugs increased macrophage phagocytic activity and enhanced macrophage-mediated activation of B cells for antibody production. Moreover, the assayed medications augmented macrophage-mediated effector phase of CHS, but suppressed the sensitization phase of cell-mediated hypersensitivity under HSD conditions. In certain circumstances, drugs with similar mechanisms of action, amlodipine and verapamil especially, exerted opposite effects.
Conclusion: Our current findings clearly support reports about high sodium-associated interference with development of hypertension and pro-inflammatory processes, which may be reversed by hypotensive drug treatment. Modulation of Th1- and Th2-dependent immune responses may have important clinical significance due to the additional anti-inflammatory effects of hypotensive drugs in the hypertension-related dysregulation of immune system.

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1269 – WS23.6

CD109 as a target for lung cancer and tumor-associated macrophages

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Purpose: Due to oncogenic mutations and resistance development, there is a persistent need for novel targeted therapies to combat lung cancer. The glycoprotein CD109 has been shown to contribute to tumorigenesis and presents an appealing target for anti-cancer therapy. We hypothesize that CD109 supports the M2 macrophage phenotype, and thereby promotes a pro-tumorous micro-environment. Herein, we apply a primary human *ex vivo* setting to reveal the expression of CD109 on tumor-associated macrophages (TAM) and explore the use of RNA therapeutics *in vitro*.
Methods: Expression of CD109 was visualized *ex vivo* in primary human lung tissue (i.e. precision-cut (tumor) lung slices, PCLS/PCTLS) using confocal microscopy. Flow cytometry was applied to determine CD109 expression on macrophages isolated from donor-paired tumor and non-tumorous human lung tissue. Macrophages with pro (M1)- and anti (M2)-inflammatory phenotypes were differentiated from either $CD14^+$ selected monocytes (M1/M2 R&D differentiation kits) or induced pluripotent stem cells (iPSC, M1-IFNg, M2a-IL4), and CD109 expression was confirmed by flow cytometry or whole transcriptome analysis.
Results: Comparison of tumor tissue and donor-matched, non-tumorous tissue confirmed a higher expression of CD109 within the tumor tissue. Additionally, a higher expression of CD109 on TAMs compared to non-tumoral macrophages (55.6%±0.1 vs. 16.4%±0.9 $CD45^+CD109^+$ cells, n=2) was revealed. Moreover, monocyte-derived M2-like macrophages (median fluorescence intensity (MFI) fold change 2,8±1.1, n=4) and iPSC-derived M2a macrophages displayed a higher CD109 expression compared to their M1-like phenotypes. Furthermore, effective CD109 downregulation was achieved using target-specific small interfering RNA (siRNA) in A549 cells (MFI protein fold change 0.21±0.07, n=5 and 96% gene knockdown, n=1), in monocyte-derived M1 and M2 macrophages (MFI fold change 0.46±0.11 and 0.28±0.03 respectively, n=2) and in iPSC-derived macrophages with a M0, M1 and M2a phenotype (MFI fold change 0.39±0.02, 0.41±0.05 and 0.50±0.06 respectively, n=2).
Conclusion: Our results confirm overexpression of CD109 in lung tumor tissue and reveal an increased expression on *ex vivo* TAMs and *in vitro* M2-like macrophages. Moreover, a decrease in CD109 expression was achieved *in vitro* via siRNA-targeted knockdown. Henceforth, further experiments will investigate the immunomodulatory effects of CD109 downregulation on both cancer as well as macrophage functionality.

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WS24 – IMMUNOMETABOLISM IN CANCER

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473 – WS24.1

The tumor microenvironment drives mitochondrial metabolic reprogramming in neutrophils during non-small cell lung cancer

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Lung cancer is among the most common and deadly cancers, and neutrophils are a key immune cell found in high abundance within lung tumors. Neutrophils are generally pro-tumoral due to their immunosuppressive functions, but display heterogeneity and plasticity. A better understanding of their roles in the lung tumor microenvironment (TME) is thus needed. In particular, how cellular metabolism dictates neutrophil function, and especially the role of mitochondria, within the lung TME is not well-elucidated. Here, we investigated the metabolic state of neutrophils from human lung and tumor tissue of non-small cell lung cancer (NSCLC) patients. Results reveal altered mitochondrial metabolism, oxygen utilization, and ROS production in tumor-associated neutrophils compared with those of non-tumor lung tissue. This correlated with changes in neutrophil subpopulations within human lung tumors, including of degranulating, immature, aged, and immunosuppressive neutrophils. Mitochondrial function, ROS, and the cellular metabolome were also altered in human neutrophils treated with NSCLC tumor-conditioned media *in vitro*, which also induced immunosuppressive functions. Further, single-cell RNA sequencing analyses revealed that specific neutrophil subsets of the NSCLC TME have modified metabolic gene expression and heterogeneous metabolic flux, including of glucose and glutamine. Finally, inhibition of the mitochondrial complexes differentially altered ROS production and immunosuppressive functions of neutrophils, suggesting that mitochondria are essential switches that dictate pro- or anti-tumoral responses of neutrophils in the lung TME. Altogether, these metabolic alterations drive the development/polarization of heterogeneic subsets that support or restrict tumor progression. Our work thus highlights potential novel therapeutic outlooks for lung cancer in conjunction with current immunotherapies.

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2155 – WS24.2

Tackling tumor microenvironment metabolism: unveiling IL4i1's role and therapeutic potential

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Purpose: The metabolism of amino acids, especially tryptophan, is strongly linked with immune tolerance and unfavorable outcomes in the tumor microenvironment (TME). IL4i1, one of the enzymes involved in tryptophan degradation, has recently been identified as a crucial factor that makes the TME less resilient. IL4i1 sustains several tumor hallmarks, including immune suppression and resistance to ferroptosis. However, the mechanism and biology of IL4i1's action are not fully understood.
Methods: A poorly immunogenic fibrosarcoma cell line was analyzed through single-cell analysis 10 days after the tumor cell inoculation. Mass spectrometry was used to conduct the metabolite analysis derived from tryptophan degradation. To investigate the role of IL4i1 in the myeloid lineage, Il4i1 flox mice were bred with specific cre mice for enzyme deletion in macrophages and dendritic cells. Lastly, bioinformatic RNAseq data analysis was carried out on a cohort of 231 patients with different sarcoma subtypes.
Results: Single-cell RNA sequencing analysis showed that IL4i1 is only expressed in regulatory dendritic cells (mregDCs) in the TME of a transplantable fibrosarcoma cell line. These IL4i1+ mregDCs are mature dendritic cells that express markers such as CCR7 and Zbtb46. We also found that the TME is rich in metabolites derived from tryptophan conversion to I3P and 3-IAld by IL4i1. When we deleted IL4i1 in mice, we observed a reduction in tumor growth and an increased CD8 T cell-mediated response. Interestingly, when we used conditional models to delete IL4i1 in mregDCs, we saw a decrease in tumor cell growth and IL4i1-derived metabolites in the TME. We also found that IL4i1 metabolites suppress CD8 T cell response and conferred resistance to ferroptosis in tumor cells. Finally, we discovered that AhR-expressing target cells respond to IL4i1-produced metabolites, expanding our understanding of how IL4i1 shapes a metabolic network with profound effects on the TME and host anti-tumor response. Our findings show that IL4i1 is an adverse prognostic factor in specific sarcoma subtypes in the TCGA-SARC dataset, further highlighting its relevance in cancer contexts.
Conclusion: Our research identifies IL4i1 as a promising target for cancer therapy in the complex interplay of metabolism within the TME.

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1037 – WS24.3

Extracellular α-ketoglutarate potentiate myeloid cells' capacity to induce Th17 and Tregs

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Purpose: Alpha-ketoglutarate (αKG) emerged as a potent regulator of energetic and redox metabolism in immune cells. However, it is still unclear how exogenous αKG affects the metabolism and functions of key myeloid cells regulating the inflammation and T cell response, such as dendritic cells (DCs) and myeloid-derived suppressor cells (MDSCs).
Methods: The models of human monocyte-derived DCs and MDSCs induced by GM-CSF/IL-4 and GM-CSF/IL-6, respectively, were used to assess the effects of exogenous αKG *in vitro*. Cell survival, OXPHOS, Glycolysis, ROS, Akt/FoxO1 signaling, autophagy, cytokines production and phenotype of MDSCs and DCs were monitored by qPCR, WB and multi-color flow cytometry, whereas their functions were analyzed in different co-culture assays with allogeneic T cells.
Results: Non-toxic doses of exogenous αKG (<50mM) acted via Akt/FoxO1 and Nrf2/KEAP1 signaling pathways in DCs and MDSCs leading to an increased HIF-1α stabilization, ROS production and autophagy. Thereby, αKG-treated myeloid cells displayed down-regulated HLA-DR and PD1L expression, as well as an increased production of IL-1β, IL-23, IL-10 and TGF-β. αKG-DCs displayed an altered differentiation pathway compared to control DCs, according to their impaired up-regulation of CD1a on $CD14^{-}HLA\text{-}DR^{+}$ DCs. Functionally, αKG-DCs displayed a reduced allostimulatory and Th1 polarization capacity, but an increased capacity to induce Th17 cells, and conventional Tregs and IL-10-producing T cells in an IDO-1 and ILT-3-dependent manner. On the other hand, αKG potentiated differentiation of $HLA\text{-}DR^{low}CD14+$ MDSCs, as well as their capacity to induce both Th17 and Tregs *in vitro*.
Conclusion: Alph-ketoglutarate displays a potent capacity to alter the redox signaling in monocyte-derived cells, thereby potentiating their capacity to induce Th17 and Treg response, which could be a relevant mechanism for the establishment of persistent and chronic inflammation.

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943 – WS24.4

Overcoming the limitations of α4-1BB/PD1 dual treatment by exploring the metabolic programming of therapy-responsive T-cells

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Purpose: Costimulatory agonists in combination with PD1/PD-L1 blockade has been an extensively studied approach in treating cancer patients. Although several clinical trials have demonstrated the potential of activating costimulatory pathways as evidenced by increased response rates the eventual clinical effectivity seems limited. To overcome the possible limitations of costimulatory agonists we explored the metabolic programming of therapy-responsive T-cells upon combinatorial PD1/PD-L1 blockade with α4-1BB agonist therapy.
Methods: *In vivo* studies were performed with colon carcinoma MC-38 bearing mice treated with either an α4-1BB agonist and/or PD1/PD-L1 blockade. The metabolic state and expression of rate-limiting enzymes of the cellular metabolic pathways were determined in circulating, tumor- draining and -infiltrating $CD8^+$ and $CD4^+$ T-cells. *Ex vivo* studies were performed with human T-cells isolated from blood and colon carcinoma to identify possible metabolic targets in α4-1BB/PD1 dual treatment. Crispr/Cas-based gene editing was used to knock-out key metabolic enzymes in T-cells.
Results: Combinatorial treatment with α4-1BB and PD1/PD-L1 blockade resulted in the induction of therapy-responsive circulating $CD8^+$ T-cells of which each unique subset expressed their own metabolic enzyme profile. Specifically, glycolysis-related enzymes and transporters were increased in $CD8^+$ T-cells by 4-1BB stimulation while PD-L1 blockade enhanced the magnitude of the circulating subsets. Moreover, T-cells isolated from the tumor and tumor-draining lymph nodes acquired similar metabolic phenotypes. Human $CD8^+$ and $CD4^+$ T-cells treated with α4-1BB and αPD1 recapitulated the profiles observed *in vivo*. However, particularly $CD8^+$ T-cells isolated from colon carcinoma were unresponsive to 4-1BB stimulation, implying dominant suppressive cues.
Conclusion: Costimulatory agonists are widely used in the clinic in combination with checkpoint inhibitor therapy, such as PD1/PD-L1 blockade. However, the clinical effects of these combinatorial immunotherapies seem limited. Here, we demonstrate that although T-cells in the circulation are responsive to α4-1BB/PD1 dual therapy, tumor-infiltrating, especially $CD8^+$, T-cells became less responsive and altered their metabolic profile. These findings may contribute to improved understanding of current immunotherapies in the clinic.

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830 – WS24.5

Assessing Immune Cell Infiltration and Metabolic Activity During Immunotherapy in vivo through Immuno-Metabolic PET/MRI Imaging

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Purpose: Immune checkpoint inhibitors (ICIs) have revolutionized cancer treatment by aiding pre-existing T cells. However, their efficacy is limited in most solid tumors. In this study, we explored the synergistic potential of combining sorafenib, a multi-kinase inhibitor (MKI), with ICIs to elicit enhanced antitumor responses. We aimed to unravel the mechanisms behind this combined therapy in triple-negative breast cancers, which are resistant to ICIs or MKIs. Additionally, we developed a non-invasive multimodal imaging methodology to enable precise image-guided therapy.
Methods: Orthotropic tumor mouse models were generated by inoculating 4T1 breast cancer cells under the mammary fat pad. Tumor-bearing mice were treated with ICIs (anti-PD-1 + anti-CTLA-4), sorafenib, or both. Isotypes-treated mice were included as a control group. Multimodal imaging methods, including [^{89}Zr]Zr-DFO-αCD8 PET, [^{19}F]perfluorocarbon (PFC) MRI, [^{18}F]FDG PET, and hyperpolarized ^{13}C MRI, were applied to detect dynamic changes in CD8+ T cells and phagocytes infiltration, as well as tumor glucose uptake, glycolysis, respectively.
Results: Combining ICIs and sorafenib drastically prevented tumor progression, while monotherapy with ICIs or sorafenib did not yield tumor growth inhibition. [^{89}Zr]Zr-DFO-αCD8 PET showed an increase of CD8+ T cell infiltration in the sorafenib group (p=0.02) and combined therapy group (p=0.02) 3 days post-treatment. From [^{19}F]PFC MRI, the combined therapy group presented the highest ^{19}F signal at the late reaction phase (p=0.009), suggesting a high phagocyte accumulation in the tumor. These results were further supported by flow cytometry. [^{18}F]FDG PET and hyperpolarized ^{13}C MRI showed a uniform FDG uptake and an increasing lac/pyr ratio trend during the combined therapy. These findings strongly indicate that the combined therapy induces a proinflammatory tumor microenvironment (increased glycolysis; immune-cell activation) compared to monotherapies (aerobic oxidation; immune-cell exhaustion), underscoring its potential to enhance immune response and improve treatment outcomes.
Conclusion: Our findings demonstrate for the first time that combining ICIs and sorafenib effectively prevents the progression of triple-negative breast cancer in 4T1 mouse model, which can be attributed to the infiltration and activation of immune cells in the tumor microenvironment. This study highlights the unique role of multimodal immune-metabolic imaging in accurately discerning the different stages of immune-systemic therapy response *in vivo*.

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1442 – WS24.6

Mitochondrial respiratory complex III sustains IL-10 production in macrophages and promotes tumor-mediated immune evasion

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The anti-inflammatory interleukin (IL)-10 is a cytokine produced by activated immune cells and it is necessary for the dampening of the immune response and the resolution of acute and chronic inflammation. Because of its ability to switch off the activation of leukocytes, IL-10 upregulation is a common feature of tumor progression and metastasis. Most recently, IL-10 regulation has been shown to depend on mitochondria and redox-sensitive signals. We have found that Suppressor of site III_{Q_o} Electron Leak (S3QEL) 1.2, the specific inhibitor for reactive oxygen species (ROS) production from mitochondrial complex III, and Myxothiazol, a Complex III inhibitor, decrease IL-10 production from macrophages activated after priming with different ligands and by affecting basal mitochondrial respiration. IL-10 downregulation is likely to be mediated by the suppression of C-Fos, which is a subunit of Activator protein (AP)-1 transcription factor. S3QEL 1.2 impairs IL-10 production *in vivo* after Lipopolysaccharide (LPS) challenge and ameliorates the survival of mice bearing B16F10 melanoma, thus lowering tumor growth. Our data identify a new link between Complex III-dependent ROS generation and IL-10 production in immune cells and suggest S3QEL 1.2 as a compound with clinical relevance for the treatment of tumors and the advancement of mitochondria targeted anti-cancer therapies.

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WS25 – AI AND BIOINFORMATICS

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1536 – WS25.1

TCRgrapher: a software for the identification of antigen-specific TCRS

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Understanding TCR specificity could open up novel avenues for target treatment of autoimmune diseases and diagnostics. Our study aims at benchmarking established tools for the antigen-specific TCR identification from a single repertoire snapshots, highlighting their limitations, and providing recommendations for analysis and experimental design.
We designed the "TCRgrapher" R library for the identification of specific TCRs. TCRgrapher includes a unified framework that can run ALICE, TCRNET, tcrdist3, and GLIPH2 with a flexible set of controls and parameters. We applied TCRgrapher to three sets of TCR repertoires: post-LCMV infection data published by Shlesinger et al., our data of mice vaccinated with Sputnik V, and our data of lung-infiltrating CD4+ T cells collected at the peak of tuberculosis infection. Each dataset had an experimentally obtained repertoire enriched with antigen-specific TCRs, used as a positive set.
Among the single-repertoire analysis methods scrutinized, ALICE and TCRNET performed best on mice TCRβ repertoires. Furthermore, we found minor differences between ALICE performance with different TCR generation models and with corrections to clonotype abundance. TCRNET performance by clonotype grouping was mostly identical. TCRNET showed higher precision in the area of low recall with real data used as the control sample compared to the OLGA-generated control. GLIPH2 gave satisfactory results only in cases of clusters formed by one nucleotide mismatch and only being run locally.
TCRgrapher provides a user-friendly way to find condition-specific clonotypes and compare the results from different tools. We hope that our work will be useful to a wide range of researchers in the field.

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720 – WS25.2

Modeling Variations in Antibody Response Magnitude and Longevity

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Long-lasting antibody responses are pivotal for both protective immunity and autoimmunity. Yet, the intricate mechanisms that dictate the duration of these responses remains only partially elucidated.
By employing an agent-based in silico model, we simulated the generation of short-lived and long-lived plasma cells during the immune response to an adenoviral COVID-19 vaccine, postulating that antigen-specific plasma cells have a certain probability of attaining an extended half-life. This hypothesis implies that the quantity of antigen-specific plasma cells generated in the initial immune response, coupled with their likelihood of becoming long-lasting, influence the magnitude of the antibody response months after immunization. Interestingly, our simulations unveiled two distinct clusters among individuals several months post-vaccination, delineating markedly divergent dynamics in antibody titers: one group exhibited sustained elevated antibody levels (sustainers), while another witnessed a decline (decayers). Notably, the absence of long-lived plasma cells in the decayers distinguished them from the sustainers. Leveraging machine learning clustering on antibody titers, we achieved an accuracy of 0.925 in identifying the decayers 28 weeks following the initial dose.
Presently, we are comparing the predictions of our model with clinical data on the antibody response to SARS-CoV-2 Nucleoprotein and Spike post-COVID-19 infection or vaccination, with the aim of validating and refining our model.
Specifically, we are harnessing machine learning methodologies on data sourced from published immunological studies to discern patterns in the dynamics of the antibody response.

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1784 – WS25.3

Unveiling the power of high-dimensional cytometry data with cyCONDOR

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High-dimensional cytometry (HDC) is a powerful technology for studying single-cell phenotypes in complex biological systems. Although technological developments and affordability have made HDC broadly available in recent years, technological advances were not coupled with an adequate development of analytical methods that can take full advantage of the complex data generated. While several analytical platforms and bioinformatics tools have become available for the analysis of HDC data, these are either web-hosted with limited scalability or designed for expert computational biologists, making their use unapproachable for wet lab scientists. Additionally, end-to-end HDC data analysis is further hampered due to missing unified analytical ecosystems, requiring researchers to navigate multiple platforms and software packages to complete the analysis.
To bridge this data analysis gap in HDC we developed cyCONDOR, an easy-to-use computational framework covering not only all essential steps of cytometry data analysis but also including an array of downstream functions and tools to expand the biological interpretation of the data. The comprehensive suite of features of cyCONDOR, including guided pre-processing, clustering, dimensionality reduction, and machine learning algorithms, facilitates the seamless integration of cyCONDOR into clinically relevant settings, where scalability and disease classification are paramount for the widespread adoption of HDC in clinical practice. Additionally, the advanced analytical features of cyCONDOR, such as pseudotime analysis and batch integration, provide researchers with the tools to extract deeper insights from their data. We used cyCONDOR on a variety of data from different tissues and technologies demonstrating its versatility to assist the analysis of high dimensionality data from preprocessing to biological interpretation.

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182 – WS25.4

Bw4 ligand and direct T-cell receptor binding induced selection on HLA A and B alleles

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Introduction: The HLA region is the hallmark of balancing selection, argued to be driven by the pressure to present a wide variety of viral epitopes. As such selection on the peptide-binding positions has been proposed to drive HLA population genetics. MHC molecules also directly binds to the T-Cell Receptor and killer cell immunoglobulin-like receptors (KIR).
Methods: We here combine the HLA allele frequencies in over six-million Hematopoietic Stem Cells (HSC) donors with a novel machine-learning-based method to predict allele frequency.
Results: We show for the first time that allele frequency can be predicted from their sequences. This prediction yields a natural measure for selection. The strongest selection is affecting KIR binding regions, followed by the peptide-binding cleft. The selection from the direct interaction with the KIR and TCR is centered on positively charged residues (mainly Arginine), and some positions in the peptide-binding cleft are not associated with the allele frequency, especially Tyrosine residues.
Discussion: These results suggest that the balancing selection for peptide presentation is combined with a positive selection for KIR and TCR binding.

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905 – WS25.5

Quantitative comparison of cellular trajectories from single-cell transcriptomics data

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Single-cell transcriptomic analysis has greatly improved our understanding of immunological processes by uncovering the detailed dynamics of cell development. Developmental trajectories, which show how cells progress along differentiation pathways, are crucial for comprehending immune cell lineage commitment, activation, and function, offering insights into genetic mutations, diseases, and external influences. Despite the availability of numerous tools for inferring developmental trajectories, quantitative comparisons remain challenging due to limitations associated with single-cell RNA sequencing data, such as its high dimensionality, complexity, and sample heterogeneity.
Our study aims to develop a method for systematically comparing trajectory graphs and obtaining quantitative measures to comprehensively analyze single-cell data. Collaborating with various research groups at the University Hospital Bonn and the University of Bonn, we established trajectories using diverse datasets, including PBMC samples from individuals with Bardet-Biedl syndrome and COVID-19 patients. While many trajectory inference tools generate graphs in the low-dimensional UMAP space, we initially focus on comparing different trajectories within this embedding. To thoroughly analyze the data, we project the graph structures back into the high-dimensional data space, compare the differences, and assess the significance of variations between graphs at lower and higher dimensions.
This framework has the potential to provide valuable insights into immunological dynamics, particularly in patient-derived time-series datasets like those from COVID-19 patients. Identifying differences in disease progression could greatly affect clinical outcomes, shaping treatment decisions and patient care. We aim to establish a reliable framework for analyzing single-cell data, enabling a more thorough examination and deepening our understanding of immunological dynamics. Ultimately, the quantitative insights gained can be leveraged to develop data-driven mathematical models to explore cell-cell communication within the immune system and conduct *in-silico* perturbation studies.

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214 – WS25.6

The National Research Data Infrastructure for Immunology in Germany (NFDI4Immuno): Building a framework for comprehensive immunological data integration and analysis, collaboration and Open Science.

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In the era of advanced single-cell technologies, including flow cytometry, mass cytometry and single cell sequencing, capable of generating very information-rich datasets, the necessity for data sharing and reuse has grown significantly in immunological research. The National Research Data Infrastructure for Immunology (NFDI4Immuno) initiative funded by the German Federal Ministry of Education and Research tries to address this need.
Here, we present how the NFDI4Immuno initiative aims to integrate immunological data and metadata from various experimental technologies, including cytometry, sequencing, immunoassays, and imaging, to provide a holistic view of immunological processes. The initiative strives to enhance scientific collaboration by harmonizing data representations, metadata standards, ontologies, and programmatic interfaces with other NFDI consortia, promoting seamless queries and cross-referencing. The project is committed to support users in effectively utilizing its resources and to foster the adoption of FAIR principles (findability, accessibility, interoperability and reusability) within the immunological community to contribute to the broader cultural shift towards Open Science. Finally, NFDI4Immuno plans to establish and manage a network of federated repositories for immunological data, to develop tools and services that facilitate standardized and reproducible data analyses, reinforcing scientific rigor and transparency.

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WS26 – IMMUNOLOGY OF SKIN DISEASE

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1886 – WS26.1

Lin-CD117+CD34+FceRI+ progenitor cells in chronic spontaneous urticaria and atopic dermatitis

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Purpose: Chronic spontaneous urticaria (CSU) is a common, debilitating skin disorder characterised by recurring episodes of raised, itchy and sometimes painful wheals lasting longer than 6 weeks. CSU is mediated by mast cells which are absent from peripheral blood. However, lineage$^-$CD34^{hi}CD117$^{int/hi}$FcεRI$^+$ cells in blood have previously been shown to represent a mast cell precursor.
Methods: We enumerated FcεRI$^-$, FcεRI$^+$ and FcεRI^{hi} lineage$^-$CD34$^+$CD117$^+$ cells using flow cytometry in blood of patients with CSU (n=55), including 12 patients receiving omalizumab and 43 not receiving omalizumab (n=43). Twenty-two control samples were studied. Disease control and patient response to omalizumab was evaluated using the Urticaria Control Test. We performed single cell RNA sequencing (scRNA-seq) on lineage$^-$CD34^{hi}CD117^{hi} blood cells from a subset of patients with CSU (n=8) and healthy controls (n=4). We also assessed lineage$^-$CD34$^+$CD117$^+$ cells in patients with atopic dermatitis.
Results: CSU patients had more Lineage$^-$CD34$^+$CD117$^+$FcεRI$^+$ blood cells than controls. Lineage$^-$CD34$^+$CD117$^+$FcεRI$^+$ cells were significantly higher in patients with CSU who had an objective clinical response to omalizumab when compared to patients who had poor disease control 90 days after initiation of omalizumab. scRNA-seq revealed that lineage$^-$CD34$^+$CD117$^+$FcεRI$^+$ cells contained both lymphoid and myeloid progenitor lineages, with omalizumab responsive patients having proportionally more myeloid progenitors. The myeloid progenitor lineage contained small numbers of true mast cell precursors along with more immature FcεRI$^-$ and FcεRI$^+$ myeloid progenitors.
Conclusion: Increased blood CD34$^+$CD117$^+$FcεRI$^+$ cells may reflect enhanced bone marrow egress in the setting of inflammatory CSU. High expression of these cells strongly predicts better clinical responses to the anti-IgE therapy, omalizumab.

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1577 – WS26.2

Single-cell responses to mTOR inhibition in patients with cutaneous sarcoidosis

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Background: In a recent clinical trial, systemic, but not topical, mTORC1 (mechanistic target of rapamycin complex 1) inhibition resulted in a long-lasting remission in a subset of patients with cutaneous sarcoidosis, a granulomatous inflammatory disease. Skin and blood samples from different timepoints of the trial were assayed by single-cell RNA sequencing and spatial transcriptomics. We are investigating this dataset to uncover the transcriptional changes in the tissue upon mTOR inhibition and underlying patterns which determine treatment response.
Methods: After thorough quality control, high-quality cell transcriptomes from skin and blood were integrated using an scVI model, clustered, and cell types were annotated based on marker genes. Changes in cell-type composition throughout treatment were assessed using the scCODA model. Differential expression analysis between timepoints per cluster was performed using limma on pseudobulked counts. The results were used for gene set enrichment analysis.
Results: We find all expected cell subsets in our transcriptomics data as well as distinct granuloma-specific cell populations, which significantly decrease in abundance throughout treatment. The expression of many genes changes in macrophages, helper T cells and fibroblasts after systemic treatment, including cytokines, chemokines, transcriptional regulators, and genes previously linked to sarcoidosis. This is not the case for topical treatment. Pathways downregulated by systemic treatment are mostly related to metabolism, antigen presentation and autoimmune diseases, with the strongest effect on metabolism observed in macrophages. While macrophages show a strong response already immediately after treatment, T cells only undergo major expression changes at a later timepoint. Comparison of responders and non-responders throughout treatment reveals differences in pathways related to metabolism but also lymphocyte chemotaxis and activation.
Conclusions: The cell composition and expression profile of granulomatous tissue appear to change markedly after mTOR inhibitor treatment. mTOR inhibition results in changes of metabolic pathways in immune and structural cells, but also immune function is strongly affected in these populations. Different cell types respond on different time scales and we will further investigate details of these specificities. Additionally, by disentangling differences between responders and non-responders we aim to predict response of future patients.
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2180 – WS26.3

The hair canal serves as an EGFR-regulated antimicrobial gatekeeper

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Hair follicles are crucial to maintain mammalian skin barriers and protect against physical stressors of the environment. They represent a unique niche for commensal skin bacteria but unchecked proliferation of microbiota within hair follicles initiates folliculitis and fosters cutaneous dissemination of infections. This clinical implication is exceptionally displayed in cancer patients receiving anti-EGFR therapy that evokes papulopustular eruptions with concomitant bacterial *Staphylococcus aureus* superinfections.
In this study, we identified the specific cell population facilitating the pivotal microbial gatekeeping function of the hair follicle. We exploited single-cell datasets, deciphered epidermal growth factor receptor (EGFR)-dependent transcriptional signatures, and conducted targeted knock-out experiments in genetically engineered mice to pinpoint the significance of the EGR2/K79-positive hair canal as essential antimicrobial bastion. EGFR orchestrates the expression of antimicrobial peptides (AMPs) such as beta-defensin1/6 and SPRR1a/4 within the hair canal via the ERK signaling pathway. Notably, our data suggests that the presence of EGFR in fully differentiated sebocytes is expendable for the homeostatic defense mechanism of the hair follicle.
Our investigation further revealed that the identified AMP profile translates to the human skin, as AMP homologues are also concentrated in K79-expressing cells, and their overexpression is evident in EGFR-ERK-dominant psoriatic skin conditions. These findings provide crucial mechanistic insights into the microbial defense strategy employed by the hair follicle, with direct therapeutic implications for addressing folliculitis associated with EGFR-inhibitor-based anti-cancer therapy.

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159 – WS26.4

Spermidine/spermine N1-acetyltransferase controls tissue-specific regulatory T cell function in chronic skin inflammation

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Regulatory T cells (T_{regs}) are a critical immune component guarding against excessive inflammatory responses, but current understanding of T_{reg} heterogeneity and function in non-lymphoid tissues in humans is limited. In this project we characterized unique types of T_{reg} signatures in the context of tissue-confined inflammatory responses in human skin and gut. During chronic inflammation, T_{regs} fail to control effector T cell responses. Causes of T_{reg} dysfunction in these diseases are poorly characterized and therapies are aimed at blocking aberrant effector responses rather than rescuing T_{reg} function. We hypothesized that impairment in tissue-specific immune regulation leads to exacerbated T cell responses and chronic inflammation in these diseases. Therefore, we utilized single-cell RNA sequencing data from patients suffering from chronic skin and colon inflammation to show that diseased tissue-T_{regs} have a strong, specific, tissue-resident signature. We uncover *SAT1*, the gene encoding spermidine/spermine N1-acetyltransferase (SSAT), as a novel marker and driver of skin-specific T_{reg} dysfunction during T_H17-mediated inflammation. T_{regs} expressing high levels of *SAT1* are specifically enriched in chronic skin inflammation and show a proinflammatory effector-like transcriptomic profile. Using CRISPRa in healthy human skin-derived T_{regs}, we validated that expression of *SAT1* leads to loss of suppressive function and a switch to a T_H17-like phenotype. This phenotype is induced by co-receptor expression on keratinocytes exposed to a T_H17 environment. Lastly, we highlight the potential therapeutic impact of targeting SSAT in a mouse model of skin inflammation by inhibiting SSAT pharmacologically, which rescued T_{reg} number and function in the skin and systemically. Together, our results reveal a novel interface of polyamine catabolism and T_{reg} function in the skin which leads to impaired control of effector T cell responses. Ultimately, our findings significantly contribute to basic understanding of T_{reg} function and shed light on immune regulation during chronic inflammation in human tissue and reveal SSAT as a potential novel target for chronic inflammatory skin disease.

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1997 – WS26.5

Single cell transcriptomics reveals two fates of Hair Follicle Stem Cells in patients with hidradenitis suppurativa and sheds light on the mechanisms of keratinisation and inflammation

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Purpose: Hidradenitis suppurativa (HS) is a devastating skin disorder that affects 1% of the world's population. Although the pathogenesis is multifactorial and poorly understood, the pathomechanisms of HS are intimately associated with aberrantly activated keratinization and autoinflammation. It is unknown whether the autoinflammatory events precede or follow the hyperkeratotic changes in the hair follicle (HF) epithelia. An intrinsic dysregulation of the hair follicle stem cell (HF-SCs) compartment leads to replication stress stimulating type I interferon responses. To further dissect the role of HF-SCs, we performed single cell RNA Seq using freshly isolated total HF cells.
Methods: HF samples were from discarded plastic surgery specimens from 2 controls and 5 HS patients. To obtain single-cell suspension, the isolated HFs were digested in Trysin-EDTA. We used the Seurat R package to analyze the scRNA-seq data.
Results: We identified three main clusters: i) HF matrix lineage enriched for *MSX2* (HF matrix marker), ii) non-matrix lineage enriched for *KRT14* and iii) immune cells enriched for *PTPRC*. To determine whether differences could be observed between HF cells from controls and HS patients, unsupervised clustering was performed based on the proportion of cells in each cluster. The most striking differences were observed in non-matrix cells and could segregate patients into 2 groups. The first group is enriched in cluster 1 (IBL), while the second is enriched in clusters 0 (mORS). These two clusters displayed fundamental transcriptomic changes. Noteworthy, GSEA revealed significant enrichment for genes involved in keratinization in IBLs, whereas mORS showed enrichment for interferon response genes. The trajectory analysis revealed that the HF-SCs differentiated in either IBL or mORS. We were able to confirm our results on 47 HS patients included in Fol-HYDRA cohort (CPP# n°2021-A02352-39) and show that these two pathways are associated with two different clinical phenotypes of patients.
Conclusion: Our results provide an unprecedented view of HS pathology and identify two pathways involved in HS, one involved in the keratinization process and the other in inflammation. Based on these pathways, two endotypes of HS patients were identified, which could serve as a potential marker for personalized medicine.

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1337 – WS26.6

Multimodal and Longitudinal Single-Cell Analysis of Local Innate Immune Response to Vaccine in Ex Vivo Natural Human Skin Modules

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Purpose: In the rapidly evolving landscape of vaccine research, the quest for innovative methodologies with single cell resolution that accurately investigate human immune responses at the site of injection prior to clinical trials is of utmost importance.
Methods: In response to this challenge, we used bio-stabilized, injectable *ex vivo* human skin modules (HypoSkin®) to closely investigate mRNA vaccine-induced immune response within its natural environment and at the single cell level. We developed a framework that combines MANTIS spatial biology and single-cell RNA sequencing to detect subtle changes in immune landscape dynamics and track mRNA incorporation after vaccination with mRNA-1273 COVID-19 vaccine.
Results: In preparatory experiments, we confirmed the transcriptomic stability of *ex vivo* human skin modules at the single cell level. To delve deeper into the innate mechanisms of the vaccine at the injection site, we conducted single-cell RNA sequencing analysis on dissociated human skin modules enriched in skin-resident CD45+ immune cells. Our findings unveiled the presence of SARS-CoV-2 spike mRNA sequences primarily in DC/macrophage and mast cell clusters at 8 hours post-injection, accompanied by significant transcriptomic changes in multiple skin-resident immune cells post-vaccination. Furthermore, to assess protein-level changes, we utilized the Multiplex ANnotated Tissue Imaging System (MANTIS®) to analyze the activation status and redistribution of skin-resident antigen-presenting cells. Our analysis revealed that, upon vaccination, LCs and Langerin+ dDCs upregulate activation/maturation markers while redistributing in specialized dermal areas and enhance their capacity to interact with neighbor CD4+ T cells.
Conclusion: In summary, our comprehensive investigation underscores the efficacy of HypoSkin® combined with single cell analysis in capturing the intricate dynamics of vaccine-induced immune responses within native tissue environments. This innovative approach not only enhances our understanding of vaccine efficacy but also holds promise for expediting the development of novel vaccination strategies, thereby propelling the field of human innate immune response to vaccines forward.

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WS27 – TISSUE MICROENVIRONMENTS-DAMAGE AND REPAIR

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1907 – WS27.1

Atherosclerotic patients at high-risk for plaque destabilization show an enhanced adaptive reconfiguration of NK cells in response to HCMV

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Purpose: Human cytomegalovirus (HCMV) is associated with atherosclerosis and destabilization of atherosclerotic plaques. In some patients, HCMV promotes a marked reconfiguration of the natural killer (NK) cell compartment characterized by a distinct phenotype and antibody-dependent enhanced functional capabilities, including antibody-dependent cell-mediated cytotoxicity (ADCC), and cytokine production. IFN-γ producing NK cells have been implicated in atherosclerosis progression, apparently in association with infection to human cytomegalovirus (HCMV). Yet, the precise role of NK cells in atherosclerotic plaque destabilization and the molecular mechanisms underlying HCMV-associated atherosclerosis progression remain open issues. This study aims to investigate the potential impact of the HCMV-induced reconfiguration of the NK cell compartment in the pathogenic mechanisms underlying atherosclerotic plaque instability.
Methods: A total of 64 patients were enrolled in a follow-up protocol for carotid artery stenosis. Patients were classified following conventional criteria as bearing high-risk plaques (High-risk patients- HR patients) or low-risk plaques (Low-risk patients- LR patients). High-risk patients underwent carotid endarterectomy according to the European Society for Vascular Surgery (ESVS) guidelines. Carotid plaques and pre-operative blood samples were obtained from all patients to be processed. Multiparametric flow cytometry was used to evaluate NK cell phenotype and functionality.
Results: Adaptive- non-conventional NK cells are enriched in high-risk atherosclerotic HCMV seropositive patients and display an increased expression of NKG2D. Remarkably, we observed that FCεR1γ^{-} non-conventional NK cells increase upon plaque destabilization in peripheral blood and accumulate in carotid plaques of high-risk atherosclerotic patients by upregulating tissue resident markers (CD103, CD49a). Moreover, NK cells from High-Risk HCMV seropositive patients have enhanced antibody-dependent effector functions compared to Low-Risk patients and antibody-dependent IFN-**γ** release correlates with the frequency of FCεR1γ^{-} in high-risk atherosclerotic patients. Remarkably, masking of NKG2D receptor significantly reduces antibody-dependent IFN- **γ** release by FCεR1γ^{-} NK cells, suggesting that NKG2D might act in conjunction with CD16 to trigger NK cell activation thus representing a crucial axis for atherosclerotic plaque progression.
Conclusion: In conclusion, we provide new data involving memory-like NK cells and NKG2D in atherosclerotic plaque destabilization, further suggesting that their analysis may provide useful indications to identify high-risk patients that might benefit from early surgical intervention and/or closer follow-up.

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1661 – WS27.2

Comparative analysis of interferon (IFN) signalling reveals an IFNy-Jak1 axis in the selective control of intestinal stem cell niche damage and fetal injury

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The Inflammatory bowel diseases (IBD: Crohn's disease and Ulcerative Colitis) are characterised by idiopathic inflammation with a central pathologic hallmark being the dysregulation of the epithelial stem cell niche (including loss of paneth cells, crypt atrophy, etc). While both infiltrating and tissue resident immune subsets play a role in the pathogenesis, how immune-epithelial stem cell crosstalk impacts on disease course is not fully defined. A defining feature of multiple clinical data sets has highlighted enhanced interferon (IFN) signalling associated with elevated disease activity and further with treatment refractory patients. This is of clinical relevance as biologic therapeutics targeting Jak-STAT signalling are in clinical use for IBD (e.g.: Tofacitinib). Yet, these therapeutics present with complications for some patients in addition to non-responsive cohorts. Currently, a clear understanding of how the different IFN classes specifically effect intestinal epithelial biology is not well defined.
To address this knowledge gap we utilised small intestinal organoid cultures to screen the effects of recombinant IFN classes (IFN-alpha, IFN-beta, IFN-gamma, IL-28a [IFN-lambda2]) and assessed the impact on growth, cell cycle, cellular composition and function. While organoids cultured in the presence of IFN-alpha, IFN-beta and IFN-lambda2 displayed no adverse effects, IFN-gamma had a marked detrimental impact on epithelial stem cell function, selectively depleting mature stem cell (*lgr5, olfm4*) and paneth cell markers (*lyz, defa21*). Notably, enterocyte, goblet cell and enteroendocrine cells were unchanged. We next performed RNA-sequencing and profiled organoids longitudinally using automated morphometric analysis to define the functional consequences of this on stem cell function. Of note, IFN-gamma selectively reverts the stem cell niche to an injury associated, fetal-like stem cell program (*ly6a, sprr1a*). We investigated the signalling events that IFN-gamma elicits to drive this gene program and performed a targeted small molecule pharmacologic screen to identify possible signalling hubs. While blocking hallmark injury, stress and damage-associated pathways had no impact, Jak1 (and not Jak2) inhibition reversed this fetal-stem cell programme and restored morphological growth patterns and organoid function. Thus, IFN-gamma has a selective negative impact on intestinal epithelial stem cell biology and further mechanistic understanding of this process has implications for IBD therapeutics.

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496 – WS27.3

Immune cells play a critical role in cytokine- and endotoxin-mediated endothelial permeability

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Purpose: Vascular leakage is a severe pathology occuring in a broad range of scenarios, e.g. as secondary disease induced by sepsis, infections, cytokine storms, or as side effect of immunotherapies. Its severity is underlined by the high lethality rate of 20-30% for the systemic capillary leakage syndrome (CLS). The unspecific symptoms during the acute phase of CLS and the rapid progression into the massive leak-phase often cause misdiagnosis or a diagnostic delay, which contributes to the high mortality. While many compounds are reported to affect endothelial cell (EC)-activation, the exact mechanisms resulting in increased permeability, including the role of immune cells, are not fully understood.
Methods: In this study, we generated a comprehensive dataset analyzing activation, vitality, cytokine secretion, and permeability of human umbilical vein endothelial cells (HUVEC) upon stimulation with 16 different stimuli (cytokines, vasoactive substances, and danger signals). HUVEC-permeability was analyzed in a trans-well-based leakage assay with or without human peripheral blood mononuclear cells (PBMC).
Results: Activation of HUVEC is characterized by upregulation of intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin with production of interleukin (IL)-8, monocte chemoattractant protein-1, and partly IL-6. Strong HUVEC-activation and reduced vitality was observed upon treatment with IL-1β, TNF-α, a TGN1412-induced cytokine cocktail ($SN_{TGN1412}$), thrombin, and lipopolysaccharide (LPS). Interestingly, only thrombin, and to a lesser extent $SN_{TGN1412}$ and VEGF, induced increased permeability of HUVEC-monolayers. On the other hand, compounds reported to be associated with vascular leakage such as TNF-α, IL-1β, or LPS had no direct effect on HUVEC-permeability. Remarkably, inclusion of stimulated PBMCs in the leakage assay induced elevated HUVEC-permeability mediated by IL-1β, TNF-α, $SN_{TGN1412,}$ and LPS.
Conclusion: Our results demonstrate that strong activation of EC not necessarily results in increased permeability. Especially for cytokine- and endotoxin-mediated vascular leakage, immune cells are required to induce increased permeability of HUVEC in our *in vitro* assay. In depth analyses of the mechanisms underlying the development of increased permeability will uncover potential treatment-targets for patients suffering from vascular leakage and help to improve safety-assessment of leakage-associated immunotherapies.

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227 – WS27.4

IL-18-induced HIF-1α in ILC3s ameliorates the inflammation of C. rodentium-induced colitis

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Group 3 innate lymphoid cells (ILC3s) are vital for defending tissue barriers from invading pathogens. Hypoxia influences the production of intestinal ILC3-derived cytokines by activating HIF. Yet, the mechanisms governing HIF-1α in ILC3s and other innate RORγt$^+$ cells during in vivo infections are poorly understood. In our study, transgenic mice with specific Hif-1a gene inactivation in innate RORγt$^+$ cells (RAG1KO HIF-1α$^{\Delta Rorc}$) exhibit more severe colitis following Citrobacter rodentium infection, primarily due to the inability to upregulate IL-22. We find that HIF-1α$^{\Delta Rorc}$ mice have impaired IL-22 production in ILC3s, while non-ILC3 innate RORγt$^+$ cells, also capable of producing IL-22, remain unaffected. Furthermore, we show that IL-18, induced by Toll-like receptor 2, selectively triggers IL-22 in ILC3s by transcriptionally upregulating HIF-1α, revealing an oxygen-independent regulatory pathway. Our results highlight that, during late-stage C. rodentium infection, IL-18 induction in the colon promotes IL-22 through HIF-1α in ILC3s, which is crucial for protection against this pathogen.

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1621 – WS27.5

Complement-mediated severe hypersensitivity reaction to Paclitaxel

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Background: Every day practice reveals the relative high frequency of non-IgE mediated severe hypersensitivity reactions (SHR). Since the 80s, Paclitaxel, more specifically it's vehicle Cremophor EL has been associated with SHR, some of them fatal, which is why patients receiving this treatment must be pre-treated with corticosteroids and antihistamines, even though this measure has not been proven 100% effective. Herein we present a case of anaphylaxis to Paclitaxel associated to direct complement activation (DCA).
Case summary: A 62 years old female diagnosed with grade IVB endometrial carcinoma (endometrioid type), underwent complete cytoreduction surgery and subsequent adjuvant treatment with Paclitaxel-Carboplatin. After first infusion of Paclitaxel she presented anaphylactic shock with skin, cardiorespiratory and digestive system involvement that reverted after treatment with corticosteroids, antihistamines, three doses of adrenaline and cardiorespiratory support. Analytical results at 20 min post-reaction showed: tryptase 8.0 ug/L, tIgE 642.0 kU/L, C3 1.13 g/L (nv 0.87-1.70 g/L), C4 0.28 g/L (0.11-0.54 g/L) and CH50 17 U/mL (nv 28-60 U/mL). Prick test and basophil activation test (BAT) to paclitaxel were negative after 1 week of the reaction. Further Complement studies in the acute phase also detected increased levels of anaphilotoxins C3a (24.4 µg/mL; nv 6.7-11.7 ng/ml) and C5a (103 ng/mL; nv 11.5-77.8 ng/mL), as well as of membrane attack complex (MAC) sC5b-9 (1298 ng/mL (nv 187-525 ng/mL). For drug readministration, desensitization could not be achieved since a new anaphylactic shock appeared. Therefore, an alternative chemotherapy with non-pegylated doxorubicin was well tolerated.
Conclusion: Based on SHR occurring upon the first contact with paclitaxel, no evidence of specific IgE could be obtained and significantly increased anaphylotoxins and soluble MAC serum levels, we consider this to be a SHR mediated by DCA. Non-IgE mediated SHR involving DCA are relatively frequent, but little considered when making in vitro diagnosis. So far there is no difference in the medical management of IgE-mediated and non-IgE-mediated anaphylaxis. Therefore, it would be important to differentiate them and to consider the possibility of implementing preventive treatments directed against complement that seem to work in small *in vitro* studies and could prove to be more effective than current preventive treatment.

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1550 – WS27.6

Anti-Ig-E treatment in patient with severe asthma and eosinophilic granulomatosis with polyangiitis: A case report

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Eosinophilic granulomatosis and polyangiitis (EGP), is a rare systemic small vessel vasculitis characterized by eosinophilia and typically accompanied by asthma. Patients typically present with symptoms related to asthma exacerbations, such as dyspnea, cough, and wheezing, as well as features of vasculitis such as neuropathy, skin lesions, and cardiac involvement. The exact cause of EGP is not fully understood. Both genetic and environmental factors are thought to contribute to its development. EGP is believed to be a T helper (TH) 2 mediated disorder, characterized by the upregulation of TH-2 related cytokines such as IL-4, IL-13, and IL-5. Eosinophilia in both blood and tissue is driven by eotaxin-3, produced by epithelial and endothelial cells. Glucocorticoids (GC) are the primary treatment for EGP and often lead to remission of the disease. Managing EGP can be challenging, particularly when patients are unresponsive or intolerant to standard therapies like GC. Omalizumab, an anti-Ig-E monoclonal antibody, is used in patients with severe asthma and can also be considered in EGP due to its ability to decrease eosinophil growth factors and increase eosinophil apoptosis. By targeting Ig-E and modulating eosinophilic inflammation, omalizumab offers a targeted approach with the potential to reduce reliance on systemic steroids and improve outcomes.
The 54-year-old patient had a history of asthma for 7 years before being diagnosed with EGP in 2014. Initially, the patient received steroid treatment for an extended period to manage EGP. However, he experienced recurrent asthma attacks and continued eosinophilia during follow-up. Due to the patient's severe symptoms, serious side effects from GC treatment, and frequent hospital admissions, omalizumab treatment was deemed appropriate. After initiating omalizumab treatment, the patient's symptoms were effectively controlled. He did not experience any serious asthma attacks requiring hospitalization during this 2 years period of time.
This case highlights the successful use of targeted therapy (omalizumab) in controlling EGP symptoms and preventing severe asthma exacerbations requiring hospitalization. The presentation of this case contributes valuable insights into the management of rare and refractory diseases, emphasizing the importance of personalized treatment approaches and the potential of novel therapies to improve patient outcomes.

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WS28 – IMMUNE SENESCENCE AND AGING

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1684 – WS28.1

IL-15 drives NK-receptor mediated cytotoxicity in senescent CD8 T cells

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Purpose: Alongside reduced proliferative capacity, senescent T cells upregulate Natural Killer receptors (NKR) and are able to kill NK-target cells. We hypothesise that within inflammatory microenvironments, T cells, can induce non-specific tissue damage via NK-like cytotoxicity. We have explored the role of IL-15 in inducing senescent T cells to perform NK cytotoxic killing as a potential mechanism for non-specific pathology.
Methods: Peripheral blood mononuclear cells from males and females (30-80 years) were sorted into CD8 naïve (CD27+CD28+) and senescent (CD27-CD28-) T cell populations, followed by proteomic analysis. Cytotoxicity assays were performed using these cells cultured with and without 10 ng/ml IL-15 for 48 hours prior to co-culture with K562 target cells. Virus-specific CD8+ T cells and autologous fibroblasts were provided by Prof. Tao Dong.
Results: Upstream pathway analysis showed that IL-15 was predicted to be the master regulator of the proteome of senescent but not naïve T cells. Incubation of naïve and senescent T cells with IL-15 increased NKG2D and DAP-12 expression, facilitating antigen-independent cytotoxicity. IL-15 induced CD8+ senescent T cells to kill the K562 NK-target cell line. We investigated if virus epitope specific CD8+ T cytomegalovirus (CMV) and influenza virus (Flu) specific cell clones, which had a similar phenotype to senescent T cells, could kill autologous fibroblast targets. These cells were able to, but only at early time points after activation. This suggests that antigen-specific CD8+ T cells may only possess NK-like activity at the height of activation. To determine if this mechanism of non-specific killing was relevant *in vivo*, we studied patients with cutaneous leishmaniasis (CL) who develop skin lesions after parasitic infection. As the disease progresses there are few parasites, but an accumulation of NK-like CD8 T cells. We showed that a cocktail of cytokines, mimicking those that were within the lesions, could induce CD8 T cells to kill autologous fibroblasts and this was blocked by preventing NKG2D signalling.
Conclusion: Therefore, senescent CD8+ T cells can perform antigen-independent cytotoxic activity. This may be a mechanism for antigen non-specific pathology in clinical situations where intense inflammation and the accumulation of senescent T cells coexist.

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2207 – WS28.2

Persistent exposure to cancer-derived granulocyte colony stimulating factor leads to aberrant bactericidal functionality of neutrophils

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Bacterial infections exert a profound impact on the morbidity and mortality rates among cancer patients. Besides the variety of disease- or treatment-dependent factors, modulation of immune responses by tumor itself potentially fosters the persistence of pathogenic microorganisms. While short treatment with granulocyte colony stimulating factor (G-CSF) effectively increases neutrophil counts and stimulates antibacterial properties of neutrophils both *in vitro* and *in vivo*, the controversial effects of persistent exposure to tumor-derived G-CSF on neutrophil functionality have been previously underestimated.
Our investigation revealed an increased susceptibility to infections associated with Gram-negative pathogens in previously untreated patients with head and neck cancer characterized with elevated expression of G-CSF. To investigate the impact of tumor G-CSF on impaired antibacterial responses, we established a murine model of oropharyngeal carcinoma characterized by low and high G-CSF production (MOPC and G-MOPC, respectively). We confirmed the role of persistent tumor-derived G-CSF stimulation in decreased resistance of such animals to lower respiratory tract infection caused by *Pseudomonas aeruginosa*.
Through analysis of proteome landscapes of lung neutrophils, we identified the affected pathways involved in cytoskeleton reorganization, response to reactive oxygen species, and regulation of aging and apoptosis in neutrophils isolated from G-MOPC-bearing animals (G-neutrophils). Accordingly, isolated G-neutrophils exhibited aberrant functionality related to these processes, with the most prominent impairment in aged cells. Notably, the alterations induced by persistent G-CSF exposure manifested at the neutrophil progenitor stage and persisted even after withdrawal of G-CSF from the system. Modulation of G-CSF downstream signaling pathways restored neutrophil maturation and functionality *in vitro*, leading to improved bacterial clearance *in vivo*.
Thus, the disregulated functionality of neutrophils, orchestrated by tumor-derived G-CSF, can be persistent over time and have an extended impact on the bactericidal clearance. Clinically, heightened attention to neutrophil functionality in cancer patients, also after surgical removal of the tumor, is required.

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2150 – WS28.3

Loss of SARM1 attenuates chemokine production & immune cell infiltration during retinal degeneration

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Retinal degenerative diseases are a leading cause of incurable vision loss worldwide. Photoreceptors are the cells responsible for sensing light and initiating visual signalling, many retinal degenerative diseases are characterised by photoreceptor cell death resulting in irreversible sight loss. SARM1 is a TLR adaptor with an additional role in induction of axonal degeneration and neuronal cell death in response to various insults. Our lab has previously described a role for SARM1 in promoting photoreceptor cell death in models of retinal degeneration. Additionally, SARM1 regulates an immune response in injured neurons prior to cell death, resulting in the release of chemokines from neurons and recruitment of peripheral immune cells to the site of injury.

We aimed to characterize photoreceptor immune responses during retinal degeneration using the sodium iodate ($NaIO_3$) model of oxidative stress. $NaIO_3$ was administered by a single intravenous injection to induce retinal degeneration in C57BL/6J and *Sarm1*$^{-/-CRISPR}$ mice. *In vivo* optical coherence tomography (OCT) imaging was carried out at day-1 post $NaIO_3$ administration to assess immune cell infiltration. Chemokine levels in retinal lysates were quantified by ELISA. Immune cell infiltration into the retina was examined by flow cytometry. Photoreceptors were isolated from the retina and qPCR analysis was carried out to assess photoreceptor chemokine production in response to $NaIO_3$.

At day-1 post intravenous administration of $NaIO_3$, photoreceptor cells upregulate a range of chemokines, including CCL5, CCL7, and CXCL12 demonstrating that photoreceptors can generate an immune response to damage. Interestingly, at day-1 post $NaIO_3$, *Sarm1*$^{-/-CR}$ mice have reduced levels of CCL2 compared to C57BL/6J counterparts, as measured by ELISA. Likely as a result of this reduced chemokine secretion we observed fewer cells infiltrating into the retina of *Sarm1*$^{-/-CRISPR}$ mice compared to C57BL/6J, following NaIO3 administration by *in vivo* OCT imaging. Notably, we found by flow cytometry analysis that *Sarm1*$^{-/-CR}$ mice had significantly decreased numbers of infiltrating monocytes and dendritic cells at day-3 post $NaIO_3$ injection compared to C57BL/6J counterparts.

Taken together these data point towards SARM1 regulating a photoreceptor immune response prior to retinal degeneration which drives immune cell infiltration into the retina via upregulation of chemokines.

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710 – WS28.4

Senescence related phenotypic alterations of lymphocytes are affected by dialysis prescription and can predict mortality in dialysis patients

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Purpose: Senescence-resembling alterations on lymphocytes of dialysis patients have been widely described. However, pathophysiology behind these phenomena has not been clarified. In this study, we examine the impact of dialysis prescription and residual urine output (RUO) on T and B lymphocytes, in dialysis patients
Methods: T and B cell subsets were determined with flow cytometry in 36 hemodialysis (HD) and 26 hemodiafiltration (HDF) patients, according to the expression of CD45RA, CCR7, CD31, CD28, CD57 and PD1 for T cells, and IgD and CD27 for B cells. Immune phenotype was associated with dialysis modality, hemofiltration volume (HFV), RUO and mortality
Results: Compared to HD, HDF patients had significantly decreased percentage of CD4+CD28-CD57- T cells [3.8(2.4-5.3) vs 2.1(1.3-3.3)%, respectively, P = 0.002] and exhausted CD4+ T cells [14.1(8.9-19.4) vs 8.5(6.8-11.7)%, respectively, P = 0.005]. Additionally, HFV was negatively correlated to CD8+ EMRA T cells (r = -0.46, P = 0.03). Moreover, retained RUO and shorter dialysis vintage were both independently associated, and also had synergistic effect, to increased count of total and naïve CD4+ T cells. Finally, exhausted CD4+ T cells percentage could predict all-cause mortality in dialysis patients, independently of age. Kaplan-Meier survival analysis for patients with proportion of CD4+PD1+ above and below median was significant (log rank P = 0.002). Moreover, significant effect of exhausted CD4+PD1+ T cells proportion remained in Cox Regression either when examined alone as a continuous variable (P = 0.003, 95% CI: 1.0, 1.2), or in combination with age (CD4+PD1+ proportion: P = 0.03, 95% CI: 1.01, 1.23, age: P = 0.02, 95% CI: 1.01, 1.44).
Conclusion: HDF especially with high HFV may have beneficial effects on senescence-related immune phenotype. RUO may also contribute to retarding immune senescence. Immune phenotype may also be a predicting factor for mortality in dialysis patients.

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2227 – WS28.5

Sustainable T and B cell immunity in long-lived rodents (Spalax)

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Healthy aging represents one of the global challenges nowadays. Preservation of immunological memory contributes to the successful survival of both mice and humans. However, in some cases, immunologic memory could lead to erroneous adaptive immune responses resulting in chronic inflammation and autoimmunity. Recently emerging data show that age-related changes in adaptive immunity include accumulation of regulatory T cells, expansion of cytotoxic memory T cells, and significant reduction of naive T cell subset within CD8+ and CD4+ T cell pool in humans and mice.
In this study, we aim to explore the adaptive immune organization of an exceptionally long-lived and cancer-resistant rodent, Spalax. In contrast to mice and humans, repertoire diversity of T cell receptors remains stable with age in Spalax. Following our previous findings on Spalax adaptive immunity (Izraelson et al., Nature Aging, 2021), we focus on the age-related changes in transcriptomic programs of T and B lymphocytes using single-cell RNA and TCR sequencing of cells from the spleen and lymph nodes of young and aged animals.
Our results show that in contrast to mice, Spalax immunity has the following features:

- Exhausted memory CD8+ T cell clones do not accumulate with age and do not express GZMK;
- T cells lack accumulation of expression signatures of immune checkpoints (*Ctla4, Pdcd1, Tigit, Lag3*) and Tregs with aging.
- B cells have stably high level of SHM that do not increase with age;
- B cells include a unique *Aicda* expressing subset. *Aicda* encodes activation induced deaminase (AID) that mediates the class switch recombination and somatic hypermutations in the V-region of BCR. The increased expression of AID in Spalax B cells might contribute to affinity maturation of immunoglobulins that apparently occurs faster than in mice.

Based on our results, we can conclude that the balanced functioning of adaptive immunity in aged Spalax is supported by several mechanisms, including maintaining a low proportion of highly specialized long-lived memory T cells - a distinct strategy that potentially underlies this animal's extraordinary longevity.

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682 – WS28.6

Aging drives dysregulation of regulatory T cells in the gut mucosa.

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Aging encompasses the gradual deterioration of physiological functions crucial for survival and reproductive capacity, driven by various factors including cellular senescence, genomic instability, and mitochondrial dysfunction. Cellular senescence, induced by diverse stress stimuli, involves intrinsic factors such as replicative stress and DNA damage, along with extrinsic factors like viral infections and UV exposure. Nuclear DNA damage activates the DNA damage response (DDR) pathway, leading to p53-mediated cell cycle arrest and senescence. With advancing age, immune senescence occurs, characterized by dysregulation of innate and adaptive immunity and a chronic low-grade inflammatory state termed inflammageing. This study aimed to evaluate the impact of aging on effector and regulatory T cell (Treg) functionality within the gut mucosa. We found that aged mice exhibited alterations in the CD4 T cell profile, including a decrease in naive CD4 T cells and an increase in effector CD4 T cells, particularly Th1 and Th17 cells. Despite an increase in Treg cell frequency, aged Treg cells showed signs of activation, with elevated expression of CD44. Furthermore, the aged gut mucosa displayed an increased population of Treg subsets, including Treg+ Helios+, Helios+KLRG1+, and RORγt+ cells. Assessment of Treg suppressive capacity in an experimental model of T-cell transfer colitis revealed diminished efficacy of Tregs from aged mice in suppressing intestinal inflammation compared to young Tregs. Additionally, aged Tregs exhibited increased DNA damage and oxidative stress, as indicated by elevated levels of H2AX and mitochondrial ROS expression. Reduced proliferation was observed in aged Tregs, indicative of senescence. Moreover, increased expression of IL-6 signaling molecules in aged Tregs suggested a potential role of IL-6 in driving Treg senescence. Further investigation into the relationship between gut microbiota composition and Treg response revealed differences in Treg accumulation between aged mice in SPF and non-SPF facilities, suggesting a potential link between gut microbiota composition and Treg alterations during aging. In conclusion, aging affects Treg functionality in the gut mucosa, leading to altered suppressive capacity and accumulation of DNA damage and oxidative stress, potentially contributing to immune dysregulation associated with aging.

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WS29 – T CELL IMMUNITY IN CANCER

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815 – WS29.1

Highly multiplexed and 3D microscopy of the bone marrow microenvironment reveals rapidly expanding immature vasculature and PD-1+CD8+ T cell niches driving leukemic progression

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Purpose: The bone marrow microenvironment (BMME) critically regulates hematopoiesis. In leukemias, the BMME is remodeled towards a pro-leukemogenic niche, and the interplay of leukemic cells with their niche is critical for leukemia initiation, disease progression, and immune evasion. Therefore, an in-depth characterization of the BMME in 3D and at spatially resolved single-cell resolution will improve our understanding of leukemic progression and antitumoral immunity.
Methods: Femoral bones from wild-type and chronic myeloid leukemia mice 7, 14 and 21 days after leukemia induction were analyzed using 54-marker CODEX highly multiplexed microscopy and full-thickness 3D light-sheet microscopy.
Results: A total of 2,033,725 cells from 3 mice per group were imaged by CODEX. Using unsupervised clustering and manual annotation, we identified 41 cell types, including myeloid, erythroid, lymphoid, vascular and stromal cells, megakaryocytes, hematopoietic stem/progenitor cells (HSPCs), and leukemic subsets. During leukemic progression, the BMME was dramatically rearranged. Besides the expected expansion of leukemic cells, we found massive increases in immature vessels and megakaryocytes, whereas B cells were significantly reduced. CD8+ T cells shifted towards an immunosuppressive phenotype and upregulated PD-1 as disease advanced. Furthermore, in advanced-stage CML, PD-L1 was not only expressed by leukemic cells but also by non-malignant antigen-presenting cells (APCs), such as macrophages and dendritic cells. Neighborhood analysis revealed 14 BMME niches, and PD-1+CD8+ T cells were mostly enriched in the leukemic niche. CD34+CD117- HSPCs were predominantly located in bone and vascular niches, whereas CD34+CD117+ HSPCs were significantly enriched in the leukemic niche. Compared to wild-type mice, PD-1+CD8+ T cells were in close contact with PD-L1+ APCs in advanced leukemia. Moreover, during disease progression, PD-L1+ leukemic cells increasingly interacted with PD-1+CD8+ T cells. Leukemic stem cells were localized closer to megakaryocytes in advanced compared to early leukemia. In addition, 3D light-sheet microscopy revealed intimate cell-cell contacts between immature vessels and megakaryocytes, which increased in advanced leukemia.
Conclusion: We created a spatiotemporal landscape of the BMME during leukemic progression at an unprecedented resolution. We demonstrate that leukemic cells remodel the BMME to form specialized niches supporting their expansion and counteracting antitumoral immunity. Our findings could pave the way for novel leukemia immunotherapies.

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639 – WS29.2

Mapping T cell signatures associated to response to neoadjuvant chemotherapy in colorectal liver metastasis.

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Colorectal cancer ranks among the most prevalent malignancies worldwide, and frequently progresses into colorectal cancer liver metastasis (CRLM), resulting in a 5-year survival rate ranging from 30 to 50%. For those patients, partial hepatectomy is the therapeutic approach with the highest success rate, but the eligibility for surgery is restricted to merely 20% of patients. Neoadjuvant chemotherapy (NAC) is used as standard of care to facilitate the feasibility of curative surgery, as it can convert up to 30% of initially unresectable CRLMs into resectable metastases. However, the underlying reasons of why some patients fail to benefit from neoadjuvant treatment are currently unknown. The success of immunotherapeutic interventions hinges upon the presence and functional activity of infiltrating lymphocytes, which is tightly governed by the metastatic microenvironment. In this study, we collected CRLM samples from patients treated with NAC and studied the unicity of three spatially distinct patient-matched regions: the metastatic nodule, the invasive margin, and the distal liver tissue. Using high dimensional flow cytometry, we revealed distinct immune landscapes characteristic of each region. As such, we observed a scarcity of effector lymphocytes in the metastatic nodule, concomitant with an enrichment of exhausted, regulatory and memory T cells. Subsequent correlations of these immune features with NAC response revealed that patients with a high tumor burden and low fibrosis exhibited a more suppressive/exhausted lymphocytic landscape. Conversely, metastases that regressed in response to NAC were highly infiltrated by unique subsets of memory T cells. The integration of whole transcriptome analysis with proteomics revealed distinct signatures for these memory T cell subsets, which predicted prognosis in publicly available data sets for metastatic CRC. Our findings provide valuable insights into the biology of CRLM and unveil immune signatures associated with response to NAC. Based on this study, we suggest new therapeutic strategies to enhance NAC efficacy in CRLM management.

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180 – WS29.3

Beyond Helpers: Unraveling the Potential of CD4+ Cytotoxic T Cells in B cell lymphoma

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$CD4^+$ T cells exhibit remarkable heterogeneity and consist of several subsets. While most $CD4^+$ T cells exhibit either helper or immunoregulatory function, emerging evidence points to the existence of a unique subset of $CD4^+$ T cells with direct cytotoxic potential. Recently, there has been growing interest in these $CD4^+$ cytotoxic T lymphocytes (CTLs) due to their potential involvement in anti-tumor immune responses and their relevance in immunotherapy.
In a recent study, we employed cutting-edge single-cell RNA sequencing (scRNAseq) and T-cell receptor (TCR) sequencing to explore the landscape of $CD4^+$ T cells in lymphoma. We included diagnostic biopsies of diffuse large B-cell lymphoma (DLBCL, n = 3) and follicular lymphoma (FL, n = 3), alongside non-malignant tonsils (n = 3). Among the $CD4^+$ T cell clusters, one was characterized by high expression of a cytotoxic gene signature (*GZMK*, *NKG7*, *CST7*, *GZMA*, *GZMB*) and high expression of checkpoint receptors (*LAG3*, *HAVCR2*, *TNFSF9*). The $CD4^+$ CTLs accounted for over 20% of $CD4^+$ T cells within DLBCL and over 10% in FL tumors. Strikingly, the $CD4^+$ CTLs were almost absent in non-malignant tonsils. Their tumor-specific nature was further highlighted by TCR clonality analysis as $CD4^+$ CTLs had the highest clonal expansion among $CD4^+$ T cell subsets.
We further validated the presence of $CD4^+$ CTLs within an independent series of DLBCL tumors using flow cytometry by identifying $CD4^+$ CTLs as Granzyme B^+ Perforin$^+$ cells within the $CD4^+CD8^-FOXP3^-CD56^-$ population, excluding cytotoxic $CD8^+$, regulatory T cells and NK cells. $CD4^+$ CTLs ranged from 1-23% of intratumoral $CD4^+$ T cells in DLBCL and consistently with the scRNAseq findings, these cells were absent in non-malignant tonsils. More extensive phenotyping of intratumoral $CD4^+$ CTLs by spectral flow cytometry and functional assessments are currently ongoing.
In conclusion, by employing state-of-the-art technologies we have characterized $CD4^+$ CTLs in lymphoma and demonstrated substantial clonal expansion in DLBCL. Our findings might have implications for therapeutic strategies that leverage the yet untapped potential of this $CD4^+$ T cell subset in cancer immunotherapy.

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2049 – WS29.4

Distinct spatiotemporal dynamics drive CD8+ T cell fate decision in tumor microenvironment

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The immune system plays a critical role in fighting cancer initiation and progression. Tumor infiltrating lymphocytes (TILs), indeed, are essential components of the tumor microenvironment and play a critical role in immunotherapy responses. However, there are still a lot of open questions about TIL heterogeneity and effector functions before and after immunotherapy treatment. Understanding lineage relationships between naïve, effectors, memory and exhausted T cell subsets, and the underlying molecular pathways that regulate gene expression programs during the transitions between these distinct states, is essential for the rational design of novel vaccines and the development of new immunotherapy protocols.
In this study, we have examined $CD8^+$ T cell heterogeneity during different stages of cancer progression, by developing an integrative approach based on the combined single cell (sc) multi-omics analysis of surface markers at protein level (scCITE-seq), gene expression and abTCR (scRNA-seq) profiles, in both highly and poorly immunogenic mouse tumour models *in vivo*. The results highlight a complex TIL heterogeneity, with the identification of new neoantigen-specific $CD8^+$ T subsets, among which, $PD1^{low}$ cycling, $PD1^{high}$ exhausted and tissue resident memory (T_{RM}) $CD8^+$ T cells. We also found that both the immunogenicity and abundance of cancer neoepitopes have an impact on the transcriptional programs associated with tumour rejection. Of note, the expression of these new intra-tumoral gene expression signatures correlates with the progression free survival in solid tumour patients.
Taken together these results highlight new inter-clonal relationships between different $CD8^+$ T subsets in tumours, with distinct self-renewal and functional properties when comparing poorly vs highly immunogenic tumours, as well as during the different phases of cancer progression.

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128 – WS29.5

Tissue resident T cell responses to immunotherapies at the patient derived tumor-lung tissue interface

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Purpose: A high infiltration of $CD39^+/CD103^+/CD8^+$ resident T cells in solid tumors is associated with improved response to immune checkpoint therapy. CD39 is an Ecto-ATPase, which contributes to an enzymatic cascade converting eATP into adenosine, thus shaping an immunosuppressive tumor microenvironment (TME). To counteract this, therapeutic anti-CD39 antibodies are being tested to drive the TME into an inflammatory state. We aim to evaluate the effectiveness of anti-CD39 and anti-PD-1 on lung cancer by analyzing patient-specific immune responses using precision-cut lung tissue slices.
Methods: Tumor far and tumor-front slices were prepared from human lung resections. Tumor-front tissue was distinguished from tumor far tissue by macroscopic and microscopic analysis. Tissue viability was assessed by metabolic activity using WST-1 assay and plasma membrane integrity by Lactate Dehydrogenase Release Assay with and without immune checkpoint treatment. After treatment with anti-CD39 (1 µg/mL) or the anti-PD1 antibody Nivolumab (50µM), we leveraged histological profiling, flow cytometry of T cell subsets, eATP levels and cytokine analysis in the culture supernatant to understand how anti-CD39 impacts immune regulation within TME after 30h *ex vivo*.
Results: Tumor slices contained significantly higher frequencies of $CD8^+/PD\text{-}1^+$ (1.6-fold) and $CD8^+/CD39^+/CD103^+$ (2.2-fold) cells. Treatment with anti-CD39 effectively inhibited CD39 activity, evidenced by a 4.3-fold increase in eATP levels in the culture supernatant of tumor-far tissue. In tumor tissue, 1 µg/mL anti-CD39 significantly increased activation of $CD8^+/CD103^+/CD39^+$ cells, denoted by an increase $CD107a^+$ expression (1.3-fold change in frequency). Additionally, $CD3^+$ T cells upregulated the early activation marker CD137 after treatment (1.43-fold) in two of four donors. As treatment reference, Nivolumab treated tumor tissue induced an increase in the activation marker CD107a on $CD8^+$ T cells (1.4-fold) in one of four donors. However, all donors displayed significantly higher total secretion of IFNγ (12-fold) and lowered secretion of TGFβ2 (1.5-fold) compared to the medium control.
Conclusion: Treatment of lung tumor tissue with anti-CD39 induces activation of T cells in and may specifically target the anti-tumor reactive $CD39^+/CD103^+/CD8^+$ T cells. Presently, we demonstrated the added value of viable patient-derived lung tumor tissue, providing data on the efficacy of immunotherapies in context of patient specific TMEs.

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1409 – WS29.6

Distinct immune resistance profiles of malignant subpopulations contribute to intra-tumoral heterogeneity of pancreatic cancer

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Purpose: Identification and characterization of hitherto unknown immune resistance genes that are actively expressed and exploited by pancreatic ductal adenocarcinomas (PDAC) to escape immune evasion and refract current immunotherapies.
Methods: To systematically identify immune resistance genes, we performed high-throughput RNA interference screens of tumor-T cell co-cultures on human primary PDAC cells alongside five other tumor entities. We explored the expression profiles of 235 validated genes by analysing publicly-available transcriptome data from primary PDAC patients. The degree of heterogeneity between tumor subjects and in comparison to control pancreases was investigated based on bulk data. Intra-tumoral heterogeneity was explored by merging and integrating single-cell RNA-seq datasets from 36 patients. Candidate genes were selected for functional in vitro testing in several primary PDAC cell lines. Murine cells were transduced with viral-based shRNA to generate the stable knockdown of promising candidates for in vivo testing and validation of the impact of immune resistance gene expression on tumor progression and response towards peptide-based nano-vaccination in syngeneic pancreatic cancer mouse model.
Results: We obtained subpopulations of malignant ductal cells reflecting distinct biological states and characterized by a differential co-expression of immune-resistance genes driven by upstream transcription factors. Candidates selected for deeper functional analyses proved to protect against the immune rejection mediated by tumor-specific cytotoxic T lymphocytes. Tested genes mediate tumor-intrinsic resistance to T cell-derived cytotoxic molecules such as TRAIL, FasL and TNFa with differential impacts on distinct downstream death-receptor signaling pathways. Downregulation of some genes resulted in elevated death receptor expression on tumor cells sensitizing them towards immune rejection. Murine PDAC cells with the stable knockdown of three candidates were tested in a KPC orthotopic mouse model. In vivo experiments proved that immune resistance gene expression promotes tumor progression in immune-competent mice. Combination of gene inhibition with peptide-based PDAC nano-vaccine improved the therapeutic efficacy over monotherapies. The tumor micro-environment of mice that received the combination therapy showed an induced T cell activation as well as cytokine secretion.
Conclusion: In this work, we have identified and validated novel therapeutic targets for inhibition in PDAC for an enhanced tumor-sensitivity towards cytotoxic immune responses.

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WS30 – NOVEL THERAPEUTICS FOR AUTOIMMUNE DISEASES

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2115 – WS30.1

Engineered nanobodies as tools to visualize and direct antigen specific tolerance

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Autoimmune disorders affect approximately 10% of the global population, presenting symptoms ranging from mild to life-threatening. Current treatments largely involve broad immunosuppression, which can leave patients vulnerable to infections. We introduce an immunomodulatory strategy that targets the immune response to specific antigens while preserving overall immune functionality.

Our work centers on developing single-domain antibody fragments (nanobodies or VHHs) derived from alpacas. These nanobodies specifically target major histocompatibility complex class II (MHCII), allowing them to interact with all professional antigen-presenting cells (APCs). We developed a method for site-specific modification of these VHHs at their C-terminus using various chemo-enzymatic techniques, enabling the attachment of a broad spectrum of autoantigens, including citrullinated peptides, and anti-inflammatory drugs, e.g., dexamethasone (DEX). We demonstrated that a single dose of a VHH_{MHCII} conjugated to myelin oligodendrocyte glycoprotein (MOG) peptides and DEX (VHH_{MHCII}-MOG-DEX) provides long-lasting protection against experimental autoimmune encephalitis (EAE), a model for multiple sclerosis (MS), and can reverse paralysis in symptomatic mice without compromising their immunity against pathogens. This approach can be adapted for other autoimmune diseases like type I diabetes and rheumatoid arthritis by simply changing the autoantigens. This strategy is also applicable in suppressing unwanted immune responses against viral gene therapy vectors, permitting gene therapy redosing. Remarkably, all these benefits are achieved with a single dose.

To explore the mechanisms behind inducing antigen-specific tolerance, we employed comprehensive flow cytometry and transcriptomic analyses, which indicated an increase in antigen-specific regulatory T cells and evidence of bystander immune suppression mechanism. Additionally, we utilized positron emission tomography (PET)-based imaging to non-invasively monitor the biodistribution and tolerogenic activity of our engineered nanobodies in real-time. Furthermore, we developed anti-idiotypic nanobodies that recognize specific T-cell receptors (TCRs), i.e., 2D2 TCR, which interacts with the I-$A^b$$MOG_{35-55}$ complex. These nanobodies were used as imaging agents in immuno-PET to detect infiltrating T cells in the spinal cord of symptomatic EAE mice, helping us trace the efficacy of our antigen-specific tolerance approach. In conclusion, our engineered nanobodies offer a promising and versatile tool for both the specific modulation and real-time tracking of immune responses in autoimmune diseases, providing targeted treatments and future diagnostic avenues.

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1988 – WS30.2

Anti-citrullinated histone antibody CIT-013: Targeting neutrophil extracellular traps as an anti-inflammatory therapy in hidradenitis suppurativa.

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Purpose: Neutrophils are scarce in healthy skin but infiltrate lesions of Hidradenitis Suppurativa (HS) patients. Activated neutrophils release neutrophil extracellular traps (NETs) which contribute to the pathophysiology of many immune-mediated inflammatory diseases, including HS. CIT-013 is a first-in-class monoclonal antibody that targets the citrullinated histones H2A and H4, specifically present in NETs. CIT-013 has a unique NET-targeting dual MoA, suppressing the proinflammatory properties of NETs, and shows therapeutic efficacy in several pre-clinical models of neutrophil associated inflammation. In this study we validate HS as therapeutic target for CIT-013 by demonstrating the presence of proinflammatory NETs in lesional and perilesional biopsies and by showing a relationship between serum NETs and increasing HS clinical score.
Methods: The possible presence of the NET component citrullinated histone H3 (citH3) was assessed in HS lesional, perilesional and unaffected skin by immunohistochemistry. Several markers of NETs (CIT-013's epitope, citH3, calprotectin, and nucleosome content) were assessed in HS serum with ELISA.
Results: The presence of the NET component citH3 is significantly increased across several layers of HS skin lesional and perilesional regions compared to unaffected skin. Furthermore, CIT-013's epitope, citH3, calprotectin, and nucleosome content were significantly elevated in the serum of HS patients compared to healthy volunteers. Detection of these markers for NETs in serum increased in association with increasing International Hidradenitis Suppurativa Severity (IHS4) clinical score.
Conclusions: NETs are abundantly present in inflamed HS skin lesions. Moreover, markers of pro-inflammatory NETs are significantly elevated in HS serum in a manner that associates with increasing HS clinical score. This data reinforces the position of CIT-013 as a therapeutic approach for NET-associated diseases with unmet therapeutic needs, such as HS. CIT-013 will enter a phase 2 proof-of-concept trial in HS during 2024.

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1616 – WS30.3

Elimination of CD45RChigh T and B cells by anti-CD45RC mAb lead to efficient control of experimental rheumatoid arthritis

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Purpose: RA is a chronic relapsing/remitting disease characterized by synovitis, joint deformity, loss of function and increased mortality involving T and B cells. We demonstrated that administration of an anti-CD45RC mAb targeting B cells and a subset of T cells (Th1 and their precursor and TEMRA cells) leads to prevention or control in preclinical models of transplant rejection, GvHD, Duchene dystrophy or APECED. In this study, we deciphered the mechanism of action of the anti-human CD45RC mAb and in vitro and in vivo and potential in an experimental model of rheumatoid arthritis.
Methods: Cells were isolated from peripheral blood. Apoptosis, ADCC, ADCP and CDC were assessed by Annexin V/DAPI staining and cell count. In vivo studies were performed in CD34+-humanized NSG mice, in a GVHD model in humanized NSG mice or in a model of collagen-induced arthritis (CIA) in rat.
Results: We demonstrated that the humanized anti-human CD45RC mAb induced CD45RC^{high} T and B cell death mainly by direct apoptosis, inducing signaling but not cytokine release and ADCP. Using in vivo studies, we showed that a single iv administration of anti-CD45RC mAb induced an efficient killing of CD45RC^{high} T and B cells as soon as day 1, this effect was dose and time-dependent and targeted cells recovered by day 24. We also showed a dose dependent efficacy in a model of GVHD. Treatment with an anti-rat CD45RC mAb in a rat model of CIA efficiently prevented weight loss, mean arthritis severity, maximum score and AUC, in contrast to control mAb. Anti-CD45RC mAb completely inhibited anti-collagen antibody production, inflammation of the paws and GM-CSF secretion in the sera. Efficacy of the anti-CD45RC mAb correlated with depletion of CD45RC^{high} T and B cells by d3 and until sacrifice with conserved Treg numbers. Analysis of RA patients showed in the blood presence of CD45RC^{high} cells and a strong infiltration by CD45RC^{high} cells of synovial tissues using IHC.
Conclusion: Our study demonstrates that anti-CD45RC mAb treatment is a potent immunomodulatory agent rebalancing the Treg/teff ratio to reduce joint inflammation and disease activity in RA and a potential alternative for first line DMARD.

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493 – WS30.4

RP23: a novel peptide-based therapeutic for inflammatory skin diseases

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Inflammatory skin diseases such as psoriasis and atopic dermatitis represent a significant and ever-increasing health burden globally. Conventional steroid therapies are ill-suited for long-term continuous usage as they are accompanied by adverse effects. While biologics such as monoclonal antibodies are highly effective in severe disease, they are not prescribed in the vast majority of mild-moderate cases and are largely inaccessible due to their high cost.
RP23 is a modified human self-peptide with novel anti-inflammatory properties. Topical formulations of this peptide effectively treat both imiquimod-induced psoriasis and oxazolone-induced atopic dermatitis models in mice. Unlike steroids which have systemic effects, RP23 only suppresses local immune responses where it is needed. Importantly, RP23 is well tolerated by mice with no adverse events unlike steroids which reduced body weight and caused skin atrophy. Using fluorophore conjugated RP23, we determined that the peptide is primarily taken up by neutrophils, macrophages, and dendritic cells. Confocal microscopy of murine bone marrow-derived macrophages (BMDM) also demonstrated that RP23 was readily taken up by these cells and co-localised with lysosomes, consistent with phagocytosis. *In-vitro* BMDM assays, as well as studies in human monocyte-derived macrophages, demonstrated that RP23 reduced IL-6 and IL-12p40 release, which likely explains its mechanism of action in these diseases.
Thus, RP23 represents a promising new therapeutic for inflammatory skin diseases. It enjoys many clinical advantages over existing therapeutics, including being a cheaper, convenient, safer, and potentially more effective treatment option for psoriasis and atopic dermatitis.

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1140 – WS30.5

Slamming the Brakes on Autoimmunity: Inhibition of the CD40:CD40L Pathway utilizing Fab Fragments and Nanobodies

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Purpose*:* Break of tolerance and production of autoantibodies by autoreactive B cells are central aspects in autoimmune diseases such as Systemic Lupus Erythematosus (SLE). Signaling through the CD40:CD40L pathway provides a pivotal co-stimulatory signal for the activation of auto-reactive B cells. We aim to inhibit the CD40-CD40L pathway to dampen the disease-causing reactions leading to symptoms in autoimmune diseases, specifically the B cell driven disease SLE. This therapeutic strategy has previously been attempted with antibodies with serious off-target effects. We aim to utilize Fab fragments (50 kDa) and nanobodies (15 kDa) to take advantage of their significantly smaller size (compared to the 150 kDa of IgG) and the possibilities for molecular manipulation of the constructs.
Methods: We have developed CD40L-targeting Fab fragments and nanobodies. To develop Fab fragments, we first developed 30 different monoclonal mouse anti-human CD40L antibodies. The antibodies were tested for reactivity to CD40L by immunoassays and flow cytometry, and the capacity for inhibiting the CD40-CD40L interaction was tested by flow cytometry. The best inhibitory candidates were redesigned as Fab fragments and tested for retained binding and inhibitory capacity in in vitro assays (TRIFMA, flow cytometry and coculture assays). We have also immunized an alpaca with human CD40L to produce anti-human CD40L nanobodies. Different nanobody candidates have been selected using yeast-surface display and phage-display. Selected nanobodies were tested for their ability to bind CD40L and to inhibit the CD40-CD40L interaction using TRIFMA and flow cytometry.
Results: The Fab fragment successfully inhibited the CD40-CD40L interaction in vitro, both preventing a soluble version of CD40 in binding to CD40L on the surface of stimulated T cells as well as in a B cell-coculture assay. We also identified nanobody candidates that demonstrate the ability to inhibit CD40-CD40L interaction.
Conclusion: In conclusion, nanobodies and Fab fragments represent a new potential treatment with definite advantages over monoclonal antibodies. The inhibitory Fab fragments and nanobodies will be tested for the ability to ameliorate autoreactive symptoms in vivo, possibly without the risk of Fc-mediated side effects. We believe this represents a new treatment strategy and expanded opportunities for patients with SLE and other autoimmune diseases.

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202 – WS30.6

An unbiased high-throughput screening identifies repurposing drugs and novel molecules that reduce proinflammatory cytokine secretion in the context of Rheumatoid Arthritis

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Rheumatoid Arthritis (RA) is an autoimmune chronic disease characterized by inflammation affecting multiple joints, ultimately resulting in deformity, pain and swelling. Available therapies target late stages of the inflammation process, when the microenvironment is highly inflammatory and therefore more challenging to control. Developing therapies targeting inflammation upstream (i.e. proinflammatory cytokine secretion) would benefit RA patients.
We have previously identified, through an unbiased high-throughput screening of small molecule chemical libraries, 27 molecules (23 novel and 4 repurposing drugs) with previously unknown anti-inflammatory capabilities.We validated their effects in PBMCs stimulated with different PAMPs/DAMPs, pharmacological concentrations and time points, and identified 14 molecules capable of reducing the secretion of TNF-α and/or IL-1β without affecting cell viability.
These molecules underwent characterization in PBMCs from healthy donors and treatment-naive RA patients. We observed that two molecules (#28 and #7, novel) reduce TNF-α secretion, another two (#1 and #15, novel) reduce IL-1β secretion, and six molecules (two of them repurposing drugs) reduce the secretion of both cytokines. Flow cytometry for lineage markers and cytokines allowed for the identification of the cell subtype(s) responsible of the decreased secretion. Molecules were prioritized based on reduction of cytokine secretion and number of donors who exhibited these effects, and proceeded to the determination of their mechanism of action (MoA) and in vivo analyses. For the novel molecules, a chemoproteomic approach is being used: compound #28, one of the best molecules, was derivatized to a fully functionalized fragment (FFF). After confirming that the FFF still maintained its anti-inflammatory properties, its MoA will be studied through chemoproteomics. In parallel, the prioritized molecules are being tested in the KxB/N serum transfer arthritis mouse model.
In summary, we developed an untargeted high-throughput drug discovery platform for the identification of novel and repurposing drugs capable of reducing the secretion of proinflammatory cytokines. The validation, characterization and prioritization steps have identified eight molecules with previously unknown immunomodulatory capabilities, which work in cells from RA patients. Therefore, our small molecule portfolio bears the potential to improve the treatment of RA, and potentially other inflammation-related diseases, by providing novel molecules capable of halting inflamamtion at early stages.

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WS31 – DIET, OBESITY AND IMMUNE MODULATION

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419 – WS31.1

Obesity-induced dysbiosis impairs type 2 immune responses

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Obesity has become a major global health concern, affecting millions of individuals directly or through associated comorbidities. Notably, obesity leads to significant changes in the cellular composition of adipose tissue and peripheral regions. These alterations subsequently influence the immune system, impacting the maintenance of adipose tissue balance. Moreover, the gut microbiome plays a crucial role in obesity and the regulation of the immune system. However, many aspects of these interactions remain poorly understood. In our study, we aimed to investigate how obesity alters the microbiome and its implications for type 2 immune responses, specifically focusing on tissue repair mechanisms.
To this end, we induced obesity in mice using a high fat diet (HFD) and analysed the microbiome by 16S rRNA sequencing. Additionally, we depleted the microbiome with broad-spectrum antibiotics before we infected the mice with the gastrointestinal helminth *Nippostrongylus brasiliensis*. Intestinal and pulmonary immune responses were assessed through flow cytometry, ELISA and qPCR.
Our longitudinal analysis of the microbiome revealed early and long-lasting changes in microbial composition induced by HFD, partly dependent on regulatory factors of the immune system. Immunopathology in the lung was increased during obesity, correlating with an exaggerated Th1-biased immune response and reduced markers for pro-resolving macrophages. Upon microbiome depletion with antibiotics, we observed significant differences between helminth-infected mice on HFD compared to those on a standard diet, both in the intestinal and pulmonary type 2 responses.
In summary, our findings demonstrate that obesity-induced dysbiosis affects type 2 immunity. Further studies will investigate the mechanisms affected by this dysbiosis, particularly those impairing wound healing during obesity thereby contributing to a deeper understanding of obesity-related comorbidities.

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1609 – WS31.2

Loss of adipose tissue protects mice from intestinal inflammation

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Background: Crohn's disease (CD) is associated with creeping fat, characterized by hyperplasia of mesenteric adipocytes and increased secretion of adipokines, including leptin. Leptin promotes intestinal inflammation by inducing pro-inflammatory cytokines such as TNFα in murine and human lymphocytes. Meta-analyses have shown that obesity increases the risk of developing CD. However, whether the surgical removal of mesenteric fat could improve the outcome of CD patients and the impact of fat-derived signals on intestinal autoimmunity remain elusive.
Method: To decipher the role of adipose tissue, we investigated here how the absence of adipose tissue affects epithelial barrier functions and immune cell homeostasis. Therefore, we studied lipoatrophic Ppargfl/fl-Adipoq-Cre mice, that completely lack adipose tissue, under steady-state conditions and after induction of chronic dextran sodium sulfate (DSS)-mediated inflammation, as well as in allogeneic fat transplantation models. We characterized our models using flow cytometry, RNA sequencing, and ELISA.
Results: At steady state, lipoatrophic mice showed a decrease in CD4 IL-17+ T cells in the ileum, and lower frequencies of CD8 T cells and CD4 FoxP3+ cells in the colon, suggesting that fat-derived signals are required for T cell homeostasis in mice. To understand how the absence of adipose tissue affects intestinal inflammation, we challenged lipoatrophic or WT littermates with 3 cycles of 1.5% DSS, which induced weight loss in both WT and lipoatrophic mice. However, only fat-proficient WT mice developed severe colitis, whereas lipoatrophic animals had significantly reduced intestinal inflammation and displayed disturbances in the differentiation and function of pro-inflammatory T cells including Th1 and Th17 cells. Importantly, these defects in T cell function could be rescued by allogeneic transplantation of adipose tissue from lean wild-type mice but not by adipose tissue from leptin-deficient ob/ob mice, highlighting that the role of the adiopokine leptin as an important pro-inflammatory factor in IBD.
Conclusion: Our data demonstrate that adipose tissue acts as an important regulator of intestinal autoimmunity by controlling the differentiation and function of pro-inflammatory T cells through the secretion of adipokines including leptin. We believe that these observations may help explain why obese individuals are at a higher risk of developing intestinal inflammation and CD.

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194 – WS31.3

Obesity-induced changes in perivascular adipose tissue fibroblasts in a mouse model of atherosclerosis

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Purpose: Atherosclerosis is a chronic inflammatory disease exacerbated by obesity. Using an atherosclerosis murine disease model, our objectives were to map the presence of immune and structural cells, to explore their potential interactions, and to investigate how obesity impacts them in different tissues.
Methods: We used ten Ldlr-/- Apob100/100 mice: five non-obese mice with a standard chow diet and five obese mice with a high-fat diet. We collected perivascular adipose tissue (PVAT), aorta, spleen, and epididymal white adipose tissue (eWAT) from each mouse, creating eight samples pooled by the tissue types. Following the tissue dissociation and enrichment of CD45+ cells from aorta samples, single-cell RNA-sequencing was performed using a 5' gene expression immune profiling protocol, combining it with a panel of 138 antibodies, resulting in a multimodal data of ~ 46k cells. For spatial validation we used immunohistochemistry and immunofluorescence in an independent cohort of similar mice.
Results: In addition to identifying various immune cell populations and their subtypes, we observed marked diversity in structural cells, with particularly notable gene expression changes in fibroblasts between the non-obese and obese states. The most substantial changes were evident in the aorta, PVAT and eWAT. PVAT- and eWAT-derived fibroblast populations were especially interesting as a gene set enrichment analysis, based on the genes that were differentially expressed between the obesity states, revealed multiple immune-related functions. We validated the location of one PVAT-derived fibroblast populations with immunohistochemistry/immunofluorescence and observed a difference in their abundance between the obesity states: these fibroblasts were more abundant in average in the non-obese mice in comparison with the obese mice. This could be due to morphological changes in the PVAT towards a white adipose tissue phenotype during obesity evident in a morphometric analysis.
Conclusion: Our findings provide novel insights into obesity-related alterations in adipose tissue-derived fibroblasts during atherosclerosis and their potential immune-related responses during the disease. Our results emphasize the significance of PVAT in atherosclerosis during obesity and how obesity might abolish the functionality of specific fibroblasts that could otherwise support normal immune responses during the disease.

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1736 – WS31.4

Structural variations and developmental characteristics of adipose-associated serosal lymphoid tissues in mice

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The peritoneal cavity in mice represents a separate lymphoid compartment with unique cellular composition and leukocyte homeostasis, dispersed into fluid and adipose-associated lymphoid congregates. In our work we studied the structural pattern, stromal organization and developmental features of adipose-associated lymphoid structures capable of binding normal as well as malignant B cells.

Using high-grade mouse B-cell lymphoma lines A20 (centroblastic DLBCL) and Bc.DLFL1 (extrafollicular DLBCL) we found that, in addition to the mesenteric fat-associated lymphoid congregates (FALC) and omental milky spots (MS) with subserosal diffuse T/B cell distribution, both lymphoma cell lines accumulated within a more compacted formation we termed foliate lymphoid aggregates (FLAgs), owing to their lymphocyte-filled body and slender stalks. The FLAg body part was arranged around reticular fibroblasts expressing VCAM, ICAM and FAP, embedded in extracellular matrix containing ER-TR7-positive Collagen type VI and fibronectin. Within FLAgs, T cells congregate in the central zone, surrounded by a B-cell-rich rim at the periphery, which pattern corresponds to the arrangement of CXCL13 and CCL21-positive chemokine domains, respectively. LYVE-1-positive macrophages sensitive to clodronate-mediated depletion mediate the binding of lymphoma cells to the FLAgs following intraperitoneal injection.

The developmental analysis of FLAgs demonstrated that these structures develop in the postnatal period. No obvious leukocyte cluster formation is observed in one-week-old postnatal mice, whereas by the second week, CD45-positive leukocytes aggregate at the bursal membrane and omentum, forming MS/FALCs at both locations. Subsequently, mature FLAgs occur. The onset of leukocyte clustering in the mesenteric adipose streaks follows a delayed appearance compared to other sites. The number of FLAgs and MS/FALCs becomes stable by week six under normal conditions.

FLAg development is absent both in RAG KO and nonobese Scid-gamma chain mutant (NSG) mice, whereas LTa deficient mice contain only small MS/FALCs; in contrast, mice with nude mutation or lacking RORgt the entire adipose-associated lymphoid tissue spectrum is present.

These observations indicate that the serosal adipose tissues in mice harbor various lymphoid structures with complex developmental determinants, and these sites contribute to the lymphoid recirculation for both normal and malignant B cells.

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188 – WS31.5

Obesity and sex differences: An overlooked reality in preclinical studies

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Obesity is a major worldwide health problem that increases the risk of developing neurodegenerative diseases including multiple sclerosis (MS) and Alzheimer's disease (AD). Current preclinical studies often only look at the effect of diet-induced obesity (DIO) in male mice as their weight gain is stable and fast. However, the risk for developing MS or AD is higher in women with obesity. Given the inflammatory changes induced by obesity, and the importance of the immune system in MS and AD, sex differences in immunological changes due to obesity should be taken into account.
Therefore, we investigated sex differences in a DIO model focusing on changes within the adaptive immune system in visceral and subcutaneous adipose tissue (vAT and scAT). We induced obesity in male and female ASC-citrine-C57BL/6J mice by feeding a high fat diet (HFD), and compared adipose tissue (AT) inflammation at the same adiposity stages (% AT/bodyweight) between both sexes. In vAT, the absolute number of T helper cells (Th, CD3+CD4+) increased sooner in females related to similar adiposity development. The same was observed for absolute numbers of the highly pro-inflammatory subsets Th17 (CCR6+) and Th17.1 (CCR6+CXCR3+). However, the effect of HFD is diminished with higher adiposity in female mice. Interestingly, only female mice showed an increase in immune cells in the subcutaneous AT after HFD. Additionally, uniquely in male mice, myeloid cells in the visceral AT showed a higher inflammasome activation upon HFD.
In summary, we showed that adiposity differently regulates immune cells in fat depots based on sex. Future experiments are warranted to assess how this affects neuroinflammation in a sex-dependent manner.

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1209 – WS31.6

High fat low carbohydrate is linked to protection against the development of CNS autoimmunity

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Multiple sclerosis (MS) is an autoimmune disease, characterised by axon demyelination, clinically leading to paralysis. The exact aetiology is still unknown but both genetic and environmental factors are involved, with the latter being the major contributors. Diets, particularly western-like diets, are emerging player in MS. However, a comprehensive understanding towards the underlying mechanisms involved is lacking.
By using state-of-the-art nutritional geometry analytic methods, we first investigated globally the association between nutrient exposure via food environments and MS disease burden. Here, increased carbohydrate supply was associated with increased MS disease burden, while fat supply had an opposite effect. Furthermore, in a preclinical MS model, experimental autoimmune encephalomyelitis (EAE), we found that an isocaloric diet high in carbohydrate aggravated disease severity, while a diet enriched in fat was fully protective. This protection was reflected by reduced neuroinflammation and skewing towards generation of anti-inflammatory regulatory T cells away from encephalitic Th1 and Th17 cell subsets. The beneficial effects of high fat feeding specifically involved transcriptomic, epigenetic and metabolic immune changes.
Together, we showcased that manipulating diets might be an efficient and cost-effective approach to ameliorate the development of EAE, potentially explaining the link between high fat low carbohydrate dietary environment and reduced MS disease burden.

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WS32 – DT CELL MEMORY IN HEALTH AND DISEASE

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982 – WS32.1

Epigenetic imprints in human T cells reveal DNA replication speed as a driver for memory differentiation

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Epigenetic mechanisms are known drivers of T lymphocyte development and function. This has been so far mainly attributed to epigenetic changes on gene regulatory elements like promoters and enhancers, which control the expression patterns of their target genes. In previous studies, we observed a surprising DNA methylation loss in large, heterochromatic parts of the genome in human T cells, which was a lasting epigenetic imprint resulting from proliferation episodes in the history of T cells. The molecular mechanisms and functional consequences of this heterochromatic DNA demethylation so far remained unclarified.
In human T cell cultures, we were now able to show that the DNA replication speed during proliferation events was the driving force for heterochromatic DNA methylation loss and that maintenance of the DNA methylation profile could be achieved by interfering with DNA replication timing. Interestingly, we observed that increased loss of heterochromatic DNA methylation levels was also a marker for TCM populations prone to undergo TEM differentiation, even in the absence of a TCR stimulus, indicating that past proliferation episodes predispose TCMs for TEM differentiation. Also, we were able to reduce the frequency of cells undergoing TEM differentiation by modulation of the DNA replication speed, indicating that the proliferation speed of T cells is causally involved in driving memory differentiation. Finally, our preliminary data suggest that this newly discovered mechanism might be exploited to improve therapeutic T cell products, as reducing proliferation-induced DNA methylation loss during the manufacturing process improved functionality of immunosuppressive regulatory T cell products.

With these data, we reveal that the proliferation behavior of T cells is monitored and recorded in the epigenome and has a causal impact on the future differentiation and functional potential of T cells.

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2248 – WS32.2

Iron deficiency and acquired immunity: novel perspectives on the effects of iron restriction on immunological memory and protective immune responses

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Immunological memory is a fundamental feature of the adaptive immune system. It allows the immune system to remember previous encounters with pathogens or antigens, which results in a faster and more effective secondary immune response. This response is facilitated by long-lived memory cells and helps manage re-infections and establish a protective state for the organism. Optimal immune responses require adequate levels of bioavailable iron, especially when T cells switch from a quiescent (catabolic) to an activated (anabolic) state for clonal expansion. However, it is not well understood how the adaptive immunity functions in the absence of adequate supply of iron. This study examines the impact of iron deficiency on CD8 T cell responses during influenza infection using *in vitro* and *in vivo* models where iron availability is restricted through genetic modifications or pharmacological inhibition of ubiquitous iron transporter called Transferrin Receptor 1 (TfR1).

Paradoxically, our *in-vivo* models showed an unexpected increase in the generation of antigen-specific ($NP_{366+}CD44^{+}CD62L^{-}$) CD8 T_{EF} cells following the primary immune response and greater survival of antigen-specific ($NP_{366+}CD44^{+}CD62L^{+}$) CD8 T_{CM} cells up to 95 days post-infection in Iron deficient mice. The primary CD8 T cell response also appeared to be delayed rather than impaired in our genetic models, suggesting that the severity of iron restriction is negatively correlated with the speed of the CD8 T cell response. Lastly, Iron-deficient CD8 T cells were more predisposed towards a memory fate over an effector fate, and this was not attributable to defects in cellular activation or apoptosis resistance during the immune contraction phase. The ongoing research aims to determine whether this increased generation and maintenance of memory CD8 T cells confer enhanced protection during a heterotypic PR8 challenge and to elucidate the mechanisms responsible for this augmented memory formation.

In conclusion, our findings contest the prevailing belief that iron deficiency necessarily impairs the primary immune response. Instead, they introduce a nuanced perspective wherein the detrimental effects of iron deficiency might exist along a continuum. Notably, there appears to be a threshold at which iron deficiency could enhance the establishment of the antigen-specific memory pool within the organism.

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1115 – WS32.3

Leveraging the strategic positioning of central memory CD8+ T cells in steady state lymph nodes for enhanced secondary recall

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Purpose: The next generation of vaccines against infectious disease, chronic infection and cancer will require robust T cell memory. Currently, vaccines that consistently induce robust and durable $CD8^+$ T cell memory remain elusive. There is increasing evidence that memory $CD8^+$ T cells are strategically positioned to enhance recall capacity. Therefore, defining the niche for central memory $CD8^+$ T cells (T_{CM}) and revealing the factors that regulate this location may provide novel insights to harness T cell memory for improved vaccine outcomes.
Methods: In this study, we combined tissue clearing and 3D-lightsheet fluorescence microscopy of intact murine lymph nodes (LNs) to determine the location of T_{CM} following the resolution of acute infection models. To identify the cellular interactions that occur within the LN memory niche, we employed high-resolution confocal microscopy, along with spatial photo-labelling, scRNA sequencing and network analysis to reveal the environmental factors that underpin T_{CM} location and longevity.
Results: In steady-state LNs, T_{CM} cells occupy a conserved niche distinct from naïve T cells. T_{CM} were quantified at a higher density in the cortical ridge and interfollicular regions than naïve T cells, which primarily reside in the paracortex. The T_{CM} niche was consistent across multiple diverse infection models, including LCMV, HSV-1 and *Listeria*. We identified a subset of dendritic cells (DCs) which specifically interact with T_{CM} cells in this niche and maintain their long-term location. Furthermore, specifically targeting this DC subset can influence the immune response to re-challenge.
Conclusion: This study lays the foundation to improve vaccine and boosting strategies grounded in robust and long-lived T cell memory.

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553 – WS32.4

Staphylococcus aureus colonisation expands respiratory tissue-resident memory CD4+ T-cells, with the potential for heterologous immunity.

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Purpose: While *Staphylococcus aureus* (*S. aureus)* is primarily recognized as an invasive pathogen, it is also known to persistently colonise the anterior nares of a significant proportion of healthy individuals. CD4+ T-cells play an important role in controlling *S. aureus* colonisation, however the impact of *S. aureus* colonisation on shaping local T-cell populations in the nasal mucosa is poorly defined.
Methods: To investigate local tissue resident immune cell development and memory responses, we used mouse models of *S. aureus* nasal colonisation. Analysis of cell functional and phenotypic changes was performed using flow cytometry.
Results: We identified CD4+ Tissue Resident Memory (TRM) T-cells in the *S. aureus*-colonised nasal tissue, which remain primed at the tissue site for rapid reactivation upon secondary exposure to *S. aureus.* Intra-nasal administration of LPS or *Klebsiella pneumonia* (*K. pneumoniae*) to *S. aureus*-colonised mice also activated nasal tissue IL-17+ CD4+ TRM cells, suggesting *S. aureus* specific CD4+ TRM cells have the capacity for bystander activation, which is driven by Th17 cell polarizing cytokines and not achieved by *K. pneumonia* antigen presentation alone. A model of persistent colonisation was developed in a population of germ-free mice, allowing us to characterize the impact of colonising *S. aureus* on nasal tissue CD4+ TRM cell responses in mice stably colonised with *S. aureus* from birth. *S. aureus* mono-colonised mice challenged with *K. pneumoniae* exhibited lower bacterial burden in the lungs than germ-free controls, as well as increased IL-17 production from nasal tissue CD4+ TRM cells, indicating enhanced protection against a novel invasive respiratory pathogen mediated by the TRM cells.
Conclusion: Our findings demonstrate that nasal colonisation with *S. aureus* can prime and expand local CD4+ TRM cells, with the potential for bystander activation to enhance heterologous immunity to an unrelated respiratory pathogen.

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1464 – WS32.5

A diverse pool of oral mucosa-resident memory T cells protects against viral infection at the site of entry

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Purpose: A reliable, long-lasting protection against viral infections including SARS-CoV-2 is particularly based on the generation of memory T cell populations within the affected tissues. As one of the first sites of contact with infectious aerosols, the assessment of specific T cell subpopulations in the oral mucosa is of utmost interest for the evaluation of adaptive cellular immune responses to vaccinations or natural infections.
Methods: We performed flow cytometry analysis and single cell RNA sequencing via the 10X-Genomics protocol on blood and oral mucosa samples from healthy, SARS-CoV2 recovered individuals one month after infection. Three donors were vaccinated against *Yellow-Fever-Virus* prior to sampling. Cell types were annotated based on widely accepted marker genes and Python package CellTypist. Differential gene expression and TCR receptor analysis were performed using the Python toolkits SCANPY and SCIRPY.
Results: We found that the majority of SARS-CoV2-specific T cells in blood samples were central memory T helper cells (TCM) and, in oral mucosa samples, cytotoxic tissue-resident memory T cells (TRM) and type-1 helper T cells. While SARS-CoV2-specific mucosa T cells had a balanced ratio of Type-1 helper cells and cytotoxic TRM cells, *Epstein-Barr virus*- and *Yellow-Fever-virus*-specific cells consisted predominantly of cytotoxic TRM cells. The differential gene expression between SARS-CoV2-specific T cells and other virus-specific T cells enabled the distinction between gene programs involved in early tissue residency, circulating virus-specific cells and genes expressed by long-lived mucosa-resident cells. Cell-cell communication demonstrated a strong incoming signaling for TRM cells and a key role for fibroblasts in outgoing signaling.
Conclusion: Our data provide valuable insights into the distribution of T cell subpopulations and their respective TCR-specificity in healthy oral mucosa shortly after COVID-19 infection. The differential gene expression and cell population distribution enabled detailed insights into the function, functional capacity and longevity of the different virus-specific T cell subpopulations. In addition, communicative networks led by oral mucosa fibroblasts supported TRM survival within the tissue. This ongoing project may contribute to further understanding of T cell responses at effector sites following viral infection and vaccination.

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743 – WS32.6

A tissue-specific molecular program governs the formation and function of brain-resident CD8+ T cells

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T cells populate the brain at steady state, where they contribute to its physiology and protect the host against reinfection. T cells residing in peripheral non-lymphoid tissues are known to adopt tissue-specific transcriptional programs shaped by the tissue microenvironment. Whether brain-resident T cells similarly acquire a tissue-specific transcriptional landscape, and to what extent this molecular signature is altered in neuropathology, remains to be determined. Here we unravel the cellular and molecular heterogeneity of brain T cells in mice using single-cell RNA-sequencing and high-parameter flow cytometry. Specifically, we profile brain T cells under homeostatic conditions and in the contexts of cerebral amyloidosis, systemic viral infection, and aging. From these studies, a framework of a predominantly tissue-specific CD8+ T cell landscape has emerged, largely defined by the expression of the inhibitory receptor PD-1, the surface molecule Ly6C, and the transcription factor TCF-1. In spite of the transcriptional overlap across conditions, CD8+ T cells adopted context-specific phenotypic and functional properties. Interrogating the molecular determinants of brain CD8+ T cell differentiation and maintenance, we found that TCF-1 and PD-1 negatively regulate the population expansion of brain CD8+ T cells. In addition, PD-1 signaling was necessary for robust effector function and antigen-specific recall response. Furthermore, the cytokine transforming growth factor (TGF)-β was required for the differentiation of brain-resident CD8+ T cells. Taken together, these findings highlight common, tissue-specific as well as context-specific features of brain CD8+ T cells, and provide insights into the molecular mechanisms governing their formation and function.

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WS33 – VACCINES FOR BACTERIAL DISEASES

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1575 – WS33.1

Inducing tick immunity by vaccination as a novel strategy to prevent Lyme borreliosis

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Lyme borreliosis is a vector-borne disease caused by *Borrelia burgdorferi* sensu lato (Bbsl) bacteria transmitted by *Ixodes* ticks. Over half a million new cases of Lyme borreliosis are diagnosed in the Northern hemisphere each year. Clinical manifestations include early and chronic skin infection as well as disseminated disease such as Lyme arthritis, neuroborreliosis, or carditis. Since there is currently no human vaccine available, we are investigating the innovative approach to prevent both Lyme borreliosis and other tick-borne diseases by targeting the tick vector, rather than targeting the pathogen itself as conventional vaccines do.
We identified and biologically validated 20 tick salivary gland genes that were consistently and abundantly expressed during early tick feeding and therefore likely to play a role in pathogen transmission. To assess their role in host-pathogen interactions and immunogenicity, 12 corresponding tick salivary gland proteins (TSGP) were produced recombinant in *Drosophila* and insect cell expression systems. TSGPs significantly inhibited human complement mediated killing of Bbsl and monocyte-derived dendritic cell activation by reducing the expression of co-stimulatory markers CD40 and CD86 as well as TNF-α and IL-6 release *in vitro*. Mice immunized with TSGPs and challenged with Bbsl-infected ticks showed no skin infections and Bbsl qPCR loads in deeper tissues such as the bladder and heart were significantly lower compared to mice receiving PBS adjuvants. These results indicate partial protection against Lyme borreliosis by an anti-tick vaccine in experimental models.
To investigate tick immunity in humans, we set up a unique human tick-challenge model in which eight human volunteers were challenged three to four times in two-week intervals with 10 pathogen free nymphal ticks per challenge, placed on the forearm and contained in a plastic eyepatch. Local skin reactions, including redness and itch significantly increased whereas tick-feeding parameters decreased after each challenge. IgG titers against TSGPs in serum were detected up till eight weeks after the last challenge. Altogether, this might indicate the development of tick immunity in humans and provide preliminary results for the use of TSGPs for an anti-tick vaccine to prevent Lyme borreliosis.

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1395 – WS33.2

Immunogenicity and efficacy of vaccination with BCGΔBCG1419c and H1-TT against tuberculosis in mice

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Purpose: A vaccine to prevent tuberculosis is urgently needed. In our group, we have developed and characterized two vaccine candidates with potential to replace and/or boost immunity against mycobacteria. In this work, we studied a combined vaccination scheme composed by BCGΔBCG1419c, a modified BCG that afford protection against tuberculosis in mice and guinea pigs, and a booster with the H1-TT subunit vaccine formulation, which has shown to induce protection against mycobacteria by inducing antibodies against EsxG/EsxH proteins in mice. We proposed that this scheme could induce cellular and humoral response resulting in better protection against tuberculosis. The purpose of our studies was to evaluate both the immunogenicity and the efficacy of the vaccination with BCGΔBCG1419c + H1-TT against tuberculosis in mice.
Methods: To evaluate immunogenicity of the BCGΔBCG1419c + H1-TT scheme, we vaccinated BALB/c mice subcutaneously with BCGΔBCG1419c and then we boosted them with H1-TT intranasally. After vaccination, we measured the cellular T response against *M. tuberculosis* antigens developed in lungs and the antibodies produced in sera and bronchoalveolar lavage (BAL). To evaluate the efficacy of protection BCGΔBCG1419c + H1-TT we challenged vaccinated and boosted mice with *M. tuberculosis* H37Rv. Here, we evaluated the effect of BCGΔBCG1419c + H1-TT on the progression of the disease by measuring the bacterial burden in lungs and we evaluated the cellular response against mycobacterial antigens in blood and lungs.
Results: The combined vaccination with subcutaneous BCGΔBCG1419c and intranasal booster with H1TT induces memory T cells responders to mycobacterial antigens in lungs, a cytolytic and helper T cell response against mycobacterial antigens in spleen and IgG, IgA, and IgM against mycobacterial antigens in sera and BAL. In the efficacy experiments, BCGΔBCG1419c + H1-TT reduced the bacterial burden in lungs in an 82% compared to unvaccinated and unboosted mice. This protection is associated with an increase of blood T cells responders to mycobacterial antigens and increased central memory T cells responders to mycobacterial antigens in lungs.
Conclusion: The combined vaccination with subcutaneous BCGΔBCG1419c and intranasal booster with H1TT induces cellular and humoral response which results in protection against the progression of tuberculosis in mice.

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457 – WS33.3

Chitin-derived polymers are promising cGAS-STING dependent mucosal vaccine adjuvants for respiratory pathogens.

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SARS-CoV-2 remains a pathogen of global significance even with the success of currently approved vaccines. Injectable vaccines have been effective at reducing mortality and morbidity, but breakthrough infections and reinfections are common. Increasingly, mucosal cell-mediated immunity (CMI), including $CD8^+$ T cells, is implicated as a critical facet of protection in individuals with asymptomatic and symptomatic COVID-19. Mucosal vaccines have the potential to curtail initial infection through induction of CMI and IgA responses at the foci of pathogen entry. However, despite significant progress the development of mucosal vaccines is hindered by the lack of effective mucosal vaccine adjuvants. Our lab has identified chitin derived polymers as effective adjuvants for injectable vaccines and demonstrated that these promote cell mediated immunity by engaging the cGAS-STING pathway. Here, chitin-derived polymers with various degrees of deacetylation were investigated as potential intranasal vaccine adjuvants for SARS-CoV-2 spike antigen *in vivo.* A fully deacetylated chitin-derived polymer, C100, emerged as a promising mucosal vaccine adjuvant capable of inducing antigen-specific $CD8^+$ T cells in the lungs, in addition to antigen-specific mucosal IgA and systemic IgG. To interrogate C100 as a potential platform-adjuvant, it was combined with the invariant NK T cell activator α-galactosylceramide (α-GalCer). The combination adjuvant further enhanced antigen-specific $CD8^+$ T cells in the lungs and spleen, and IFNγ recall responses in the lungs. Our findings suggest that C100 is a highly promising mucosal vaccine adjuvant capable of inducing mucosal immune responses increasingly correlated with protection from SARS-CoV-2. Moreover, C100 emerges as a potential platform adjuvant for the design of mucosal vaccine adjuvant systems with increased potency.

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464 – WS33.4

Preclinical immunological characterization of GMMA-based vaccine candidates against invasive nontyphoidal Salmonellosis

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Purpose: *Salmonella enterica* serovars Typhimurium and Enteritidis are responsible for invasive non-typhoidal Salmonellosis (iNTS), a leading cause of bloodstream infections in <5-year-olds in sub-Saharan Africa. The emergence of multidrug-resistant non-typhoidal *Salmonella* (NTS) strains urges the development of affordable, effective vaccines.
GSK Vaccines Institute for Global Health (GVGH) is developing two vaccines: "iNTS-GMMA", a Generalized Modules for Membrane Antigens (GMMA)-based vaccine against iNTS, composed by GMMA purified from *S.* Typhimurium and *S*. Enteritidis; and "iNTS-TCV", a combination of iNTS-GMMA vaccine with a WHO-prequalified typhoid conjugate vaccine component (TCV), Vi-CRM_{197}, against both iNTS and typhoid fever. GMMA are outer membrane vesicles able to present multiple antigens in their natural conformation, derived from Gram-negative bacteria engineered to provide over blebbing and reduce potential systemic reactogenicity.
This work characterizes the immunological response of the NTS components of the two candidate vaccines and demonstrates their broad coverage.
Methods: CD1 mice were immunized intraperitoneally, New Zealand female rabbits intramuscularly; both with two doses, 4 weeks apart, of the two vaccines independently.
Sera were collected the day before and 2 weeks after the second immunization and were analyzed by ELISA to quantify antigen-specific immunoglobulins (IgG) and by luminescence-based SBA to assess antibody functionality.
Results: Immunogenicity studies showed the ability of iNTS-GMMA and iNTS-TCV to induce high IgG levels against the active ingredients of the NTS vaccine components, the O-antigens, and against the Vi of the TCV component. Both vaccines induced all the antigen-specific subclasses in mice against the NTS vaccine components. The antibodies induced complement-mediated killing against vaccine homologous NTS strains, and also against a broad panel of vaccine heterologous epidemiologically relevant *Salmonella* strains, including isolates associated to bloodstream infections and gastroenteritis, African and Southeast Asian representatives and strains belonging to different *S. enterica* serovars other than Typhimurium, Enteritidis and Typhi.
Conclusion: Preclinical data results strongly support the development of broad coverage GMMA-based vaccines to prevent iNTS. A Phase 1/2a for safety and immunogenicity of iNTS-TCV vaccine in healthy European and African adults is ongoing. For iNTS-GMMA, a Phase 2 age de-escalation dose-finding study for safety and immunogenicity in African adults, children and infants, has recently started.

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2045 – WS33.5

The inhibition of IL-10 during immunization with a TLR agonist-based vaccine improves vaccine efficacy against the opportunistic pathogen *Staphylococcus aureus*

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Staphylococcus aureus infections are a major global burden. Despite *S. aureus'* infectivity, it is also an important part of the natural human microbiome, primarily colonising the anterior nares. An effective anti-*S. aureus* vaccine remains elusive despite decades of research. A potential bottleneck is immunosuppressive imprinting during colonisation. *S. aureus* can induce immunosuppression to facilitate persistence by impeding effector T cell responses. The aim of this work is to investigate if commensal-driven immunosuppression impacts systemic antigen-specific T cells responses and whether targeting immunosuppression during immunization improves vaccine efficacy.
PBMCs and nasal fluid were isolated from a human cohort of healthy persistently colonised and uncolonised adults, and were assessed for alterations in antigen-specific T cell responses by flow cytometry and cytokine expression within the nasal mucosa by V-PLEX assay and RT-PCR. IL-10 production was significantly increased within the nasal tissue of colonised individuals, suggesting local *S. aureus*-induced IL-10 creates an immunosuppressive micro-environment promoting bacterial persistence. These local changes coincide with reduced systemic *S. aureus*-specific Th1 responses, which could be a significant challenge for effective vaccine function.
Using a murine immunization and infection model this study determined if modulating IL-10 signalling during vaccination could improve *S. aureus*-specific memory T cell responses during subsequent infection. C57BL/6 Wild-type mice were vaccinated with ClfA(of *S. aureus*) + CpG(TLR9 agonist) in the presence/absence of αIL-10. On day 3 & 7 post-immunization mice were challenged with intraperitoneal or subcutaneous infection. IL-10 inhibition during vaccination led to significantly improved IFN-γ+ IL17+ "Th1"-skewed T cell responses and enhanced bacterial clearance. In the subcutaneous setting, where an IL-17/IL-22 response is critical for efficient bacterial clearance, a novel Th17-skewing TLR2/STING agonist adjuvant was used. IL-10 inhibition led to significantly enhanced IL-17+ CD4, CD8 and γδ T cell responses and improved bacterial elimination.
This study reveals *S. aureus* nasal colonisation impacts systemic *S. aureus* antigen-specific T cell recall responses, and provides for the first time proof-of-concept that targeting IL-10 during immunisation may be a useful approach to improve vaccine efficacy against *S. aureus*. These results highlight how colonisation status may influence vaccine function and a novel strategy for improving anti-*S. aureus* vaccine efficacy.

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1230 – WS33.6

Bacteria-targeting vaccine in prevention of cardiovascular and cardio thrombotic diseases

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Purpose: Cardiovascular diseases (CVDs), such as myocardial infarction and stroke, are the leading cause of death in the world, causing ~18 million cases annually. Although high levels of LDL-cholesterol and unhealthy lifestyle are known risk factors for CVDs, also bacteria have been demonstrated to induce atherogenesis and platelet aggregation *in vivo*. In addition, molecular microbial techniques have been used to identify several DNA sequences from oral and gut bacteria in atherosclerotic plaques and thrombus aspirates of patients with myocardial infarction or acute ischemic stroke. Here our aim is to develop bacteria-targeting vaccines that could prevent cardiovascular complications.
Methods: Bacterial targets were selected based on metagenomic and immunohistochemical findings from human coronary artery plaques and thrombus samples. Antigens were chosen based on literature, and online databases of protein sequence and structure were used for the design of vaccine candidates. Recombinant proteins were produced in *E. coli* and their immunogenicity was studied using BALB/c mice. B cell response was studied from the serum by determining antigen-specific IgG endpoint titers with ELISA, and T cell response was determined from splenocytes using cytokine based FluoroSpot assay. SpyTag-decorated norovirus-like particles (noro-VLPs) will be used to display the antigens and improve their immunogenicity.
Results: We designed and produced five bacterial surface proteins from selected species to be used as antigens. Protein purity (~100%), endotoxin (<1.5 EU/µg) and DNA levels (<10 ng/dose) were acceptable for preclinical studies. While antigen-specific T cell responses were not detected in our immunizations, we obtained high IgG titers with three antigens (end point titer >100,000) and detectable levels of antibodies with the other two antigens.
Conclusion: For targeting selected bacteria, we designed and produced five vaccine candidates and achieved antigen-specific IgG antibodies against them in mice. Next, we aim to improve the immunogenicity of the antigens using VLP display and establish an infection model in mice to study whether the formation of atherosclerotic plaques or thrombi can be prevented by vaccination. Our study can lead to novel bacteria-targeted vaccine against cardiovascular diseases and have significant socioeconomical impact around the world.

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WS34 – IMMUNE IMPAIRMENT AND EXHAUSTION

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54 – WS34.1

Role of Foxo1 and inflammation on T-cell exhaustion in the course of anti-tumor responses

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T-cell exhaustion is a phenomenon commonly observed during aging and in persistent inflammatory conditions such as cancer and chronic infections. It is characterized by a progressive loss of effector functions and the expression of numerous immune checkpoint inhibitory receptors. Over the past decade, antibodies targeting these immune checkpoints have enabled considerable advances in cancer treatment. Unfortunately, these treatments are not efficient in all cancer types and only in a minority of patients. Our recent findings suggest that age-related inflammation (like exposure to pro-inflammatory cytokines such as type I interferons (IFN) and TNF-α) promotes the development of T-cell exhaustion via decreased expression of a transcription factor called Foxo1 (Durand A *et al*, Nat Commun 2024). This project aims to determine whether pro-inflammatory cytokines and Foxo1 downregulation could also be involved in T-cell exhaustion in the tumor microenvironment.
We observed a decrease in Foxo1 expression in intra-tumoral effector T cells, associated with an increase in the expression of several immune checkpoint receptors, in all mouse tumor models studied (transplanted, induced and spontaneous). We also studied tumor growth and T-cell infiltrate in different tumor models in mice deficient in type I IFN receptor expression ($IFNAR^{KO}$ mice) or TNFα expression (TNF^{KO} mice) compared with control mice.
We demonstrated the dual role of type I IFN in the MC38 transplanted tumor model by identifying accelerated tumor growth and reduced immune infiltrate in tumors in $IFNAR^{KO}$ mice compared to controls, but also a downregulation of exhaustion markers associated with increased Foxo1 expression in intra-tumoral $IFNAR^{KO}$ CD8 T cells.
We observed slower tumor growth in TNF^{KO} mice than in controls and a higher density of intra-tumoral CD4 T cells with a less exhausted phenotype in the MC38 tumor model.
Taken together, these results suggest a key role for pro-inflammatory cytokines in intra-tumoral T-cell exhaustion with a possible Foxo1-dependent effect for type I IFN. We would now like to test antibodies targeting these inflammatory signals to reduce T-cell exhaustion and improve anti-tumor immune responses.
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9 – WS34.2

T cell Immunotherapy: thinking beyond inhibitory receptors.

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Although immune checkpoint blockade (ICB) such as anti-PD-1 has represented a turning point in cancer care, clinical responses are not observed in the majority of cancer patients. The mechanisms underlying this lack of responsiveness are still poorly understood and finding additional signals that regulate CD8+ T cell anti-tumor functions has become a major priority. While most studies focus on inhibitory receptors, signals transmitted through activating receptors also critically impact CD8+ T cell cancer immune surveillance and ICB efficacy. Indeed, we recently discovered that the loss of the activating receptor CD226 (DNAM-1) is a critical immune escape mechanism restraining CD8+ T cell function and affecting the clinical efficacy of cancer immunotherapy (Weulersse et al, Immunity. 2020, Braun et al, Immunity. 2020). We identified a subset of dysfunctional CD226-negative CD8+ T cells that progressively accumulated in the tumor microenvironment through a mechanism involving the transcriptional regulator Eomesodermin (Eomes). We found that CD226 loss was a critical mechanism altering LFA-1 functions and CD8+ T cell responsiveness to TCR stimulation. Finally, we demonstrated that the absence of CD226 was significantly limiting the therapeutic efficacy of ICB in preclinical mouse models.
More recently, we focused on CD137 (4-1BB) activating receptor, an enigmatic yet, promising target for immunotherapy. Using T cell-specific deletion and agonist antibodies, we found that CD137 modulates tumor infiltration of CD8+ exhausted T (Tex) cells expressing PD1, Lag-3 and Tim-3 inhibitory receptors (*Pichler et al, immunity. 2023*). T cell-intrinsic, TCR-independent CD137 signaling stimulated the proliferation and the terminal differentiation of Tex precursor cells through a mechanism involving the RelA and cRel canonical NF-kB subunits and the Tox-dependent chromatin remodeling. While Tex cell accumulation induced by prophylactic CD137 agonists favored tumor growth, anti-PD-1 efficacy was improved with subsequent CD137 stimulation in pre-clinical mouse models. Better understanding of T cell exhaustion has crucial implications for the treatment of cancer and infectious diseases.
Understanding the cellular process that drive T cell dysfunction has crucial implications for the treatment of cancer and infectious diseases. Thus, our study, that uncovers the importance of CD226 and CD137 pathways in T cell dysfunction, could have broad applications for immunotherapy.

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25 – WS34.3

Imiquimod induces gamma-delta T cell exhaustion in mouse psoriatic inflammation

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Purpose: Complex pathogenesis of psoriasis involves yet understudied activities of Th1, Th17 and γδ T cells linked by cytokine circuits. Psoriatic dermatitis could be induced in mice by topical administration of imiquimod (IMQ) or intradermal administration of IL-23, and current studies aimed to compare these model mechanisms focusing on locally and systemically activated subpopulations of αβ and γδ T lymphocytes.
Methods: Psoriatic dermatitis was induced by IMQ administration for 7 consecutive days or ten intradermal injections of IL-23 performed every other day and assessed macroscopically by PASI scoring and ear swelling measurements in C57BL/6 mice. Serum cytokine concentrations were measured in ELISA and immune cells infiltrating lesional skin, draining lymph nodes and spleens were analyzed cytometrically.
Results: Mice treated with IMQ developed a more severe skin reaction than mice treated with IL-23, as reflected in significantly higher ear swelling responses and PASI values. Moreover, IMQ application caused the systemic inflammatory reaction with significant weight loss and severe splenomegaly. While serum levels of IL-12/IL-23p40 were similar in IL-23 and IMQ-administered mice, the latter treatment resulted in higher IL-17A and IFNγ concentrations. Moreover, IMQ increased the proportion of myeloid cells to T cells in lesional skin but not in draining lymph nodes and enlarged spleens. IFNγ-positive cells predominated among CD3ε-positive αβ and γδ T lymphocytes, while CD3ε-negative γδ T lymphocytes with impaired activation status were found more numerous than their CD3ε-positive counterparts, in enlarged spleens of IMQ-treated mice especially.
Conclusion: Our present findings suggest that intradermally injected IL-23 activates effector Th17 cells indirectly by stimulating the release of IFNγ by Th1 cells, and the initial activity of IFNγ-positive αβ and γδ T lymphocytes appears to be the common feature of both models studied. Moreover, the presence of CD3ε-negative γδ T cells with impaired activation status provides the first experimental evidence for γδ T cell exhaustion in psoriasis. Finally, one can conclude that IL-23-induced psoriasis model allows to study skin-related immune mechanisms with lower impact on animal welfare, while the IMQ-induced model enables holistic studies of psoriasis as a systemic disease with many comorbidities.

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1090 – WS34.4

Activated hepatic stellate cells promote the retention and functional impairment of tissue-resident CD8+T-cells in the fibrotic liver

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The function and phenotype of liver compartmentalised T-cells - tissue-resident T-cells (T_{RM}) - have been well-defined in hepatotropic infections and cancer. We have defined a population of highly functional IL-2-producing intrahepatic $CD69^+CD103^+CD8^+T_{RM}$ that contribute to the control of hepatitis B infection. While in primary liver cancer the presence of T_{RM} associates with improved overall patient survival. However, whether T_{RM} are involved in the dysregulated cycles of tissue damage and repair characteristic of liver fibrosis remains unclear.

We are becoming increasingly aware that the liver stroma can orchestrate local immunity - imprinting cellular fate, producing immunomodulatory mediators, and promoting leukocyte tethering. Here we show that hepatic stellate cells (HSCs; liver fibroblasts, the master mediators of fibrosis) dictate the derivation and retention of hepatic $CD8^+T_{RM}$. As fibrosis progresses, primary HSCs increase secretion of active-TGFb, driving the increased retention of $CD8^+$T-cells with a T_{RM} profile ($CXCR6^{hi}CD69^+CD103^+$) in the fibrotic liver. Accordingly, hepatic $CD8^+T_{RM}$ exhibit an integrin profile capable of facilitating interaction with, and increased tethering to, activated HSCs and the extracellular matrix they lay down. For example, we show $CD8^+T_{RM}$ upregulate a1b1/CD49a and aLb2/CD11a, that bind to collagen-IV and HSC-expressed ICAM respectively, and downregulate a5b1/CD49e, that binds fibronectin. Additionally, our data suggest that activated HSCs not only imprint a program of residency on $CD8^+$T-cells, but also a functional impairment. *In vitro* primary fibrotic HSCs limit the functionality (IFNg/TNFa/CD107a) of peripheral $CD8^+$T-cells and highly functional hepatic $CD8^+T_{RM}$ from individuals without fibrosis. Correspondingly, *ex vivo* $CD8^+T_{RM}$ from individuals with fibrosis express more PD-1, produce less pro-inflammatory cytokines and have a reduced capacity for degranulation upon stimulation. Significantly, we also show increased PD-L1 expression on HSCs in advanced fibrosis, suggesting a potential axis for the functional impairment of intrahepatic $CD8^+T_{RM}$ in chronic liver disease.

Advanced liver fibrosis can lead to an increased infection risk, the development of primary liver cancer and ultimately liver failure if not addressed. Our data suggest that blocking the PD1/PD-L1 pathway may represent a therapeutic strategy to reinvigorate the function of these highly efficient antiviral/antitumour immune sentinels within the liver, therefore helping to reduce the risk of disease complications.

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1381 – WS34.5

Immune checkpoint molecules control the local antigen-specific immune response in the liver

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Purpose: Antigen presentation is regulated in the liver to limit inflammation and preserve liver functions in healthy condition. There is an increased incidence of conditions where the hepatic tolerance is altered, such as autoimmune liver diseases or immune checkpoint (IC)-related immunotherapies for cancer patients. Autoreactive $CD4^+$ T cells are considered as playing an active role in these alterations, however studying their emergence and biology is technically challenging. The objectives of this work were to define signaling pathways regulating antigen-specific immune response in the liver, with a special focus on $CD4^+$ T cells.
Methods: We developed a new murine model, in which the model antigen hemagglutinin (HA) expression is restricted to hepatocytes, thanks to inducible Cre recombinase under the control of hepatocyte-specific promotor (HA/iCre mice). Intraperitoneal injections of tamoxifen induce HA expression specifically in hepatocytes. Peripheral immunization is obtained by intramuscular injection of HA-coding plasmid. The role of IC in the liver antigen-specific response was investigated using IC blocking antibodies *in vivo*. Injections of depleting anti-CD4 antibodies were used to study the involvement of $CD4^+$ T cells in the induced immune responses. Hepatitis scoring method on liver sections was implemented to measure liver inflammation. HA-specific $CD4^+$ and $CD8^+$ T cells were tracked, measured, and characterized through tetramer staining, RNA-sequencing and flow cytometry analysis.
Results: HA-specific $CD4^+$ and $CD8^+$ T cells were detected in the spleen of mice after peripheral HA-specific immunization. After tamoxifen-induced HA expression by hepatocytes, a transient accumulation of HA-specific $CD4^+$ and $CD8^+$ T cells in the liver was detected. Flow cytometry and RNA-sequencing analysis of liver HA-specific $CD4^+$ T cells revealed up-regulation of IC molecules (PD-1, CTLA-4, TIGIT). In this condition, blockade of PD-1 and CTLA-4 induced severe hepatitis, including massive infiltration of HA-specific $CD8^+$ T cells, an effect reversed by $CD4^+$ T cell depletion.
Conclusion: Using a mouse model, we demonstrated that antigen-specific $CD4^+$ T cells acquire an immuno-exhausted profile, with up-regulation of IC molecules, when they accumulate in the liver after local antigen reactivity. Inhibition of IC pathways is associated to increased liver inflammation in a $CD4^+$ T cell dependent manner.

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1168 – WS34.6

Circulating Immune profiling in people living with HIV (PLHIV) reveals that a differential pattern of exhaustion and activation is associated with viral control

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Purpose: HIV controllers (HIC) consist of a heterogeneous group of individuals who exhibit the capacity to sustain low plasma viral load with high CD4+ T-cell counts without antiretroviral therapy. Specifically, elite controllers (EC) show undetectable plasma viral load for several years. Which immunological mechanisms play a role in HIV control is not fully understood. We aimed to characterize the composition of circulating immune cells to identify an immune signature of HIV control.
Methods: High dimensional flow cytometry was performed to phenotype the major circulating immune cells and their maturation, activation, and exhaustion status, in 95 HIC and 1044 non-HIC (normal progressors on ART) of the 2000HIV study (NCT03994835). Differences in frequencies of the immune cell populations between HIC and non-HIC were investigated using a linear regression model.
Results: HIC were less often of European ancestry and showed higher CD4 nadir and HIV duration compared to non-HIC ($p<0.05$), while age and sex did not differ. HIC showed higher frequencies of total CD4+T-cells as well as several lineages of naïve cells (CD4+T, Treg and B cells) and CD8+TEMRA cells. In contrast, lower frequencies of CD4+ Th17, total CD8+T cells and memory subsets: CD4+Tcm, mTreg, CD8+Tcm, CD8+Tem and switched memory B cells were seen in HIC. Regarding immune cell status, CD4+T-cells of HIC were characterized by a reduced activation and exhaustion profile, evidenced by lower frequencies of cells positive for CCR5, HLA-DR+, CD38+ and PD1 ($p<0.05$). To dissect the populations linked to the long-term viral control, we independently analyzed the immune cell composition of 21 elite HIV controllers (EC, HIV-RNA <75 copies/mL/no-ART) and sought which cell subsets were enriched in EC. Notably, EC exhibited a higher frequency of TCRgdv2 cells and lower TCRgdv1 cells, as well as reduced exhaustion and activation within the T cell compartment, in comparison to non-HIC.
Conclusion: Our extensive immune cellular profiling suggests that HIC are characterized by increased proportions of naïve immune cells, reduced cellular exhaustion and activation with lower CCR5 expression. Increased proportions of TCRgdV2 cells in EC may contribute to maintaining the elite HIV controller status, highlighting their potential as a therapeutic target.

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WS35 – TOLERANCE AND IMMUNE REGULATION

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472 – WS35.1

Understanding immunity at the site of embryo implantation – implications for women with recurrent pregnancy loss

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Introduction: At the beginning of human pregnancy, an embryo implants into the endometrium, the inner most lining of the uterus. Like all mucosal tissues, the endometrium is enriched with immune cells providing pathogen defence, however alterations in the immune environment have been identified in patients with various disorders with associated subfertility. Recurrent Pregnancy Loss (RPL) effects 1-4% of couples and is defined as the consecutive loss of two or more pregnancies before 24 weeks' gestation. Although a significant proportion of RPL is linked with the autoimmune disorder antiphospholipid syndrome (APS), for the majority of patients (>50%) no reason is found but the immune system is implicated.
Methods: This study utilises a 35-parameter full spectrum flow cytometry panel (Cytek®Aurora), both bulk and single-cell transcriptomic sequencing (10X Genomics; 5'GEX/TCR/BCR immune-profiling) and Chip-cytometry (ZellScannerONE®) to characterise the T and B cell populations in the endometrium and blood of women with RPL. Samples were obtained when most relevant to embryo implantation, 7–11 days post Luteinising-Hormone surge.
Results: We analysed endometrial (n=10; 97K CD45+cells) and peripheral blood (n=10; 700K CD45cells) samples from RPL patients with miscarriage versus live birth in the subsequent pregnancy, and identified novel immune cell phenotypes, activation/regulatory markers, and cytokine receptors in matched samples. Endometrial naïve CD8+ T cells were increased in RPL patients that miscarried in their next pregnancy versus those with a live birth (p-adj = 0.021) and CD8+ T cell transcriptome was significantly altered with genes associated with chemotaxis, angiogenesis and vascular development. We present a detailed single cell transcriptomic analysis of the T and B cell compartments (n=8). Histology revealed CD8T cells were intraepithelial lymphocytes or in association with endometrial 'lymphoid aggregates', comprised of T, B, and macrophage cells.
Conclusions: This is the first study of endometrial mucosa using full-spectrum flow cytometry in combination with transcriptome analysis to identify immune modulation in RPL. Identification of the modulated pathways is key to the development of evidence-based treatments and diagnostics to enable detection of patients who would benefit from immunomodulators.

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1052 – WS35.2

Studies to Determine Myelin Peptide-Specific Nanoparticle Tolerance Mechanisms and Clinical Translation

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Purpose: Self-antigen-specific T cells are the underlying cause of many autoimmune diseases. T cells are found at the site of tissue destruction, resulting not only in the exacerbation of disease, but also the release of self-epitopes in the context of inflammation resulting in the activation of additional T cell populations, *i.e.*, epitope spreading, thereby perpetuating disease. Treatment with Ag-containing biodegradable poly(lactide-*co*-glycolide) (PLGA) nanoparticles, *i.e.* tolerogenic immune-modifying particles (TIMP, also denoted as CNPs), has been shown to be both safe and induces Ag-specific tolerance in mouse models of autoimmunity and allergy, as well as in a celiac disease Phase I/IIa clinical trial. The present studies addressed three key objectives. (1) To further identify the cellular populations and molecular pathways required for Ag-specific TIMP-induced tolerance. (2) To investigate the ability of TIMP-induced tolerance to modulate spread epitope-specific CD4+ T cell activation, which is critical for the inhibition of autoimmune disease progression. (3) The present studies further identified novel pathways required for Ag-specific TIMP-induced tolerance.
Methods: Tracible TIMPs were used to determine the cell type that first phagocytosed TIMPs following injection in vivo, and confocal microscopy and flow cytometry used to determine the fate of these cells. A combination of in vitro and in vivo studies were completed to identify the requirement for STING/IFN-alpha receptor pathway for antigen-specific TIMP-induced tolerance utilizing transferred PLP139-151-specific and MOG35-55-specific T cell receptor transgenic CD4+ T cells in SJL and C57BL/6 mice respectively.
Results: The present data show that myeloid cells phagocytose TIMPs and undergo apoptosis via a caspase-dependent pathway. Subsequently, TIMP-induced Ag-specific tolerance therapy increases both $FoxP3^+$ regulatory T cells and $IL\text{-}10^+$ Tr1 cells in naïve mice and diseased mice via a STING/IFN-alpha receptor-dependent pathway. Furthermore, these data show TIMP treatment modulates T cells specific for spread epitopes associated with disease progression, but not encapsulated within the TIMP, *i.e.* tissue-specific bystander suppression.
Conclusion: Therefore, treatment activates various Ag-specific regulatory T cell subsets capable of regulating responses to disease-associated autoantigens not encapsulated within the TIMP via release of immunoregulatory cytokines, and treatment with multiple myelin-derived peptide epitopes has proven efficacious in preclinical mouse models of multiple sclerosis.

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676 – WS35.3

Tolerogenic dendritic cell-based immunotherapy to prevent unwanted immune response in enzyme replacement therapy for lysosomal storage disorders

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Lysosomal storage disorders (LSDs) are caused by the lack of lysosomal enzymes. When available, enzyme replacement therapy (ERT) is the standard treatment for LSDs, although immune responses towards the infused enzyme can limit its therapeutic efficacy. Tolerogenic dendritic cells (tolDC) are pivotal in promoting antigen(Ag)-specific tolerance. We developed a protocol to generate tolDC using lentiviral-vectors encoding for human IL-10 and selected Ag-derived epitopes ($DC^{Ag/IL\text{-}10}$) and showed their efficacy in modulating Ag-specific T and B cell responses. With the aim of developing tolDC as enzyme-specific adjuvant immunotherapy for LSD patients undergoing ERT, we dissected the immune status of ERT-treated LSD patients (Mucopolysaccharidosis-IVA, Pompe disease and alpha-mannosidosis), generated tolDC from patients' monocytes, and tested the ability of $DC^{Ag/IL\text{-}10}$ to modulate adaptive responses to ERT (a-L-iduronidase, IDUA) in a mouse model of Mucopolysaccharidosis-I (MPSI).
We measured cytokines/chemokines in patients' plasma by Luminex, performed flow cytometry profiling of peripheral blood cells and generated tolDC from $CD14^+$ monocytes of LSD patients. We found increased inflammatory cytokines and chemokines in the plasma of LSD patients, compared to healthy controls (HC). In line with this, we observed the upregulation of activation markers on monocytes and $CD8^+$ T cells and down-regulation of inhibitory markers on T cells. The frequency of granulocytes, in particular neutrophils, and antibody-producing B cells was higher in LSD patients compared to HC. Despite the activated phenotype, patient-derived $CD14^+$ cells differentiated into functional tolDC that modulate allogenic $CD4^+$ T cells, and promoted Tr1 cells as efficiently as those differentiated from HC. In a preclinical ERT model in which MPSI mice received IDUA treatment, we detected IDUA-specific $CD4^+$ T cell responses early after ERT initiation and the induction of anti-IDUA antibodies. Repetitive injections of engineered DC expressing IDUA and IL-10 ($DC^{IDUA/IL\text{-}10}$) reduced circulating anti-IDUA antibodies, compared to control mice injected mice with DC^{IDUA}.
Our data indicate that, despite the pro-inflammatory signature of ERT-treated LSD patients, monocytes can differentiate into functional tolDC. Preliminary *in vivo* data suggest that tolDC immunotherapy effectively limit the immune response elicited by ERT, thus potentially fostering its efficacy.

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2188 – WS35.4

Tolerogenic dendritic cells are strong producers of extracellular vesicles with immunomodulatory function

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Introduction: While the potential of tolerogenic dendritic cells (tolDC) for the regulation of pro-inflammatory pathogenic responses in multiple sclerosis has been recognized, their mode-of-action (MOA) remains elusive. With preliminary data pointing towards an increase in the production of extracellular vesicles (EVs), we hypothesize a role for EVs in the MOA of tolDC.
Methods: tolDC-derived EVs were isolated from conditioned media using tangential flow filtration, characterized by nanoparticle tracking analysis (NTA), high-sensitivity flow cytometry (HS-FCM), transmission electron microscopy (TEM) and western blot and compared to EVs derived from conventional DC (convDC). The suppressive function of tolDC-EVs was evaluated using an allogeneic mixed lymphocyte reaction (allo-MLR) and evaluated for differences in T cell and B cell responses.
Results: Staining of the EV membrane with Cell Mask Green showed the successful isolation of EVs from cell culture medium with a higher number of EVs retrieved from tolDC culture media compared to convDC. The median size of tolDC-EVs seems to be larger as compared to convDC-derived EVs, while tolDC produced significantly more EV. Characterization of EVs was done according to the current MISEV guidelines. Furthermore, tolDC-EV were able to suppress the allo-MLR as shown by a significant decrease in secreted interferon-gamma, decreased expression of cellular activation markers by allogeneic T cells, and changes in the B cell compartement as compared to the allogeneic lymphocytes alone. Moreover, our assay shows that tolDC-EV were able to suppress the allo-PBL reaction to a similar extent as tolDC.
Conclusion: Altogether, our data show the secretion of EVs by tolDC and suggest an immunosuppressive role of tolDC-EV. More research is needed to characterize tolDC-EV according to MISEV guidelines and further explore their immunosuppressive function. Ultimately, we aim to deliver new insight in the mode-of-action of tolDC and the induction of tolerance. In addition, this research could lead to the development of a new EV-based therapeutic agent for the treatment of MS.

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2245 – WS35.5

A new paradigm for the role of AIRE in the regulation of cytoskeleton

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Autoimmune regulator (Aire), one of the most studied transcription factors, is well known for its critical contribution to the establishment of immunological tolerance, a phenomenon that takes place in the thymus. Since Aire loss-of function mutations can lead to the onset of multi-organ autoimmunity, Aire has been studied for nearly three decades almost exclusively in the context of medullary thymic epithelial cell (mTEC) function where it regulates mechanisms involved in the prevention of autoimmunity. Recently, we and others have shown that Aire also fulfils an analogous immune function in a rare subset of lymph node cells. In addition, accumulating evidence suggests that Aire is also expressed in other non-immune cell types and tissues. Our newly acquired data now provides unambiguous proof of the novel role of Aire in testes. In the absence of Aire, the process of spermatogenesis is spatially disordered resulting in markedly reduced and malformed sperm leading to sterility. This aberrant phenotype is not only caused by transcriptional deregulation but also a previously undescribed cytoplasmic function of Aire in relation to essential microtubule cytoskeleton regulation. Importantly, we have evidence that AIRE is also associated with microtubules in autoimmunity-preventing mTECs as well as rapidly dividing cancer cells. This strongly suggests that AIRE could have a much broader spectrum of activities implicated in the modulation of physiological as well as pathological processes. We believe that this data invites for a revised view on the function of the Aire protein and could provide novel approaches for therapeutic interventions in processes linked to sterility, autoimmunity, and malignancy.

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1584 – WS35.6

The embryonic lymphoid cells permanently imprint the thymic epitelial compartment

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Thymic epithelial cells (TEC) play an essential role in central tolerance, guided by medullary (m)TEC driving negative selection of self-reactive newly generated thymocytes. Between days 12-15 of gestation, the thymus is colonized by a first wave of hematopoietic thymic seeding progenitors (TSP) that have the unique capacity to generate invariant gd T cells and lymphoid tissue inducer (LTi) cells. These embryonic populations are the first to express RANK ligand (L) in the thymus. RANK-RANK-L interactions are required for the expression of autoimmune regulator (AIRE) by mTEC, which is essential for the negative selection of self-reactive T cells. Previous analyses showed that injection of anti-IL-7Ra antibody in mid-gestation largely decreased the first wave of TSP and resulted in a strong reduction of invariant gd T cells and LTi cells, as well as of mature mTEC around birth. We are now investigating the impact that the absence of the first wave of TSP has on thymic function later in life. At post-natal day (P)15, anti-IL-7Ra antibody-treated (aIL7Ra-AT) mice exhibit reduced numbers of thymic regulatory T (Treg) cells and normal numbers of other populations of thymocytes. By contrast with control mice that show a peak of mTEC at P30, aIL7Ra-AT mice maintain constant the numbers of mTEC between P15 and P270. Transcriptional analyses of TEC in aIL7Ra-AT mice showed the virtual absence of AIRE-expressing mTEC and a decreased population of immature mTEC at P30. aIL7Ra-AT mice also showed an abnormal thymic architecture with increased numbers of small medullary areas that fail to coalesce, a feature that persists in older mice. aIL7Ra-AT mice showed delayed thymocyte recovery and a lack of capacity to regenerate their TEC compartment when subjected to whole-body sublethal irradiation or dexamethasone treatment. 50% of the aIL7Ra-AT mice died before the age of 9 months and the survivors showed immune infiltrates in several organs, indicative of autoimmunity. We conclude that interactions between embryonic thymocytes and TEC are essential for normal thymic function in adults and their perturbation will result in life-long consequences for the immune system.

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WS36 – T CELL REGULATION AND FUNCTION I

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1370 – WS36.1

Inheritance of old mitochondria controls early CD8 T cell fate commitment and is regulated by autophagy

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T cell immunity is impaired during ageing, particularly in memory responses needed for efficient vaccination. Autophagy and asymmetric cell division (ACD) are cell biological mechanisms key to memory formation, which undergo a decline upon ageing. Thus, we aimed to decipher whether autophagy regulates the early-rise of asymmetric T cell fates and investigate whether there is a causal link between ACD and *in vivo* T cell fate decisions, as evidence has remained highly correlative. Our results are consistent with the concept that initiation of asymmetric T cell fates is indeed regulated by autophagy.
Firstly, by analysing the proteome of first-daughter $CD8^+$ T cells following TCR-triggered activation, we observed that mitochondrial proteins rely on autophagy for their asymmetric inheritance and that damaged mitochondria are polarized upon first division. These results led us to evaluate the functional impact of unequal inheritance of different mitochondrial populations on T cell function. To achieve that, we used a novel mouse model that allows sequential tagging of mitochondria in mother and daughter cells, enabling their isolation and subsequent *in vivo* analysis of $CD8^+$ T cell progenies based on a pre-mitotic cell cargo. Autophagy-deficient $CD8^+$ T cells showed impaired clearance and symmetric inheritance of old mitochondria, suggesting that both segregation and degradation events promote asymmetry and are needed to generate T cells devoid of old organelles. Daughter cells inheriting old mitochondria are more proliferative, glycolytic and show poor survival in absence of TCR stimulation. Adoptive transfer of cells followed by antigen-specific infection revealed that progenies inheriting old organelles have reduced memory potential, whereas daughter cells that have not inherited old mitochondria from the mother cell are long-lived, able to re-expand upon cognate-antigen challenge and produce effector cytokines upon re-stimulation. Proteomic and single-cell transcriptomic analysis of cells inheriting aged mitochondria suggest that their early fate divergence relies on one carbon metabolism as a consequence of poor mitochondrial quality and function.
These findings increase our understanding of how T cell diversity is early-imprinted and will help foster the development of strategies to modulate T cell function, which is particularly relevant in the context of immune rejuvenation and regenerative medicine.

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1142 – WS36.2

A subset of αvβ8-expressing CD4+ effector memory T cells restrain anti-viral CD8+ T cell responses and prevent tissue pathology via activation of latent TGFβ.

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Eliciting rapid responses to previously encountered antigen underpins immunological memory and is a cornerstone of durable immunity. Central to this are long-lived memory T cells, which rapidly respond following re-encounter with cognate antigen and have been shown to be indispensable for protection following heterologous Influenza challenge. Hence understanding the mechanisms regulating immune memory is essential to identify candidate targets for therapies aimed at boosting antigen-specific cellular immunity.
Transforming growth factor β (TGFβ) is a key regulator of mucosal homeostasis and has several cellular targets, including effector T cells. However, due to its potent immune-suppressive functions, together with the fact it is a mediator of tissue fibrosis, TGFβ is secreted as an inactive complex which must be activated extracellularly, a process in which cell surface integrins are integral. However, the role of such molecules during an active memory recall response remains unresolved. Using heterologous *in vivo* viral infection models, we show that a population of $CD4^+$ T_{EM} can activate latent TGFβ via expression of the integrin molecule αvβ8. This pathway appears crucial *in vivo*, as mice lacking αvβ8 on $CD4^+$ T_{EM} ($CD4.Cre^{\Delta Itgb8}$) exhibit exacerbated virus-specific effector $CD8^+$ T cell responses and enhanced viral clearance following a secondary influenza infection. However, this enhanced anti-viral recall response in the absence of αvβ8-expressing $CD4^+$ T_{EM} is associated with enhanced lung pathology following secondary Influenza infection, a phenotype which can be completely reversed via adoptive transfer of αvβ8-expressing $CD4^+$ T_{EM} into $CD4.Cre^{\Delta Itgb8}$ mice prior to rechallenge. Furthermore, we demonstrate that antigen-specific $CD8^+$ T cells in the small intestine are enriched in mice lacking αvβ8 on $CD4^+$ T_{EM} following heterologous viral challenge with LCMV, indicating that this pathway is not solely restricted to the lung. Finally, we also show the presence of αvβ8-expressing $CD4^+$ T_{EM} in human peripheral blood.
Taken together, our findings suggest that the activation of TGFβ by $CD4^+$ T_{EM} is crucial to prevent exacerbated, tissue damage-inducing effector responses and that's this feature of some $CD4^+$ T_{EM} may also serve as a viable therapeutic target to harness the potent cytotoxicity of $CD8^+$ memory T cells, a correlate of protective anti-viral and anti-tumour immunity.

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1876 – WS36.3

Highly-functional myeloid-instructed CD8+ T-cells accumulate in the peritoneal cavity

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Peritoneal leukocytes play a role in immunoregulation, tissue homeostasis and repair. When peritoneal homeostasis is disturbed leukocytes are recruited to restore balance. Previously we described the reprogramming of liver-resident $CD8^+$T-cells upon interaction with neighbouring myeloid cells (Pallett_etal.,_Nature_2023). Upon interaction resident $CD8^+$T-cells 'steal' fragments of the plasma membrane acquiring constitutive immunomodulatory features at rest and an enhanced antiviral/antitumour capacity when activated through their T-cell receptor (TCR). Crucially, we can detect these 'super-responder', myeloid-instructed $CD8^+$T-cells by co-staining for CD8 and CD14.
In advanced chronic liver disease a build-up of fluid (ascites) can occur within the peritoneal cavity. Here we hypothesise that myeloid cells within the cavity reprogramme $CD8^+$T-cells to help maintain peritoneal homeostasis, providing local antiviral/anti-tumour immunosurveillance. Co-staining for CD8/CD14, we show myeloid-instructed $CD8^+$T-cells accumulate in ascites. Peritoneal $CD14^+CD8^+$T-cells produce more pro-inflammatory cytokines (IFNg/TNFa/IL-2) when TCR-stimulated than their CD14-negative counterparts despite unexpectedly high Tim3 expression, often considered a co-inhibitory receptor via interaction with ligand galectin9. However, another major Tim3 ligand is phosphatidylserine (PS) that when co-expressed with Tim3 promotes NFkB signalling and cytokine production following TCR stimulation. To investigate whether the Tim3/PS axis was responsible for the increased functionality of $CD14^+CD8^+$T-cells we assessed expression of PS by measuring annexinV, which in the presence of Ca^{2+} binds to anionic PS. $CD14^+CD8^+$T-cells express more PS than $CD14^-CD8^+$T-cells. Notably the plasma membrane is regulated to maintain asymmetric distribution of phospholipids, with anionic phospholipids (e.g. PS) restricted to the cytoplasmic leaflet. During apoptosis loss of plasma membrane integrity triggers translocation of PS to the outer leaflet, however, importantly we established that peritoneal $PS^+CD14^+CD8^+$T-cells were not apoptotic by staining for active caspase3. Instead, we propose the anionic PS has likely been removed from the surface of the instructing myeloid cell. Furthermore, the addition of a Tim3 antibody known to block PS binding diminishes the capacity of $CD14^+CD8^+$T-cells to produce cytokines when TCR-activated.
$CD14^+CD8^+$T-cells represent a resident T-cell capable of enhanced responses within the peritoneal cavity. By exploiting the ability of PS/Tim3 to augment T-cell signalling, myeloid-instructed $CD8^+$T-cells may better contribute to the maintenance of immune homeostasis, representing an important immune sentinel providing critical antiviral/antitumour immunosurveillance.

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1541 – WS36.4

Long noncoding RNA-124 regulates CD8+ T cell response to infection

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Recent studies have identified long noncoding RNAs (lncRNAs) in key functions of immune cell regulation. However, the role of lncRNAs in regulating $CD8^+$ T cell responses against pathogens and tumors *in vivo* remains incompletely understood. Deep RNA-seq profiling of mouse $CD8^+$ T cell subsets at different stages of bacterial infection *in vivo* allowed us to identify differentially regulated lncRNAs, such as *lncRNA-124*. By using CRISPR/Cas9-mediated genome engineering, we generated *lncRNA-124* knockout (KO) mice. To analyze the *in vivo* function of *lncRNA-124* we performed adoptive co-transfer of *lncRNA-124* WT and KO T cells followed by infection with listeria monocytogenes. Our results demonstrate that *lncRNA-124* regulates effector $CD8^+$ T cell expansion as well as effector-to-memory $CD8^+$ T cell differentiation. We also show that *lncRNA-124* is evolutionarily conserved and the human ortholog of *lncRNA-124* is expressed in human $CD8^+$ T cells. In conclusion, *lncRNA-124* is a novel long noncoding RNA that regulates distinct phases of $CD8^+$ T cell differentiation *in vivo*.

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848 – WS36.5

Exploring biomarkers of clinical response to immunotherapy in lung cancer: insights from peripheral CD8 T cell profiles and ctDNA dynamics post-PD-1 blockade

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Background: Programmed Death-1 (PD-1) blockade has transformed the treatment of late-stage metastatic cancer, particularly for patients with non-small cell lung cancer (NSCLC). Indeed, real-world data report durable clinical responses for 10-15% of cases, in part due to the reinvigoration of exhausted CD8 T cells in the tumour microenvironment. However, failure to reinvigorate terminally exhausted CD8 T cells could lead to clinical relapse. The characterization of the exhausted CD8 T cells in peripheral blood holds promise as a biomarker of clinical response, informing treatment changes during relapse. Furthermore, the relationship between circulating immune cell profiles and circulating tumour DNA (ctDNA) remains largely unexplored. Our aim was to characterize the phenotype and function of peripheral CD8 T cells while concurrently monitoring ctDNA dynamics after PD-1 blockade in patients with NSCLC.
Methods: Peripheral blood mononuclear cells were collected from 35 NSCLC patients pre- and post-treatment. Flow cytometry and CITE-seq was performed for phenotypic and transcriptomic analysis of CD8 T cells. Plasma ctDNA analysis was performed using SiMSen-seq, a simple multiplexed PCR-based barcoding of DNA for ultrasensitive mutation detection by next-generation sequencing. Response assessment (responder versus non-responder) was based on CT scan results and followed the iRECIST criteria with a cut-off of 9 months. All patients provided informed consent prior to enrolment to the study.
Results: Responders had higher baseline expression of TIGIT and PD-1 on CD8 T cells and an increased frequency of stem-like TCF-1+PD-1+ CD8 T cells post-treatment compared to nonresponders. Functional differences in peripheral CD8 T cells based on the transcriptional profile, between responders and nonresponders may explain treatment outcome. At baseline, nonresponders had high plasma ctDNA levels, compared to responders who had decreased or undetectable levels. Concurrent profiling of T cells and ctDNA analysis at matched time points within the same patient revealed a correlation between reduction in ctDNA load and an increase in the frequency of stem-like CD8 T cells following PD-1 blockade in a subset of patients.
Summary: The results of our study in NSCLC patients provide insights into the identification of biomarkers of clinical response in late-stage cancer, guiding treatment decisions and the development of combination therapies.

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Activation phenotypes defined by coordinated expression of activation markers discriminate TCR-mediated and bystander T-cell responses

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Identifying antigen-specific T-cell responses following T-cell receptor (TCR)-mediated activation during viral infection is fundamental to understanding virus-specific immunity. Using flow cytometry, activation of virus-specific T cells is commonly marked by the induction of activation-related CD-molecules and cytokine production. However, responses by non-specific bystander T cells to cytokines produced by T cells and infected cells complicate identification of antigen-specific T-cell activation. Since activation markers are sensitive to bystander activation, they lack specificity for antigen-activated T cells. Here, we aimed to gain insight on how activation markers can be applied to discriminate antigen-specific TCR-activated T cells from bystander-activated T cells. To this end, we studied how various markers are induced in response to stimulatory components of viral infections, and questioned if coordinated expression of markers could provide an activation phenotype that discriminates bystander-activated T cells.
We stimulated peripheral blood mononuclear cells (PBMCs) with anti-CD3 antibody to mimic TCR-mediated activation, supernatant of anti-CD3 activated T cells, or IL-2, IL-15, or IFNα as bystander cytokines. We then measured various activation-related characteristics in T cells, including CD69, CD25, CD137, CD154, IFNγ, and CD107a induction by flow cytometry. Based on coordinated expression of these markers, we defined activation phenotypes that pinpoint TCR-mediated and bystander T-cell responses. Subsequently, we translated our findings to anti-viral T-cell responses by stimulating PBMCs with peptides of influenza virus and SARS-CoV-2.
We observed significant induction of activation markers in response to both TCR-mediated and bystander stimulation. In comparison to TCR-mediated responses, bystander responsiveness was marked by lower co-expression of the different activation markers, and mainly included CD69 and CD107a expression. Such expression of markers without other markers being co-expressed defined activation phenotypes that indicated which T cells were activated by viral antigens or as bystander cells.
Together, our data show that defining activation phenotypes by coordinated expression of activation markers aids in pinpointing TCR-mediated versus bystander T-cell responses. The finding that a significant number of T cells respond as bystander cells indicates the relevance of considering such definition of bystander T-cell activation when studying antigen-specific T-cell responsiveness in viral infections.

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WS37 – B CELLS IN HEALTH AND DISEASE

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1561 – WS37.1

Antigen-presenting autoreactive B cells activate regulatory T cells and suppress autoimmune arthritis in mice

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B cells undergo several rounds of selection to eliminate potentially pathogenic autoreactive clones, but in contrast to T cells, evidence of positive selection of autoreactive B cells remains moot. Using unique tetramers, we traced natural autoreactive B cells (C1-B) specific for a defined triple-helical epitope on collagen type-II (COL2), constituting a sizeable fraction of the physiological B cell repertoire in mice, rats, and humans. Adoptive transfer of C1-B suppressed arthritis independently of IL10, separating them from IL10-secreting regulatory B cells. Single-cell sequencing revealed an antigen processing and presentation signature, including induced expression of CD72 and CCR7 as surface markers. C1-B presented COL2 to T cells and induced the expansion of regulatory T cells in a contact-dependent manner. CD72 blockade impeded this effect suggesting a new downstream suppressor mechanism that regulates antigen-specific T cell tolerization. Thus, our results indicate that autoreactive antigen-specific naïve B cells tolerize infiltrating T cells against self-antigens to impede the development of tissue-specific autoimmune inflammation.

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629 – WS37.2

Elucidation of antigen-specific B cell dynamics after vaccination reveals activated B cell states that precede the classical memory B cell and plasmablast compartments

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T-cell driven B-cell responses in extrafollicular and Germinal Center (GC) reactions are needed to induce antibody secreting cells (ASC) that produce protective antibodies upon infection and vaccination. It remains to be elucidated how B-cell differentiation occurs after antigen exposure, and how formation and maintenance of the different extrafollicular and GC B-cells subsets relate to each other and to formation of actual memory B-cells (Bmem) and ASCs. In this study, we characterized antigen-specific B responses longitudinally in PBMCs of persons receiving SARS-Cov-2 mRNA vaccinations or experiencing infection by using multiparameter high-dimensional flow cytometry. This deep immune phenotyping approach allowed us to distinguish different B-cell subsets and their dynamics.
Recently, we and others have described a CD71+ IgG+ Activated B-cell (ActBC) population that arises shortly after antigen stimulation and found indications that these might be B-cells that were derived from recent GC reactions. ActBC were also observed to be strongly expanded shortly after vaccination and could be further subdivided in the different B-cell states based on the deep profiling. Interestingly, the ActBC showed close phenotypic and dynamic relationships to CD11c+ B-cells previously described as 'atypical' B-cells. While the atypical and GC-derived B-cell populations have been described as separate entities, our data strongly point to interconnections between defined CD11c+ and classical populations in ongoing GC reactions. While it is unknown why B-cells differentiate towards a CD11c+ expression, these cells thus seem part of the normal immune response and together with the ActBC form the B-cell states that precede classical Bmem and/or ASC formation.
Currently, we are investigating the functional properties of ActBC and CD11c+ B-cell populations under influence of different antigenic and cytokine stimulation. In addition, the evolution of the BCR repertoire and clonotypes are being analysed over time along the various stages of B-cell differentiation. For this we link BCR analyses and transcriptomic expression to the defined proteomic phenotype of single cell sorted B-cells. These data may pave the way for strategies to target specific B-cell populations before Bmem or ASC formation has occurred in order to inhibit undesired humoral responses in transplantation or autoimmune diseases.

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772 – WS37.3

The brain-infiltrating B cell in multiple sclerosis: identifying triggers and receptors contributing to its effector program

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Purpose: The effectiveness of anti-CD20 therapies highlighted B cells as a driving force behind multiple sclerosis (MS). Our group has identified distinctive, CXCR3$^+$ memory B cells that are triggered to infiltrate the MS brain and locally mature into antibody-secreting cells (ASCs). We aim to elucidate how T cell-dependent and –independent triggers mediate this process and which receptors enable CNS maintenance. This could aid in identifying MS patients who will benefit most from brain-penetrating drugs, such as next-generation Bruton tyrosine kinase (BTK) inhibitors.
Methods: In-depth *ex vivo* spectral flow cytometry analyses were performed with B cells from fresh post-mortem CNS compartments of MS donors and from the blood of healthy donors and untreated MS patients. B-cell responsiveness was determined based on phosphoflow cytometry (anti-BCR, sCD40L/IL-21) and *in vitro* T cell-dependent (CD40L/IL-21) differentiation assays, both with and without a BTK inhibitor. Single-cell RNA sequencing was performed for (precursors of) CXCR3$^+$ memory B cells at baseline and after *in vitro* differentiation.
Results: As compared to CXCR3- counterparts, CXCR3$^+$ memory B cells were more responsive to CD40L/IL-21-than BCR-related stimulation (phosphoflow), with an increased ability to mature into (CD38^{high}CD27^{high}) ASCs *in vitro*. Single-cell RNA-sequencing of *in vitro*-induced memory B cells confirmed these findings, showing a relatively enriched ASC cluster with co-expression of *CXCR3* and *ITGB1* (CD29, integrin-beta 1; VLA-4 subunit). BTK inhibition reduced induction of this ASC cluster. *Ex vivo* analyses revealed increased CD29 expression on CXCR3$^+$ versus CXCR3$^-$ memory B cells from untreated MS patients, which was even more pronounced in the CSF. This increase in CD29 expression was not seen for circulating CXCR3$^+$ ASCs. In single cell suspensions of post-mortem CSF, meninges, and brain tissues from 10 MS donors, CXCR3 and CD29 co-expression was found primarily on ASCs, in contrast to paired blood.
Conclusion: This work reveals enhanced sensitivity of CXCR3$^+$ memory B cells to T cell-dependent triggers, which likely underlies their increased ASC maturation capacity. Furthermore, we show that CD29 could serve as a phenotypic marker for their recruitment, persistence, and maturation into long-living ASCs in the MS brain.

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1484 – WS37.4

Age-associated B cells drive loss of CD8+ T cells in systemic lupus erythematosus

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Age-associated B cells (ABCs) are more prevalent with aging, infection, and autoimmunity. ABCs are characterized by their expression of T-bet and CD11c in mice and contribute to disease and inflammation by producing autoreactive antibodies, secreting inflammatory cytokines, and activating self-reactive $CD4^+$ T cells. However, no interplay between ABCs and $CD8^+$ T cells has been identified so far. In this study, epicutaneous application of resiquimod, a TLR-7 agonist, induced a systemic lupus erythematosus (SLE)-like autoimmune response in mice. This mouse model exhibits an increased frequency of plasma cells and germinal center B cells, increased levels of anti-DNA antibodies, and immune complex deposition in the glomeruli. The treatment also causes a loss of $CD8^+$ T cells confirmed by both flow cytometry and histology. Surprisingly, the $CD8^+$ T cell loss was rescued by a conditional knock-out of T-bet in activated B cells. Histological analyses showed that the loss of $CD8^+$ T cells could not be explained by homing to the resiquimod-treated areas. Single-cell sequencing indicated a hyperactivated or exhausted double-negative phenotype of the $CD8^+$ T cells. Ongoing experiments are aiming to elucidate whether the mechanistic link between ABCs and $CD8^+$ T cells depends on cytokines, cell-to-cell contact, or antibody production, leveraging additional genetic models, flow cytometry, cytometric bead arrays, and histological evaluation. Samples from SLE patients are also being examined to assess the applicability of these findings to clinical settings. This novel link between ABCs and $CD8^+$ T cells could give mechanistic insight into the heightened susceptibility of autoimmune patients to cancer and infection.

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601 – WS37.5

Contribution of memory B cells to the pool of bone marrow plasma cells through follicular and extrafollicular immune responses in health and disease

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Memory B cells play a pivotal role in adaptive immunity by preserving immunological memory, yet the complexities of their organization and maintenance over time remain elusive. In previous work, we demonstrated that the majority of murine isotype-switched memory B cells localise to the spleen or bone marrow, with only a minority (20%) being in circulation. Whether this distribution holds true in humans remains unknown. Also unknown, is the real importance of memory B cells for plasma cell generation. Our analyses of bone marrow plasma cells (BMPC) revealed a distinct BMPC compartment characterised by a gene signature indicating an origin from memory B cell extrafollicular reactivation. These BMPC not only retain CD19 expression, but also exhibit higher levels of somatic hypermutation, raising questions about which memory B cells contribute to the pool of BMPC and the underlying processes involved. To address these questions, we analysed single-cell transcriptomes and B cell receptor repertoires of memory B cells and plasma cells isolated from paired blood and bone marrow samples. These samples were obtained from both healthy individuals and systemic lupus erythematosus (SLE) patients, the latter known for their propensity towards extrafollicular responses. Indeed, similar to mice, we report a considerable degree of heterogeneity among human memory B cells. Notably, selective repertoire overlaps in blood and bone marrow confirmed the probability of extrafollicularly reactivated memory B cells contributing to bone marrow plasma cell compartments. Utilising it as a model antigen, we exploited spike-specific public clones across individuals and devised a novel approach to analyse antigen-specific plasma cells. By "digitally sorting" bone marrow plasma cells expressing public clones with experimentally validated spike protein-binding BCR sequences, we investigated shared repertoires with blood and bone marrow memory B cells. Our findings edge closer to validating the intriguing hypothesis: do memory B cells undergo local differentiation into plasma cells within the bone marrow?

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674 – WS37.6

Distinct transcriptional profile drives enhanced activation responses in memory B cells compared to naive B Cells

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Long-lived memory B cells (Bm) arise from the differentiation of naive B cells (Bn). Antigen-experienced memory B cells are geared towards enhanced effector functions compared to their naive counterpart and provide long-term humoral immunity. To investigate the differences in responsiveness of naive and memory B cells, we profiled the transcriptome at the single cell level of human naive ($CD20^+CD27^-IgG^-IgA^-$) and unswitched memory B cells ($CD20^+CD27^+IgG^-IgA^-$) of four healthy donors. Cells were profiled in a resting state as well as at early and late stages upon in vitro T-dependent stimulation. Although both cell types underwent rapid proliferation and isotype switching, Bm proliferated more compared to Bn cells. However, isotype switching was more frequent in Bn cells. Already at a resting state as well as shortly after stimulation, Bn and Bm had considerably different transcriptional signatures suggesting that naive and memory B cells were in two different states poised to differentiate differently upon activation. At later time points, both Bn and Bm acquired multiple cell states thus recapitulating the highly dynamic process of immune response. To dissect the transcriptional determinants distinguishing Bn and Bm cells, we tested for differential expression analysis and we detected 2549 differential genes in resting cells and 1891 at day six following stimulation. Moreover, differential genes were highly time point specific, with only 32% of them overlapping between the time points. This suggests dynamic and large transcriptional differences in two similar B cell states. Interestingly, transcription of the secreting isoform of IgM was higher in the day six activated Bm cells compared to Bn, suggesting an intrinsic mechanism of fast differentiation of Bm cells to antibody producing cells. Moreover, the machinery required to sustain the high biosynthetic requirements such as genes involved in unfolded protein response, was highly expressed in memory B cells compared to naïve. Our data highlights differences in activation dynamics between naïve and memory B cells and sheds light on distinct cellular states that naive and memory B cells acquire during activation.

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WS38 – IMMUNOMETABOLISM

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282 – WS38.1

Mitochondrial perturbation in immune cells enhances cell-mediated innate immunity in Drosophila

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Purpose: Mitochondria are key players in various cellular processes including energy metabolism, apoptosis, production of reactive oxygen species, stress responses, inflammation, and immunity. However, the role of mitochondrial metabolism in the innate immune responses is not yet fully understood. Our project investigates the effects of tissue-specific mitochondrial dysfunction on immune responses utilizing the fruit fly *Drosophila melanogaster* as a model.
Methods: We modelled mitochondrial dysfunction by knocking down oxidative phosphorylation (OXPHOS) complex genes in the two main immune tissues of *Drosophila*, the fat body and the immune cells (hemocytes). We used parasitoid wasp infection model to study the cell-mediated immune responses, as well as flow cytometry based assays to quantify immune cell populations and measure physiological parameters such as mitochondrial membrane potential and reactive oxygen species production. We utilized RNA sequencing to investigate the transcriptional response to OXPHOS knockdown in hemocytes.
Results: While OXPHOS disruption in the fat body was detrimental for the host viability and caused weakened immune responses, hemocyte-specific perturbation led to enhanced immunocompetence upon parasitoid infection, formation of melanotic nodules (immune cell aggregates, which are considered a sign of activated immune system) as well as immune cell activation and proliferation prior to immune challenge. Neither viability nor development of animals with hemocyte-specific OXPHOS knockdown was compromised. The hemocyte-targeted OXPHOS perturbation resulted in mitochondrial membrane depolarization and activation of the mitochondrial unfolded protein response, which is likely the reason for enhanced immune responses. The difference between the effects of mitochondrial dysfunction in these two key immune tissues is likely due to threshold effects and the tissue-specificity of mitochondrial metabolism.
Conclusion: Our results show that when mitochondrial dysfunction is mild enough, it can be beneficial in certain context, i.e. infection. We also show a link between the mitochondrial unfolded protein response and immune system activation, a phenomenon that appears to be conserved across various taxa. While the exact mechanisms are still unclear, the data we have obtained provides more insight into the tissue-specific role of mitochondrial metabolism in immune responses.

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1699 – WS38.2

Glutamate dehydrogenase-Prostaglandin E2 axis controls Treg cell development with distinct features in maintaining immune tolerance

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Purpose: Distinct tissue-derived signals control regulatory T cell (Treg) induction in tissues and shape their heterogeneity and functionality in chronic inflammation. In the inflammatory parasitic brain disease, neurocysticercosis (NCC), we have recently identified that the lipid mediator PGE2 secreted from glutamate dehydrogenase (GDH)-modulated monocyte and microglia is a central driver of Treg development, essential to control disease in asymptomatic, non-epileptic NCC patients. Here, we characterize the epigenetic and transcriptional determinants of GDH-PGE2-modulated Treg cell development and the clinical implications in brain inflammation and silencing.
Methods: Targeted lipidomics and extensive LC/MS/MS profiling of lipid mediators in controls, asymptomatic and patients with epilepsy and neurological symptoms guided the identification of disease- and GDH-remodeling of eicosanoids, precursors and metabolites. In-depth immunophenotyping and pulsing of controls' and patients' peripheral cells with recombinantly expressed parasite GDH or PGE2 revealed the context-dependent Treg development with unique features. Subsequent mechanistic pathways of lipid mediator regulation of Treg induction were elucidated by transcriptional profiling of ex vivo sorted monocytes. The epigenetic and transcriptional determinants of Treg development and landscapes in asymptomatic patients were furthermore assessed via ATACSeq and RNASeq as compared to in vitro induced Treg and ex vivo sorted Tconv from healthy individuals
Results: Targeted lipidomics revealed a bias arachnoid acid metabolism in NCC patients with a pronounced COX-PGE2 pathway in asymptomatic disease. This is associated with distinct features of enhanced CNS migration and endothelial cell adhesion potency ($CD69^{hi}$, $CCR7^{+}$, VLA-4^{hi}, LFA-1^{+}) of $ST2^{+}$Tregs, with significant reduction of CD226 expression. The marked increase of PGE2 and precursor metabolites in patients' sera is positively correlated with pronounced EP2 and EP4 receptor expression on peripheral naïve $CD4^{+}CD25^{-}$ T cells. Integrative sequencing analyses interestingly revealed the non-canonical TNFR2-NF-kB and the JAK-STAT signaling pathways as important regulators controlling GDH-PGE2-driven Treg differentiation and thus a potential role during NCC.
Conclusion: This work highlights important insights into lipid mediators as novel regulators of Treg cell development with distinct features to maintain immune tolerance in NCC with relevance for other inflammatory brain diseases.

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376 – WS38.3

Targeting amino acid uptake on NK cells to limit pathogenesis of bacterial infections

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Purpose: NK cells contribute to the pathogenesis of infections by producing IFNg, which is important for control of the pathogen, but its excessive production may lead to "cytokine storm" and tissue damage. Thus, moderating IFNg production by NK cells represents a therapeutic strategy for treatment of acute infections. Multiple studies demonstrate critical role of metabolic reprogramming for activation of immune cells. In particular, amino acid transport via Slc7a5 and glutamine uptake (mediated by Slc1a5 in NK cells) are essential for NK cell function *in vitro*. In this project we aim to test whether targeting amino acid uptake on NK cells suppresses IFNg production and thus limits pathogenesis of bacterial infections in mouse models.
Methods: We employ mouse models of peritoneal LPS-induced inflammation as well as bacterial infections with *E. coli* and *S. aureus*. Peritoneal NK cells are analyzed using flow cytometry, including click-based nutrient uptakes, mitochondrial assessment, as well as functional antibody staining. To dissect the role of amino acid transporters for NK metabolism and function, we employ mice with conditional inactivation of Slc7a5 or Slc1a5 in NK cells as well as pharmacological inhibitors of those transporters.
Results: In response to LPS challenge peritoneal NK cells underwent dynamic activation with early response (2-6 hours after LPS), indicated by upregulation of IFNg, CD69, and GzmB, as well as late response (24-48 hours after LPS), indicated by accumulation of perforin. Importantly, early response was associated with increased amino acid uptake via Slc7a5, while at the later timepoints uptake via Slc1a5 was significantly increased. Inactivation of Slc7a5 in NK cells resulted in suppressed metabolic and functional activation at both early and late timepoints, indicating crucial role of this transporter for NK activation. Furthermore, in bacterial infection models we observed a similar metabolic and functional activation of peritoneal NK cells in response to *E. coli* challenge, but not to *S. aureus* infection.
Conclusion: Altogether, our results indicate dynamic metabolic and functional response of NK cells in the context of inflammation and bacterial infections and provide novel strategy to limit pathogenic activation of NK cells via inhibiting amino acid uptake.

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1746 – WS38.4

Deciphering the immunomodulatory effects of 4-hydroxynonenal in human monocyte-derived macrophages: size does not matter if the impact is radical

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Purpose: Sepsis is a life-threatening syndrome and is a main cause of death worldwide. In 2017, 11 million people died of sepsis. Half of the 48.9 million reported cases in 2017 were children, with 2.9 million global deaths in children. Treatment and diagnostic options are limited, and morbidity and mortality remain high. Macrophages are important in sepsis. During inflammation, macrophages produce increased levels of pro-inflammatory cytokines and reactive oxygen species (ROS). ROS are essential in clearing pathogens and are highly reactive.
Lipid oxidation by ROS produces 4-hydroxynonenal (4-HNE). 4-HNE interacts with biomolecules, impacting crucial cellular processes. Considering the 4-HNE increase in inflammatory diseases, it raises the question of how 4-HNE affects the innate immune system to regulate inflammation. My project aims to identify modulatory properties of 4-HNE on human monocyte-derived macrophages (hMDM), hypothesizing that high concentrations of 4-HNE propagate inflammation as modified protein and biomolecules are expected to accumulate progressively.
Methods: Monocytes were isolated from the blood of healthy volunteers and differentiated into macrophages. Naïve macrophages were pre-treated with or without 4-HNE before adding lipopolysaccharide (LPS). RNA sequencing data were validated using reverse transcription-quantitative polymerase chain reaction. Cytokine production was measured by ELISA, and 4-HNE protein adducts and signaling were analyzed using flow cytometry and Western blot.
Results: LPS treatment significantly increased 4-HNE production. RNA sequencing revealed that 4-HNE treatment provokes a pronounced transcriptional reorganization of hMDM, including a decrease in Interleukin (IL)-10 and Interleukin-1 receptor antagonist production. Moreover, 4-HNE increased pro-inflammatory p38 signaling and blocked anti-inflammatory STAT3 activation. However, exhibited no impact on tumor necrosis factor-α and IL-6 production.
Conclusion: Thus, our data suggest that 4-HNE is an important immune system modulator that can stimulate inflammation by suppressing the IL-10 pathway. Understanding the immunomodulatory mechanisms of 4-HNE aids in understanding its role in ROS-related diseases, like sepsis, and identifying targets to restore damage by oxidative stress.

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1243 – WS38.5

Metabolic shift of human (super)antigen-induced T cells in an ex vivo lymphoid cell culture based on tonsils.

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Objective: Key metabolic processes such as glycolysis, fatty acid and mitochondrial metabolism are now recognized as crucial players in T cell activation and differentiation, and their modulation can differentially affect the development of T helper cell lineages. Especially memory T-cells (Tmem) are important for adaptive immunity since they assure long-lasting protection against pathogens, however, can also be harmful in chronic inflammation and autoimmunity. Until now, most studies addressed the metabolic phenotype of human T cells in peripheral blood, although most inflammatory and immunological processes occur in local tissues. We therefore made use of an ex vivo lymphoid cell culture based on tonsils. This ex vivo culture was recently established by the group of Baumjohann and in an analogous way by Wagar et al. and allowed us to metabolically profile memory T cells in a more physiological setting.
Methods: We metabolically analyzed CD4+ Tmem cells from adenoids after adenotomy and compared them with Tmem from peripheral blood.
Tissues from adenoids were cut and incubated as single cell cultures in well plates. They were then stimulated with different antigens including Pertussis Toxin and TSST-1.
Mitochondrial activity, fatty acid and glucose uptake and activation markers were measured using ex vivo assays and flow cytometry.
Results: Adenoids comprise of a remarkably higher CD4+ T memory population compared to peripheral blood CD4+ T cells. Fatty acid uptake is rather low with high mitochondrial activity when cells are freshly isolated in the non-activated state. Metabolism shifts upon reactivation from mitochondrial dependence to higher glucose demand. Compared to anti-CD3/CD28 stimulated cells which are fully dependent on Glucose, Pertussis Toxin and TSST-1 stimulated cells showed metabolic differences, including a higher fatty acid uptake and need for glutamine.
Conclusion: Metabolic characterization indicates a significant role of glucose dependent metabolism together with increased demands for fatty acids and glutamine during activation of adenoid derived CD4+ Tmem cells. Targeting metabolic pathways specifically in CD4+ Tmem cells can be a promising approach to improve vaccination or to treat autoimmune diseases.

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679 – WS38.6

ANGPTL3 deficiency-induced hypolipidemia triggers spontaneous interferon response

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Purpose: Familial Combined Hypolipidemia (FHBL2) is a genetic disorder characterized by low levels of plasmatic lipoproteins and caused by loss-of-function mutations in the Angiopoietin-like 3 (ANGPTL3) gene. This condition is not associated with an increased risk of immune-mediated diseases, and thus represents a unique contest to study the link between systemic lipid metabolism and the immune system.
Our purpose is to determine whether hypolipidemia impacts on monocyte energetic metabolism and interferon (IFN) response.
Methods: First, we studied monocyte frequency, lipid content and ISG15 expression *ex vivo* in FHBL2 subjects by flow cytometry. RNASeq analysis of peripheral blood mononuclear cells (PBMCs) of FHBL2 subjects was performed. To recapitulate *in vitro* the effects of poor lipid exposure on monocyte response, we cultured monocytes in conditions of LDL deprivation or supplementation. In this setting we studied the glycolytic and mitochondrial metabolism through the analysis of active mitochondrial mass, mitochondrial reactive oxygen species (ROS) production, and glucose uptake. To investigate how prenylation affects type I IFN signaling we analyzed IFNAR, STAT1 and phosphorylated STAT1 (pSTAT1) expression in presence or not of geranylgeranyltransferase I inhibitor (GGTI), to check the impact of mevalonate pathway-derived isoprenoids in immune signaling. Finally, we analyzed IL-1β production by lipid deprived monocytes in vitro in presence or not of type I IFN and/or LPS.
Results: We detected a trend for higher monocyte frequency containing lower amount of intracellular lipids in FHBL2 subjects *ex vivo*. RNASeq analysis of PBMCs of FHBL2 subjects showed that IFN and monocyte signatures were upregulated while genes encoding for mitochondrial electron transport chain were reduced.
Under lipid deprivation in vitro, monocytes showed lower levels of intracellular lipid accumulation, higher ISG15 expression and STAT1 phosphorylation. Mechanistically, lipid-deprived monocytes exposed to type I IFN showed higher glucose uptake and reduced active mitochondrial mass and mitochondrial ROS production. We observed a prenylation-dependent increase in IFNAR-STAT1 signaling and a specific suppression of IL-1β production in lipid-deprived condition.
Conclusion: In summary, we found a link among energetic metabolism and IFN response that could help understanding the immunoprotective effects of hypolipidemia.

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WS39 – NOVEL APPROACHES TO VACCINOLOGY

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1244 – WS39.1

Limited boosting and long-term immunity of SARS-CoV-2-specific CD8+ T cell responses after COVID-19 mRNA vaccination in people living with HIV

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Purpose: People living with HIV (PLWH) are at higher risk to get severely sick after SARS-CoV-2 infection and have been therefore prioritized to get the COVID-19 vaccination. However, data on vaccine-elicited immune responses in PLWH is limited, in particular, about their virus-specific T cell responses induced by vaccination. In this study we, therefore, investigated whether SARS-CoV-2 spike-specific T cells are efficiently induced in PLWH after COVID-19 mRNA vaccination. Moreover, we also examined the fate and long-term SARS-CoV-2 spike-specific T cell immunity in PLWH focusing on the most vulnerable group with CD4 counts <300 cells/µl and, thus limited CD4+ T cell help.
Methods: SARS-CoV-2 spike-specific CD8+ T cell responses were followed in PLWH (n=18), receiving antiretroviral therapy, and healthy controls (HCs; n=24) throughout 3 to 4 mRNA vaccinations and breakthrough infection. Deep profiling of SARS-CoV-2 spike-specific CD8+ T cells was performed *ex vivo* by applying peptide-loaded MHC I tetramer technology and after *in vitro* expansion and cytokine production assays. SARS-CoV-2 spike-reactive CD4+ T cells (HCs n=20; PLWH n=22) and IgG levels (HC n=26; PLWH n=26) were determined by activation-induced marker (AIM) assay and ELISA, respectively.
Results: Our studies revealed that spike-specific CD8+ T cell responses are induced early after prime vaccination in PLWH and follow similar kinetics, however with reduced amplitude, after each vaccination as in HCs. Overall, PLWH with a CD4 count <300 cells/µl exhibited lower frequencies and a restricted breadth of the SARS-CoV-2 spike-specific memory T cell response compared to HC. The dampened SARS-CoV-2 spike-specific CD8+ memory T cell response was also reflected by a diminished induction of CD8+ T memory stem cells (T_{SCM}) in PLWH. Still, the vaccine-elicited SARS-CoV-2 spike-specific memory CD8+ T cells showed robust functional characteristics after *in vitro* recall response. In contrast to spike-specific IgG titers, CD8+ T cell frequencies were not durably boosted by repeated antigen contact and remained lower in the PLWH compared to HC.
Conclusion: Taken together, our findings demonstrate that mRNA vaccination elicited limited SARS-CoV-2-specific CD8+ T cell immunity in PLWH with low CD4 counts underscoring their vulnerability and indicating the potential requirement of different vaccination/boosting strategies.

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433 – WS39.2

Time of day influences the CD8+ T cell response to mRNA vaccination

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mRNA vaccines have recently emerged as a promising vaccine technology. $CD8^+$ cytotoxic T cells can play a central role in the immune response following mRNA vaccination and form protective immunological memory. Despite increasing evidence for circadian rhythmicity in $CD8^+$ T cell immunity, it remains unknown how the time of immunization affects the T cell response following mRNA vaccination. To investigate this, mice were vaccinated with the Spikevax SARS-CoV-2 vaccine at six different times across day and night and the Spike-specific $CD8^+$ T cell response was analyzed. We observed 24-hour rhythmicity in the expression of effector differentiation markers on Spike-specific $CD8^+$ T cells, with the highest expression after vaccination during the resting phase. Correspondingly, in animals vaccinated during the resting phase, Spike-specific $CD8^+$ T cells exhibited the highest capacity to produce IFN-γ upon restimulation. As costimulatory signals provided by dendritic cells (DCs) during T cell priming are known to shape effector T cell formation, we assessed the contribution of the molecular clock of DCs using a DC-specific conditional knock-out of the transcription factor BMAL1, which is essential for maintaining molecular circadian rhythms. We find that the time-dependent effects on $CD8^+$ T cell differentiation rely on the DC clock, indicating that a DC-intrinsic mechanism regulates rhythmic T cell responses. We show that CD80 and CD86, ligands for the costimulatory receptor CD28, regulate Spike-specific $CD8^+$ T cell differentiation and function. However, CD80 and CD86 expression does not exhibit 24-hour rhythmicity and the effect of vaccination time on T cell differentiation is maintained upon CD80 or CD86 blockade, indicating that CD28 ligation does not mediate time-dependent alterations in $CD8^+$ T cell responses. Collectively, these data demonstrate that the time of mRNA vaccination affects the effector quality of vaccine-specific $CD8^+$ T cell responses and provide insight into the underlying mechanisms. This could facilitate the development of a strategy in which timed administration is used to increase the protective capacity of mRNA vaccination.
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569 – WS39.3

Immunization with BM325, a preS-based recombinant grass pollen allergy vaccine can overcome non-responsiveness to HBsAg-based hepatitis B virus vaccines

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Purpose: Non-responsiveness to HBsAg-based vaccination against hepatitis B virus (HBV) exceeds 10%. BM32 is a recombinant grass pollen allergy vaccine consisting of the HBV-derived preS antigen and hypoallergenic peptides from four major timothy grass pollen allergens. We have shown previously that allergic patients who received allergen-specific immunotherapy with BM32 developed antibodies capable of neutralizing HBV *in vitro*. Here we investigated if immunological non-responsiveness can be overcome by immunization with BM325, a component of BM32 in a subject with more than 30 years history of unsuccessful HBV vaccination.
Methods: A subject with a history of repeated vaccinations with HBsAg-based vaccines without developing HBV-specific immunity received five monthly injections of 20 microgram BM325 adsorbed to Aluminum hydroxide. preS-specific antibody responses were measured using preS and preS-derived peptides by ELISA and microarray technology. preS-specific T cell responses were measured by CFSE staining. HBV-neutralizing capacity was determined using cultured HBV-infected HepG2 cells.
Results: Subcutaneous vaccination with BM325 induced a strong and sustained preS-specific IgG response peaking already after the third injection. The antibody response was composed mainly of the IgG_1 subclass which reached 1.9 mg/ml preS-specific IgG_1 one month after the immunization and remained at a level of 0.7 mg/ml one year after the treatment. preS-specific IgG antibodies were mainly directed to the N-terminal part of preS which contains the attachment site of HBV to its receptor Na+-taurocholate co-transporting polypeptide (NTCP). IgG reactivity to peptides from the NTCP attachment site was comparable for HBV genotypes A-H. Continuous assessment of the preS-specific T cell proliferation showed a pronounced reactivity of CD3+CD4+ lymphocytes after the complete injection course which was still present one year after the last injection. The induction of preS-specific cytotoxic CD8+ T cells was low. Maximal HBV neutralization (98.4%) was achieved 1 month after the 5^{th} injection which correlated with the maximal IgG reactivity to the N-terminus of preS.
Conclusion: Our data suggest that the BM325 vaccine may be used as a preventive vaccination against HBV even in non-responders to HBsAg-based vaccines.

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1867 – WS39.4

Development of an immunocompetent 3D-bioprinted skin-on-chip model

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Due to the complexity of the skin, the development of *in vitro* models to study skin biology is challenging. Commonly used human skin equivalents consist of collagen with fibroblasts topped with epidermal layers composed of keratinocytes. We are interested in developing a 3D-bioprinted skin-on-chip model that will contain dendritic cells (DC) to develop an immunocompetent skin model that can be used as surrogate for human skin and replace animal models in future.
Currently, we are developing different chip designs allowing bioprinting of a dermal compartment containing fibroblasts in a gelatin-methacrylate (GelMA) mix as bio-ink. On top of this dermis we microjet-print a keratinocyte cell layer that after airlift develops into a multi-layered differentiated epidermis. We also investigated how DC can be embedded into the dermal compartment. For this purpose, we mixed human blood monocytes or in vitro differentiated monocyte-derived DC (moDC) together with fibroblasts in the GelMA bio-ink that upon blue light exposure polymerizes forming stable hydrogels. With flow cytometry analysis we investigated viability, differentiation and maturation of DC in hydrogels.
First of all, we observed that moDC stayed viable in hydrogels and displayed an immature phenotype when analyzed by flow cytometry and could be activated within hydrogels by addition of a maturation stimulus. When CD14+monocytes were differentiated by GM-CSF and IL-4 in the hydrogels for a week, they upregulated HLA-DR but still showed some low level of CD14, resembling an inflammatory monocyte or the recently described DC3. These preliminary results suggest that moDC are viable and monocytes can be differentiated into DC in hydrogels.
In the next step, we will add moDC and CD34+derived DC subtypes into our 3D-bioprinted skin-on-chip model to establish an immunocompetent skin model. The differentiation of DC from monocytes but also CD34+precursors directly within the bioprinted skin model will be examined. In future we hope to use the immunocompetent human skin model for drug testing and vaccine developments.

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2228 – WS39.5

Skin routes of immunization with Langerin-targeted HIV1-gp140 vaccine impacts on germinal center activation and tailors antibody response

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Background: Targeting dendritic cells (DCs) with antigens is a promising strategy for modulating T follicular helper (Tfh) cells and germinal center (GC) reactions in lymphoid organs, enhancing the specific adaptive immune response to vaccines. Preclinical studies from our lab and others suggest a major role of Langerhans cells (LC) for inducing HIV-1 specific antibody responses. Our study explores: i) the immunogenicity of a Langerin-targeting vaccine consisting of an anti-Mouse Langerin mAb fused to HIV-1/gp140ZM96 (Env) (aLang.Env, Kervevan PLoS Path 2021) administered through different routes of skin immunization in mice; ii) the role of epidermal LC versus dermal cDC1s.
Methods: The lymph nodes (LN) draining each site of immunization were first assessed in C57BL/6 mice with ovalbumin (OVA) surrogate antigen after topical application (t.a.) or administration by subcutaneous (s.c.), intradermal (i.d.) and transcutaneous (t.c.) route through laser-guided microporation (PLEASE technology). The aLang.Env mAb (10mcg equivalent of Env) was administered without adjuvant in a Prime-Boost scheme (day 0 and day 21). Serum Env IgG titers were followed by Luminex overtime and GC/Tfh reactions were assessed by FACS phenotyping of dLN (day 28).
Results: All methods of administration proved effective, as evidenced by the proliferation of OVA-specific CD8+ T cells (OT-I) and CD4+ T cells (OT-II) in the dLN. While aLang.Env induced Tfh cells, GC B cells, and Env-specific GC B cells regardless of the immunization route, only the intradermal route resulted in a significant increase in systemic Env-specific IgG response. The GC/Tfh reaction and humoral responses through i.d. route were further validated in the Xcr1DTA mouse model, where cDC1 cells were depleted, suggesting that epidermal LCs were the primary DC subsets triggering these responses. However, we observed a reduction in effector CD8+ T cells in the absence of dermal cDC1.
Conclusion: Our study finds that i.d administration of HIV-1 Env antigen targeted to Langerin without adjuvant leads to GC reaction in proximal lymph nodes, and effective Ab responses. This effect was specifically related to the target of LCs since depletion of cDC1 did not impact humoral responses whereas $CD8^+$ effector response were predominantly mediated by dermal cDC1.

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517 – WS39.6

Exploring cross-functionality elicited by altSonflex1-2-3 vaccine candidate in different animal models

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Purpose: *Shigella* is a major cause of mortality especially in children in low-middle income countries (LMICs) and has been prioritized among the most dangerous antibiotic-resistant pathogens by the WHO. The O-antigen (OAg) component of the lipopolysaccharide is considered a protective antigen, posing challenges to vaccine development since more than 50 *Shigella* OAg structures have been identified. No vaccine eliciting broad protection against Shigellosis is currently avaiable. GVGH has developed a 4-component GMMA-based vaccine, altSonflex1-2-3, covering *S. sonnei* and *S. flexneri* 1b, 2a and 3a, identified among the most prevalent serotypes in LMICs.
Aim of this work is to investigate the ability of altSonflex1-2-3 vaccine to elicit cross-functional antibodies in different animal models and to match preclinical and clinical data. Sera from mice, rats and rabbits immunized with altSonflex1-2-3 vaccine candidate have been examined for their ability to bind to and kill relevant *S. flexneri* subtypes different from those included in the vaccine.
Methods: Animal sera were analysed by a Luminex-based multiplex binding assay and by FACS-based binding assay for the evaluation of antibody binding to purified OAg and to whole bacteria.
The functionality of antibodies present in sera from animals and human subjects was assessed using a high-throughput luminescence-based Serum Bactericidal Assay.
Results: Results clearly show ability of the antibodies elicited by the altSonflex1-2-3 vaccine to bind to and kill the tested *S. flexneri* X, Y, 6, 4a and 5b strains due to serotype-common epitopes within the OAg structure of the different *S. flexneri* serotypes. The 4-component vaccine shows a broader coverage compared to each corresponding monovalent formulation, confirming that all components included in the vaccine are necessary to achieve a good coverage. Numerically the relative level of bactericidal activity observed is different in the different animal species both against the vaccine homologous and heterologous strains.
Conclusion: This study demostrates the potential for altSonflex-1-2-3 vaccine to be broadly-protective. It will be important to understand if the preclinical results will be confirmed in humans, thus allowing validation of preclinical results with clinical data.

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WS40 – BIOINFORMATICS IN IMMUNITY AND DISEASE

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2055 – WS40.1

Unveiling tissue niches: Integrated analysis of gut resident T cells in humans using single-cell technologies

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Purpose: The gastrointestinal tract contains a vast population of T cells, localized in the Gut-Associated Lymphoid Tissue (GALT), draining lymph nodes, and scattered in the lamina propria, and intestinal epithelium. These cells play crucial roles in promoting tolerance to dietary antigens and microbiota, as well as mounting protective immune responses against pathogens. Despite their importance, our understanding of the development, maintenance, and functional adaptations of diverse populations of gut resident T cells remains limited. While studies in mice have shed light on intestinal T-cell development and specialization, translating this knowledge to humans presents significant challenges. To address this gap, we constructed an integrated atlas of the T cell compartment across the human gastrointestinal tract.
Methods: Integrating publicly available datasets with newly generated multiomics single-cell and spatial data, we compiled an atlas spanning the entire intestinal tract, including different site-specific gut lymph nodes. We incorporated newly generated single-cell data from a unique transplanted setting allowing us to track resident T-cell maturation over time. In addition, we performed scRNA-seq from different intestinal regions from matched donors and included paired TCRαβ and TCRγδ sequencing to study the clonotype dynamics and T cell trafficking across regions.
Results: In summary, we have integrated a multiomics single-cell atlas of intestinal T cells providing a comprehensive analysis across regions of the gastrointestinal tract. Our analysis reveals unique regional adaptations and high degree of specialization, dissecting the heterogeneity of different populations of circulating and resident T cells, regulatory T cells and innate-like T cells.
Conclusion: A better understanding of the heterogeneity and regional adaptations of intestinal T cells holds promise for identifying therapeutic targets for inflammatory gut diseases and advancing vaccines and cancer immunotherapies aiming at the intestinal mucosa.

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2161 – WS40.2

The frail immune system: a frailty DNA methylation surrogate in circulating immune cells predicts preclinical ageing and future clinical outcomes.

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Demographics are globally shifting towards older age. Frailty is an age-related state of functional decompensation characterized by decreased reserve and heightened vulnerability to stressors. Frailty encompasses physical, cognitive, and psychosocial domains. It also captures population-level immune variability associated with accelerated biological ageing, as indexed by inflammation, reduced anti-viral immunity, and vaccination efficacy. As populations age, variability increases in population health and immunity, underscoring the need to dissect underlying relations between frailty and immune dynamics.
We hypothesise that modelling population frailty dynamics using large-scale, longitudinal clinical data and system-wide immune metrics will facilitate profiling of the ageing immune system and assessment of immune-mediated ageing, particularly among individuals at higher risk of later-life poor health compared to peers of the same age with similar lifestyles.
This study utilizes extensive clinical and immune data collected during a 10-year, nationally-representative study, The Irish Longitudinal Study on Ageing (TILDA, n = 8,500; age 50y+). Elastic net modelling was employed to derive an epigenetic frailty surrogate using immune cell epigenome-wide methylation profiling and a validated 31-item Frailty Index. The frailty surrogate was then evaluated in Milieu Intérieur (MI, n = 1,000; age 20 – 69y) and public datasets as a predictor of accelerated ageing and used to investigate the ageing immune landscape.
The immune-based frailty surrogate consistently predicted frailty in test datasets (R = 0.46, $P = 2.2 \times 10^{-10}$), and fully adjusted models. The frailty surrogate identified non-frail individuals at risk of 10y frailty transitions. In independent human cohorts including MI, this immune-based frailty signature reflected variability in the methylome, circulating cytokine profile and viral infection status, identifying immune changes associated with accelerated ageing. Additionally, the score predicted health status at 10y follow-up in this healthy, mid-life population. Alongside modelling frailty transitions, comorbidities, and mortality in TILDA, the immune-based frailty surrogate characterised cellular changes associated with accelerated ageing in MI, including a strong neutrophil signature (β = 0.21, $P = 1.1 \times 10^{-5}$). Ongoing analyses are investigating viral infections in immune-mediated ageing. Overall, this research aims to leverage longitudinal clinical, epigenetic, and immune profiling to understand why the ageing process differentially manifests among members of society.

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244 – WS40.3

Diffusion-limited cytokine signaling in T cell populations

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Purpose: Effective immune-cell responses depend on collective decision-making mediated by diffusible cytokines. However, it remains poorly described how cytokines are spatially distributed in tissues, and how paracrine signaling efficacy is affected.
Methods: Based on response-time modeling [1], we designed a three-dimensional spatio-temporal modeling framework, to systematically investigate the origin and consequences of spatially inhomogeneous cytokine distributions in lymphoid tissues [2]. Further, we developed an open source, modular and scalable software package for quantitative analysis of CODEX multiplexed histology data.
Results: We found that spatial cytokine inhomogeneities are critical for effective paracrine signaling, and they do not arise by diffusion and uptake alone, but rather depend on properties of the cell population such as an all-or-none behavior of cytokine secreting cells. Furthermore, we assessed the regulatory properties of negative and positive feedback in combination with diffusion-limited signaling dynamics, and we derived statistical quantities to characterize the spatio-temporal signaling landscape in the context of specific tissue architectures. Our software package for processing multiplexed histology data allowed to derive cell-segmentation and subsequent feature annotation to individual cells for complex tumor tissue, which was not possible with available methodology and will set the stage for comparison to mathematical models.
Conclusion: Our simulations highlight the complex spatiotemporal dynamics imposed by cell-cell signaling with diffusible ligands, which entails a large potential for fine-tuned biological control especially if combined with feedback mechanisms. Moreover, insights from spatio-temporal simulations will be critical for interpretation of multiplexed histology and spatial transcriptomics data sets such as from lymphoid tissues and the tumor microenvironment.

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535 – WS40.4

Deep immune phenotyping of the ageing immune system

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Purpose: Population ageing is a prominent feature of the global demographic change. Elderly people represent a vulnerable population group with an increased risk for infections, cancers, and other diseases. Studying the ageing immune system is important to understand biological ageing and age-related diseases, but challenging due to the high number of immune parameters and large inter-individual variability.
Methods: We conducted an in-depth immune system profiling of 550 elderly participants (≥60 years) and 100 young controls (20–40 years) of the RESIST Senior Individuals (SI) cohort. Participants were randomly selected via the local residents' registry office. We collected extensive demographical, clinical, and laboratory data and performed multi-color spectral flow cytometry and 48 plasma cytokine multiplex assays for a deep immune phenotyping, and analyzed the data using an unsupervised, computational clustering approach. This was complemented by a multi-omics integration analysis to jointly analyze cytometry, cytokine, and laboratory data.
Results: Our analysis approach allowed us to study 97 distinct innate and adaptive immune cell populations. We monitored their frequencies across ages and related them to clinical, laboratory, and cytokine data. Substantial age-related changes were observed in selected cytokines (e.g., CCL27 and CXCL10), and in both innate and adaptive immune cell compartments. The in-depth profiling revealed that often particular subsets within sub-lineage cell populations displayed age-related changes, while others remained stable, e.g. only two of five naïve CD4+ T cell clusters decreased with age. Furthermore, known associations (age, sex, CMV status, smoking) could be assigned to individual of the 97 immune cell subpopulations with high precision and novel associations (e.g., gout, COPD, obesity) were unveiled. Finally, using the multi-omics integrative analysis to combine the different datasets, we could identify immune signatures for common age-related diseases such as heart failure, gout and many others, enabling a systems biology view on disease-associated changes.
Conclusion: This study is one of the largest immune profiling studies conducted so far and the first one to focus on the elderly immune system. We provide a high-resolution picture of age-related immune system changes and unveil important associations with other clinical and laboratory data as well as diseases.

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245 – WS40.5

Pancreas-infiltrating T cells in Type 1 diabetes: an integrated investigative approach using spectral flow cytometry and bioinformatics analysis

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Type 1 diabetes (T1D) is an autoimmune disorder in which immune cells target the insulin producing β cells of the pancreas leading to hyperglycaemia. CD28 co-stimulation blockade has been tested as a therapeutic for T1D with mixed clinical results. A better understanding of the T cell populations present at the site of the autoimmune attack, and the extent to which these populations are sensitive to co-stimulation blockade or other interventions, would be helpful in designing approaches to inhibit T1D development.
In this study, we have compared mice that develop diabetes with their non-diabetic littermate controls over the timecourse of disease development. We designed and optimised two 33 colour spectral flow cytometry panels to perform detailed immunophenotyping of pancreas-infiltrating T cells. Diabetic mice were also treated with co-stimulation blockade reagents to assess the dependence of different immune populations on CD28 signalling.
The traditional method of manual gating proves to be insufficient for effectively analysing the complex datasets generated by spectral flow cytometry. Here, we have optimised a bioinformatics pipeline for the identification of novel pancreas infiltrating T cell populations and for the assessment of their sensitivity to co-stimulation blockade. After quality control of the data, we perform unbiased cell clustering and use marker enrichment modelling analysis for cell identification and explore cell population dynamics with differential abundance and state analysis. We further validate our results using an algorithm-guided gating strategy.
Overall, we have developed a workflow for the detection of candidate T cell populations in T1D and highlight the importance of using bioinformatic tools for the analysis of large spectral flow cytometry datasets.

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672 – WS40.6

Dissecting chromatin regulatory landscape during B cell activation

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Impaired B cell activation results in high susceptibility to infection, immune deficiency and autoimmune diseases. Genetic variants linked to immune-mediated diseases show enrichment in enhancers and promoters specifically active in B cell activation. Considering the dynamic nature of B cell activation and the diverse population of B cells, we mapped gene expression regulation and chromatin accessibility using single-cell transcriptomics (scRNA-seq) and single-cell sequencing assay for transposase-accessible chromatin (scATAC-seq) across three different stages of B cell activation. We stimulated human naïve and memory B cells with anti-IgM, CD40L and a cocktail of cytokines and performed immune profiling in the early and late stages of activation. The stimulation cocktail triggered B cell activation, proliferation and antibody class switching *in vitro*. As expected, the majority of activated memory B cells proceeded to later cell divisions than naïve B cells. Furthermore, memory B cells expressed a higher level of antibodies, suggesting a higher effector capacity. The chromatin accessibility and transcriptomic analysis of 64.513 high-quality cells demonstrated that a large number of transcriptional changes are detected in the early time point of activation. In addition, naïve and memory B cells clustered separately along activation, suggesting different differentiation paths. This demonstrated a rapid activation of B cells following activation, and a large diversification of activated naïve and memory B cells. Finally, to investigate transcription factor activity and gene-regulatory networks driving the differences in the activation responses between naïve and memory B cells we linked peaks of open chromatin to the activation-induced genes. Our results demonstrated cell type specific chromatin elements affecting differences in gene expression in naïve and memory B cells. This functional map of active chromatin regions, coupled with gene expression dynamics will inform prioritisation of autoimmunity-associated genetic variants and provide further insight into the dynamics of naïve and memory B cell activation.

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WS41 – MOLECULAR MECHANISMS IN INNATE IMMUNITY

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754 – WS41.1

The human leukocyte antigen (HLA) locus harbors translated lncRNAs with immunomodulatory functions

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The evolution of the human leukocyte antigen (HLA) locus has helped promote immune adaptation specific to the human species. However, for many recently evolved genes in this locus it remains unknown whether they exert immune-related functionality. These include 72 long noncoding RNAs (lncRNAs) with unknown function and coding potential. Here, we combined RNA-seq and Ribo-seq data from human tissues and cell lines to detect new coding sequences in the HLA locus. We found 18 lncRNA-ORFs translated from 7 out of 72 lncRNAs and observed that the putative microproteins produced from these ORFs emerged *de novo* during primate evolution. One key example is a 42-amino acids long, and potentially secreted, alpha-helical microprotein, which shares genomic regulatory elements and a signal peptide nearly identical to classical HLA proteins. Silencing of this microprotein in immune cells impacts inflammatory cytokines production, such as IL-12, as well as the expression of the immune checkpoint receptor ligand PD-L1 and HLA class I proteins. Our work illustrates that evolutionarily dynamic genomic loci can harbour novel, potentially human-specific regulatory factors that modulate immune cell functions critical to cancer recognition by the immune system.

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447 – WS41.2

Study of the non canonical signalling induced by the binding of small molecules to CXCR4

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Purpose: The chemokine receptor CXCR4 is historically known to be involved in cell migration and adhesion. Our recent reports describe CXCR4 as a potentially promising target in immune regulation. Indeed, small molecules targeting the minor pocket of CXCR4, called minor pocket agonists (MiPAs), have been demonstrated to have anti-inflammatory activities, in particular reversing pathogen or small molecule mediated toll-like receptor (TLR) activation. This anti-inflammatory effect is probably mediated through a non-canonical signalling pathway that is further explored in this study.
Methods: The broad-spectrum inhibitory effect of MiPAs was confirmed by monitoring IRF7 phosphorylation using flow cytometry and NF-kB activation via HTRF following TLR activation with a TLR7/8 ligand. RNA silencing techniques were employed to identify potential key components.
Phosphoproteomics and cytometry by time of flight (CYTOF) techniques were utilized to get a broader view of cell activation states. A phosphoproteomics study examined the phosphorylation status of proteins in the presence and absence of MiPAs at different time points. Similarly, a panel of proteins involved in immune regulation was investigated using CYTOF. These techniques will allow us to identify prominent targets, subsequently validated by western blotting, flow cytometry and RNA silencing.
Results: MiPAs induced a reduction in IRF7 and NFkB phosphorylation, confirming their broad-spectrum anti-inflammatory effect. The phosphoproteomics study revealed approximately 100 proteins with altered phosphorylation status between treated and untreated conditions. Key targets identified were validated using western blotting and flow cytometry. CYTOF on PBMCs allowed us to get a clearer picture of the proteins involved in the TLR pathway under different treatments.
Conclusion: Our results indicate that MiPAs downregulate both TLR7/8 mediated phosphorylation of transcription factors such as NFkB and IRF7. This regulation of innate immune cell activation by MiPAs appears to be activated through a non-canonical pathway. Phosphoproteomics and CYTOF studies provided initial insights into possible key proteins involved in this pathway. Future efforts should focus on identifying points of cross-talk between the CXCR4 and TLR pathways.

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1553 – WS41.3

Concentration dependent pro- and anti-inflammatory signaling by the SCFA butyrate

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Butyrate is a four-carbon short-chain fatty acid produced from microbial fermentation of dietary fibers in the colonic lumen. Current studies show anti-inflammatory properties of butyrate and suggest that it might have therapeutic applications in inflammatory bowel disease (IBD) and colonic cancer. The widely recognized molecular mechanisms causing the anti-inflammatory effects of butyrate include histone deacetylase (HDAC) inhibition and activation of G protein-coupled receptors (GPCRs). However, recently, butyrate has been reported to also bind to the transcription factor PPARγ, which might also result in immunomodulatory effects. Therefore, we hypothesized that butyrate activates PPAR signaling in human monocyte-derived macrophages of the immune system. Indeed, RNA-seq showed that butyrate induced the expression of PPARγ and CD36, a well-known gene downstream of PPAR signaling. In line with literature, ELISA showed that low concentrations of butyrate (0.1-1mM) can suppress production of the proinflammatory cytokine TNF-α by blood-derived macrophages. This was at least partly attributable to PPAR signaling, because the immunosuppressive effect of low concentration of butyrate was reversed by knockdown of PPARγ. However, high concentration of butyrate (10mM) surprisingly promoted the expression of TNF-α and IL-1β. Furthermore, the inhibition of the GPCR signaling pathway alleviated the secretion of IL-1β induced by 10 mM butyrate, but had no effect on TNF-α. Instead, our data show that butyrate promoted TNF-α production through CD36 and SRC signaling. Ultimately, the divergent immunomodulatory effects of butyrate on macrophages may be the result of a combination of mechanisms. As the concentration of butyrate varies in different regions of the human gut, and the concentrations of butyrate in the gut and tissues depend on health and disease, exploring the immunological mechanisms of different concentrations of butyrate will be helpful for the treatment of intestinal diseases and peripheral inflammation.

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340 – WS41.4

Identification of novel interactors of T cell specific adaptor protein TSAd - an adaptor molecule with elusive function

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Signals from the extracellular milieu are received via cell surface receptors and relayed along a complex network of interconnected intracellular signalling pathways. T cell functions depend on tight regulation of these signalling pathways to respond in a flexible, dynamic and adequate manner. Adaptor proteins play a central role in modulating and fine-tuning intracellular signalling pathways by bridging protein-protein interactions, one of which is T cell specific adaptor protein (TSAd). Following activation of naïve T cells, TSAd is upregulated and believed to influence T cell receptor signalling by modulating Src family tyrosine kinase activity. TSAd-deficient mice display accelerated allograft rejection, suggesting that TSAd mediates regulatory T cell activity. However, the defined role of TSAd in T cell activation and function and the molecular details of the underlying mechanisms remains elusive. Here, we employ two independent approaches with an aim to better understand TSAd biology in an unbiased manner. Firstly, using affinity-purified mass spectrometry (AP-MS) and reverse genetics, we identified two novel ligands of TSAd, namely DOK2 and PTPN11. Binding of these ligands to TSAd was dependent on a functional TSAd Src homology 2 (SH2) domain binding to DOK2-pTyr402 and PTPN11-pTyr580 respectively. The interactions were further characterised and validated on the cellular level using bimolecular fluorescence complementation (BiFC) and proximity ligation assay (PLA). Secondly, using a publicly available dataset of healthy PBMCs, we identified genes co-expressed with TSAd. In particular, DOK2 expression correlated with TSAd expression in lymphocytes. Using CRISPR/Cas9 mediated ablation of TSAd and/or its ligands in primary T cells, we are currently exploring the affected signalling pathways and downstream events that these interaction partners are implicated in.

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1769 – WS41.5

Peroxiredoxin 4 links redox homeostasis to innate antiviral immunity

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Purpose: Viral infection can lead to cellular oxidative stress through excessive production of reactive oxygen species (ROS). ROS homeostasis, regulated by ROS production and antioxidant enzymes, is crucial for modulating cell signalling and avoiding cell damage. ROS also appear to have important functions in regulating the innate immune response. However, how the redox system and the antioxidant enzymes are involved is not fully understood. Here we sought to determine how the antioxidant enzyme peroxiredoxin 4 (PRDX4) modulates RNA sensing and activation of the antiviral response.
Methods: We analysed publicly available RNA-seq and proteomic datasets of an *in vivo* hepatotropic infection model (LCMV) to identify differentially expressed genes involved in redox homeostasis. Using *in vitro* models (HEK293T and Huh7 cells) we assessed how PRDX4, whose expression was decreased in the viral infection model, modulates the antiviral response. We measured type I and III interferons, inflammatory cytokines, and interferon stimulated gene (ISG) expression upon stimulation with dsRNA or viral infection with Dengue virus in PRDX4-silenced cells.
Results: We found that expression of several antioxidant enzymes was altered following LCMV infection. PRDX4 was among those mostly strongly decreased at both the gene expression and protein level. Our in vitro studies showed that PRDX4 silencing leads to an increased antiviral response after stimulation with dsRNA or infection with the RNA virus Dengue. Moreover we found that PRDX4 silencing did not alter the activation of dsRNA sensors such as protein kinase R or RIG-I-like receptors, but it increased the IRF3 and IRF7-mediated interferons induction. Mechanistically, we found that PRDX4 deficiency upregulates ROS levels which lead to increased Activator Protein 1 (AP-1) activation, acting as enhancer for type I and III interferons expression and subsequent ISGs induction.
Conclusions: In our study we identified a novel role for the antioxidant enzyme PRDX4 in restraining the antiviral immune response by limiting AP-1 activation. Our results highlight the possibility of targeting PRDX4 as an antiviral immunomodulator.

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1154 – WS41.6

A role for Natural Killer cells in establishment of non-reactivating latent HSV-1 reservoirs

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Herpes simplex virus type 1 (HSV-1) is a common neurotropic human pathogen that establishes a life-long latent infection in sensory neurons. Recurrent outbreaks of a diverse range of disease stems from reactivation of latent reservoirs. Experimental HSV-1 vaccines elicit robust antibody responses, but are yet to achieve beneficial clinical outcomes, while available antiviral drugs offer only partial protection. Therefore there is a need to broaden our understanding of mechanisms that govern the latency and reactivation process; how to control reactivation may be crucial for developing effective vaccines or therapies. Using our clinically-relevant HSV1 mouse model, and reactivation triggering by explant culture, we previously defined a protective state that provokes the establishment of non-reactivating latent reservoirs. This is elicited by primary infection of mice with a thymidine kinase-deficient (TKdel) mutant before virulent WT challenge. Non-reactivating reservoirs of persisting HSV1 genomic DNA differ from classical latent reservoirs, by a lack of latency-associated mRNA transcription and loss of T cell accumulation. Herein we demonstrate an essential role for a subset of NK cells in the process that elicits non-reactivating latent reservoirs. Their depletion rescued the ability of challenge HSV1 to reactivate in TKdel-primed animals, which also induced a compensatory increase in B and CD4 T cell responses during early challenge. Phenotypic and functionality of NK cells compared between establishment of different latent states. Meanwhile, TG proteome analysis during the establishment of the different latent HSV1 reservoirs identified novel proteins and biological pathways associated with establishment of non-reactivating vs classic latent HSV1 reservoirs, and changes in the absence of the NK subset. These included suppression of inflammatory, metabolic and wound healing/repair pathways of the establishing classic reservoir, to pathways involving mRNA message and signalling regulation during non-reactivating latent reservoir establishment. This study demonstrated that TK-deficient primary HSV1 infection elicits NK cell-dependent immune responses during acute-phase challenge that dictate the reactivatability of establishing latent HSV1 reservoirs. These findings have direct implications for understanding the mechanisms that govern the latency and reactivation process, and their exploitation to extinguish HSV1 reactivation.

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WS42 – NK-BASED CANCER IMMUNOTHERAPIES

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1921 – WS42.1

CAR-NK cell therapy combined with checkpoint inhibition induces a NKT cell response in glioblastoma

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Background: Glioblastoma (GB) is the most common and aggressive primary brain tumor with limited efficacy of standard therapy. Analysis of the GB tumor microenvironment (TME) has shown prominent immunosuppression with PD-L1 expression on glioma cells and exhaustion of infiltrating immune cells. In early-stage tumors from the syngeneic GL261/HER2 mouse model, monotherapy with off-the-shelf NK-92/5.28.z CAR-NK cells targeted against HER2 was highly effective. However, CAR-NK cell monotherapy did not overcome the pronounced immunosuppressive TME of large tumors. Hence, we explored if combination of CAR-NK cells and anti-PD-1 checkpoint inhibition (ICI) induces tumor regression in advanced tumors.
Methods: Combination therapy with systemic ICI and local CAR-NK cell treatment were explored in subcutaneous and orthotopic GL261/HER2 tumors. Effects on tumor growth and survival were determined. The TME and infiltrating lymphocytes were characterized with highplex multi-spectral flow cytometry, multispectral histology and bulk RNAseq.
Results: Combination therapy with CAR-NK cells and systemic ICI resulted in strong synergistic effects, with tumor regression and long-term survival observed even in advanced-stage tumor-bearing mice. Analysis of tumor tissues revealed alterations in lymphocyte infiltration and TME upon combination therapy. Specifically, infiltrating lymphocytes displayed a more activated phenotype with predominance of NKT cells and reduction of regulatory T cells.
Conclusions: The data obtained demonstrate that efficacy of HER2-specific CAR-NK cells is increased in large tumors by combination with checkpoint blockade. The combination therapy reverted the immunosuppressive TME, resulting in successful treatment even of advanced tumors refractory to CAR-NK monotherapy. We are currently translating this combination therapy concept into early clinical testing by including a respective patient cohort in our ongoing CAR2BRAIN phase I clinical trial (NCT03383978).

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289 – WS42.2

Defective NK cell function in metastatic melanoma patients underpinned by metabolic abnormalities

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Metastatic Melanoma (MetMel) is an aggressive cancer with a poor 5-year survival rate of less than 30%; however, the introduction of immunotherapy has contributed to dramatic improvements in survival. Previous data from our group demonstrated NK cell dysfunction in the circulation of metastatic breast cancer patients including reduced IFN-γ production and cytotoxicity, along with deficits in NK cell metabolism. Identifying NK cell dysfunction in other cancers such as MetMel is integral for the introduction and successful implementation of new NK cell-based immunotherapeutic approaches. We hypothesised that patients with MetMel have dysregulated circulating NK cell phenotype, function and metabolism.

Peripheral blood samples were collected from newly diagnosed MetMel patients prior to commencement of treatment, and healthy donor (HD) controls. PBMC were isolated and stimulated with IL-2 or IL-12/15 for 18 hours and profiled by flow cytometry or confocal microscopy.

Circulating NK cell frequencies were significantly lower in MetMel patients while the proportion of $CD56^{dim}$ and $CD56^{bright}$ NK cells between HD and MetMel patients was not different. MetMel patient NK cells upregulated activation markers CD69 and CD25 in response to cytokine, however their production of IFN-γ and TNF-α were significantly diminished. Furthermore, NK cell degranulation following stimulation was significantly lower on MetMel patient NK cells compared to HD. Upregulation of CD98, a nutrient receptor, was not significantly increased following stimulation with IL-12/15 on MetMel NK cells relative to HD NK cells. These parallel decreased mitochondrial mass and hints at mitochondrial dysfunction in these patients as mitochondrial mass and mitochondrial membrane potential do not correlate in MetMel patients.

Our data demonstrate that NK cells two key functions; production of cytokines and cytotoxicity are dysfunctional in MetMel patients. Our data provide the first indications that NK cell dysfunction in the circulation of MetMel patients extends to metabolic defects and highlight the need to explore methods to restore NK cell function in these patients. These data highlight some general and cancer specific facets of NK cell dysfunction in metastatic cancers and emphasises the need to identify methods to target these mechanisms to improve NK cell functionality in metastatic cancer patients.

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1734 – WS42.3

Targeting intrinsic inhibitory checkpoints using nano-carriers to unleash NK cell anti-tumor activity

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Purpose: Current Natural Killer (NK) cell-based immunotherapy relies heavily on adoptive transfer and ex-vivo manufacture of NK cells, which has major limitations in achieving therapeutic impact. Moreover, NK cells express multiple inhibitory checkpoint receptors. Therefore, even if a given receptor is effectively blocked, NK cells may still be inhibited via alternative pathways, compromising the efficiency of this approach. These current limitations call for novel approaches for targeting prevailing intracellular inhibitory signaling cascades shared by multiple surface inhibitory receptors to unleash NK cell's anti-tumor responses.
Methods: In this study, we developed novel non-viral lipid-based nanoparticles (NPs)-based delivery system encapsulating small interfering RNAs (siRNAs) targeting three key negative regulatory genes SHP-1, Cbl-b, and c-Cbl. We validated the cytotoxic potential of the modified NK cells by degranulation, granzyme B, and killing assays. Furthermore, we established an in vivo system to assess the efficacy of these NPs in activating NK cells.
Results: We demonstrate that these NPs effectively enhance NK cell activity against HLA-matched cancer cells. Our data demonstrates the potential of NPs treated NK cells to exhibit increased cytotoxicity and moreover these NPs also provide an effective in vivo delivery system to enhance NK cytotoxicity in the tumor microenvironment (TME). Targeting NK cells in-vivo bypasses the need for ex-vivo isolation of NK cells.
Conclusion: This technology provides an innovative and broad therapeutic approach that includes both the active-modulating compounds and the systemic delivery platform. NK cells are attractive immunotherapeutic candidates due to their innate ability to target tumors without the requirement for prior antigen exposure or antigen specificity. They can complement T-cell immune surveillance, and recent data also demonstrate their ability to trigger substantial adaptive immune responses through crosstalk with dendritic and T-cells. Therefore, Utilizing NPs to enhance the cytotoxic capacity of NK cells by downregulation of key intrinsic inhibitory checkpoint molecules may provide a robust immunotherapeutic approach.

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Modulation of intrinsic inhibitory checkpoints using nano-carriers to unleash NK cell activity (2021). EMBO Mol Med. 14(1):e14073.

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782 – WS42.4

Dual blockage of PD-L/PD-1 and IL33/ST2 axes improves antitumor immunity by enhancing NK cells' tumoricidic potential

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Purpose: During last decade, blockage of PD-L/PD-1 axis has had a great impact on immunotherapy of tumors. On the other hand, blockage of IL33/ST2 axis has shown to be benefitial in supressing tumor oncogenesis. However, co-blockage of these axes has not been thouroghly studied yet.
Methods: Breast cancer and colon cancer were inducted in BALB/C wild type and BALB/C ST2 knockout mice, after which mice underwent anti PD-1 and anti IL-33 treatment.
Results: Simultaneous blockage of IL33/ST2 and PD-L/PD-1 significantly postponed tumor appearance and slowed down breast cancer and colon cancer growth. We showed that enhanced NK cell cytotoxicity against breast cancer cells in ST2 knockout anti-PD-1 treated mice was followed with overexpression of miRNA-150 and miRNA-155, upregulation of NFκB and STAT3, increased expression of activation markers and decreased expression of immunosuppressive markers in splenic and tumor-infiltrating NK cells. In addition, NK cells from ST2 knockout anti-PD-1 treated mice tend to proliferate more and are less susceptibile to apoptosis. On the other hand, accumulation of immunosuppressive myeloid derived suppressor cells and regulatory T cells was significantly impaired in spleen and tumor microenvironment of ST2 knockout anti-PD-1 treated mice.
Conclusion: Simultaneous blockage of IL3/ST2 and PD-L/PD-1 axes stimulates anti – tumor immune response more efficiently, and therefore impedes tumor progression more efficiently in comparison to single blockage of either axes, which offers a new perspective to immunotherapy of tumors.

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2014 – WS42.5

The NKG2A-HLAE immune checkpoint: novel-IC therapy based on mAb to specific HLAE-peptide complexes

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Purpose: HLA-E, a non-classical MHC class Ib molecule, is overexpressed in Multiple Myeloma (MM). HLAE:NKG2A is one of the ICs that leads to the suppression of NK and $NKG2A^+$ T cells activity and enhances tumor progression. Currently, Immune Checkpoint Inhibitors (ICIs) target the NKG2A receptor which has a low specificity to cancer and high toxicity to the patient. nonetheless, a new ICI targeting the $HLAE^{pHLAG}$ complex could potentially improve cancer therapy outcomes.
Methods: mAb "4D7" was developed using a hybridoma system. mAb 4D7 affinity was assessed in-vitro with recombinant proteins and various cell lines that expressed zero to high levels of HLAE. Its functional impacts were examined in a system where MM cell lines were co-cultured with primary NK cells. pNK were isolated from the peripheral blood of 5 healthy donors, and their activity was examined in the presence of mAb 4D7 by CD107a degranulation assay. Also, the functional impacts of the mAb 4D7 on allogenic pNK and MM blast cells, isolated from the bone marrow of 6 MM patients were assessed.
Results: mAb 4D7 was determined to be an IgG-kappa isotype showing a high specific binding to recombinant $HLA\text{-}E^{pHLA\text{-}G}$. Cell lines with overexpression of membranal $HLA\text{-}E^{pHLA\text{-}G}$: RPMI8226, U266, 721.221 HLA-E, and 721.221 HLA-G positively stained with the mAb 4D7. Cell lines with low to zero expression of HLA-E like 721.221 WT showed low or negative staining with the mAb 4D7. The degranulation of $NKG2A^+$ pNK subset cells against MM target cells increased in the presence of mAb 4D7. However, adding mAb 4D7 to $NKG2A^-$ pNK subset cells co-cultured with MM target cells did not affect the pNK activity. Similar results were obtained when the target and effector cells were isolated from the bone marrow of active treatment-naive MM patients.
Conclusions: MAb 4D7 demonstrates a remarkably high affinity for binding to the $HLAE^{pHLAG}$ complex, ensuring the safety of its application. The ability of 4D7 mAb to effectively block the $HLA\text{-}E^{pHLA\text{-}G}$ complex and counteract the inhibition signal in NK cells. mAb 4D7 is a promising monoclonal antibody with substantial potential to serve as an ICI for clinical use in MM patients.

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1039 – WS42.6

Primary NK cells modified by CD33-CAR insertion and CRISPR/Cas9-based disruption of the KLRC1 gene mediate high efficacy against acute myeloid leukemia

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Purpose: Chimeric antigen receptor (CAR)-modified natural killer (NK) cells show strong antileukemic activity against acute myeloid leukemia (AML). However, NK cell-mediated tumor killing is often impaired by tumor-mediated immune suppression. We previously observed increased IFN-γ secretion by CAR-immune cells following target cell contact, which can induce upregulation of inhibitory molecules such as HLA-E. Here, we report a novel strategy to overcome CAR-NK cell inhibition mediated by the HLA-E-NKG2A immune checkpoint.
Methods: CD33-targeting CAR-NK cells were generated by lentiviral transduction of primary NK cells as recently reported (Albinger et al., *Blood Cancer J* 2022). Deletion of the NKG2A-encoding *KLRC1* locus was performed by nucleofection using CRISPR-Cas9 technology. The CAR33- and NKG2A-expression as well as cytotoxicity were analysed. *In vivo*-efficacy was evaluated in OCI-AML2 xenografted NSG-SGM3 mouse models. Furthermore, single-cell multi-omics analyses were used to characterize the genetically engineered NK products in comparison to non-transduced NK cells.
Results: Lentiviral transduction of NK cells resulted in up to 60% CAR33-positive cells, while *KLRC1* gene disruption resulted in a significant reduction of NKG2A cell surface expression. CAR33-*KLRC1*^{KO}-NK cells showed significantly higher elimination of CD33^{+}/HLA-E^{+} OCI-AML2 cells in *in vitro* cytotoxicity assays compared to *KLRC1*^{KO}- or CAR33-NK cells. Furthermore, a reduction of leukemic burden was observed *in vivo* following a single injection of a low dose of CAR33-*KLRC1*^{KO}-NK cells compared to *KLRC1*^{KO}-NK or CAR33-NK cell treatment in an NSG-SGM3 AML-xenografted mouse model. Two injections of 3x10^{6} CAR33-*KLRC1*^{KO}-NK cells each led to a complete elimination of AML and leukemia-initiating cells in the bone marrow, which was confirmed by bone marrow re-engraftment analysis. Finally, we were able to unravel transcriptional features of activation and maturation on single cell level in the CAR33-*KLRC1*^{ko}-NK product, which were preserved following exposure to AML cells.
Conclusion: Removing an inhibitory receptor in CAR-NK cells showed a highly beneficial effect for the treatment of AML. Finally, we conclude that dual-modified NK cells have the potential not only to bypass immune suppression in AML but also in a broad range of other cancer entities.

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WS43 – CONTROL OF TISSUE INFLAMMATION AND REPAIR

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619 – WS43.1

Macrophage-fibroblast crosstalk in the rheumatoid arthritis synovium is defined by sexually dimorphic extracellular matrix patterning and drives disruption of tissue homeostasis.

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Purpose: Tenascin-C (TNC) is an immunomodulatory extracellular-matrix protein, that is expressed during the onset of rheumatoid arthritis (RA). High TNC-serum levels are associated with hard-to-treat disease and predicts patients whose pain will not improve with anti-TNF therapy. Conversely, in patients with spontaneously resolving synovitis, TNC levels decrease. Our lab has developed TNC-targeting antibodies, that were shown to prevent disease progression in a rodent arthritis model. The aim of this project is to determine which patients would benefit most from these antibodies, by better understanding the role of TNC in human RA pathology.
Methods: Single-cell RNA sequencing data was used to investigate the cellular source of TNC in RA and OA synovium. Multiplex imaging was used to determine the localisation and cellular targets of TNC in synovial biopsies. Spatial transcriptomics was used to investigate how TNC affects cells in the synovium, and these results were validated using confocal imaging and qPCR analysis of matrix-activated human macrophages.
Results: TNC is expressed exclusively by stromal cells, particularly fibroblasts, in the synovium. Gene expression amongst fibroblast subsets changes with disease type and stage. Multiplex imaging shows two distinct groups of patients, characterized by the presence or absence of TNC in the lining-layer. Female patients showed significantly higher levels of TNC in the lining-layer, compared to male patients. Moreover, male patients showed increased proportion of T-cells in lymphoid aggregates, and seropositive patients showed increased proportion TNC+ B-cells in the lymphoid aggregates. Regardless of tissue-niche, TNC-rich areas contained significantly more immune cells, particularly macrophages, compared to TNC-poor areas. GPNMB, an immunomodulatory protein, was down-regulated in TNC-rich areas. GPNMB is expressed in the lining-layer on remission-associated macrophages, and we confirmed that expression of GPNMB is regulated by TNC, through activation of TLR4.
Conclusion: There is a large variation in the location and synovial tissue levels of TNC. TNC was found to be associated with distinct cell neighbourhoods that drive the development and persistence of inflammation. Moreover, varying levels of TNC in the synovium may contribute to differences in the course of disease and treatment response between male and female RA patients, and seropositive and seronegative RA patients.

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1350 – WS43.2

Elucidating the role of interleukin-4 receptor alpha signalling in the pathogenesis of haemophilic arthropathy.

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Purpose: Haemophilia A is a rare X-linked congenital bleeding disorder caused by mutations in the *F8* clotting factor gene. These mutations lead to a deficiency in Factor VIII resulting in prolonged bleeding time. Repeated bleeding into major articular joints may led to the development of haemophilic arthropathy (HA), a degenerative chronic joint disease characterised by synovitis, cartilage degeneration and bone remodelling. Currently, there are no available disease modifying treatments for haemophilic arthropathy with patients with severe HA requiring joint replacement surgery.
Methods: A new *F8* deficient (*F8$^{em1-/-}$*) mouse model of severe haemophilia A, generated by CRISPR-Cas9 targeting of Exon 1 of *F8*, was developed that recapitulated the spontaneous HA observed in patients with haemophilia. To define the immunological basis of HA, *F8$^{em1-/-}$* mice were subjected to sub-patellar needle injury of the knee joint to induce synchronized acute articular bleeding leading to hemarthrosis. The synovium from damaged knee joints were analysed during acute and chronic disease phases to characterise cell populations and inflammatory gene expression in the joint by flow cytometry and NanoString technology.
Results: Needle injury to the knee joint of *F8$^{em1-/-}$* mice, but not to wild-type mice, led to local bleeding and acute inflammation in the synovium. This was associated with acute pro-inflammatory cytokines, such as IL-1β and TNF-α, and changes in immune cell populations and genes networks as injury resolved. KEGG pathway analysis revealed marked increases in the IL-4Rα signalling axis in the synovium of *F8$^{em1-/-}$* mice after injury. We defined the IL-4Rα expressing cells in the joint during HA. A dual IL-4Rα$^{-/-}$ x *F8$^{em1-/-}$* mouse was generated that confirmed IL-4Rα expressing cells have a role in the immunopathogenesis of HA.
Conclusion: A functional role for IL-4Rα axis in the pathogenesis of HA in a novel mouse model was demonstrated. The study raises options for therapeutic interventions addressing disease modifying inflammatory targets to treat HA.

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689 – WS43.3

Staphylococcal Enterotoxin B Binding to CD28 mediates inflammatory T cell-dependent intestinal epithelial barrier dysfunction.

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Purpose: *Staphylococcus aureus* is a gram-positive bacterium that may cause intestinal inflammation by secreting enterotoxins, which commonly cause food-poisoning and gastrointestinal injuries. Staphylococcal enterotoxin B (SEB) acts as a superantigen by stimulating T lymphocytes to produce inflammatory cytokines. We recently highlighted that SEB may induce the massive production of inflammatory cytokines by binding in a bivalent manner the TCR and CD28 costimulatory molecule. Here, we investigated the role of inflammatory cytokines elicited by SEB-mediated stimulation of T cells in the dysregulation of Caco-2 intestinal epithelial barrier function.
Methods: Primary T cells, isolated from the peripheral blood of healthy donors, were stimulated with SEB and co-cultured with of Caco-2 cells in trans-well plates and the following analyses were performed: 1) Inflammatory cytokine production in culture supernatants was quantified by ELISA; 2) actin cytoskeleton modifications, the localization of cell-cell junctions and the nuclear translocation of pSTAT3 and NF-kB transcription factors (TFs) were analysed by confocal microscopy; 3) the expression of epithelial markers, mesenchymal markers epithelial to mesenchymal transition TFs (EMT-TFs) were evaluated by western blotting and real time PCR; 4) the specific recruitment of pSTAT3 and NF-kB on the proximal promoters of EMT-TFs was analysed by chromatin immunoprecipitation.
Results: We demonstrated that inflammatory cytokines produced by T cells following SEB stimulation induce dysfunctions in Caco-2 intestinal epithelial cells by promoting actin cytoskeleton remodelling and epithelial cell-cell junction down-regulation. Moreover we also found that SEB-activated inflammatory T cells promote the up-regulation of SNAIL-1, TWIST-1 and ZEB-1 EMT-TFs in a NF-kB- and STAT3- dependent manner. Finally, by using a structure-based design approach, we identified a SEB mimetic peptide that, by blocking the binding of SEB to CD28, dampens inflammatory-mediated dysregulation of intestinal epithelial barrier.
Conclusion: Our results provide new insights into the enterotoxic activity of SEB during *S. aureus* infections, which may lead to the exacerbation of chronic inflammatory diseases, and we identified of a novel mimetic peptide able to attenuate inflammatory-dependent epithelial barrier dysfunctions.

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1172 – WS43.4

Exploring the roles of γδ intraepithelial lymphocytes in gut physiology

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Purpose: The murine intestinal intraepithelial lymphocyte (IEL) compartment is dominated by Vγ7$^+$ γδ T cells, which rely on neighbouring epithelial proteins, Btnl1 and Btnl6 for their development. The role of intestinal Vγ7$^+$ T cells is still unclear but of note, mice lacking Vγ7$^+$ cells are more susceptible to colorectal cancer, while patients with Crohn's disease are more likely to develop penetrating B3 disease if they are homozygous for an hypomorphic allele of *BTNL3* and *BTNL8* that are the human homologues of *Btnl1* and *Btnl6.* In this context, we hypothesise that Vγ7$^+$ T cells promote tissue integrity at steady-state and tissue repair post-challenge. This essential property will be dependent on the cells' regulation by Btnl proteins.
Methods: The Btnl-dependent gene network regulating Vγ7$^+$ cells is investigated by analysing the immunophenotype, the functional response and the transcriptomic profile of Vγ7$^+$ cells after acute loss of *Btnl1*. Additionally, small intestinal organoids will be used to develop a system for quantifying IEL-IEC interactions at steady-state. The impact of Vγ7$^+$ cells post-challenge will be investigated by examining the susceptibility of Btnl1-deficient mice to gut-damaging insults that require epithelial repair.
Results: Acute loss of Btnl1 does not induce an immediate change in Vγ7$^+$ IEL frequency, but it does alter the cells' phenotype. Specifically, following acute loss of Btnl1, Vγ7$^+$ cells show diminished expression of CD69, commonly considered to be downstream of TCR signalling, but also associated with tissue-residence. Additional changes will be presented. Beyond the impact on T cells, acute Btnl1 loss seems to induce enterocyte apoptosis.
Conclusion: These results, which will be expanded upon, are already sufficient to point to a key importance of the Btnl-gd axis for steady-state intestinal physiology. Given the association of this axis in human IBD, specific findings in the mouse model system described might identify actionable targets.

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132 – WS43.5

Utilizing integrated spatial transcriptomics to elucidate localized immune responses within human coronary arteries throughout the progression of atherosclerosis

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Aims: The contribution of immunity to human atherosclerosis is increasingly recognised. However, the anatomical organization and the specific roles of immune cells in the various stages of disease in the different vascular layers remain underexplored, underscoring the necessity for research to elucidate their function throughout disease progression. Moreover, research on human atherosclerosis has predominantly relied on samples obtained from endarterectomies of carotid arteries, representative of end-stage disease and confined solely to the plaque, offering little insight into disease progression.
Methods: Here, we performed combined Nanostring GeoMx® DSP and CosMx™ SMI on serial cross-sections of coronary arteries from human explanted hearts from subjects that underwent heart transplantation at University Hospitals Birmingham NHS Foundation Trust. The cross-sections include the full thickness of the vessel wall and surrounding adipose tissue with vasa vasorum. These show atherosclerosis progression from near-normal vessels to severe lesions, corresponding to AHA type II-V lesions. For GeoMx®, FFPE sections were stained with morphology markers (SYTO13, CD45 and CD4) and hybridised with the Whole Human Transcriptome Atlas RNA panel. A total of 55 Regions of Interest (ROIs) were placed across the cross-sections covering anatomical locations that were CD45+CD4$^-$ or CD45+CD4$^+$ in the adventitia or the atherosclerotic plaque. The collected barcodes were processed for Next Generation Sequencing (NGS). The NGS readout data was converted from FASTQ to DCC format, and the data uploaded to the GeoMx® DSP. Data analysis was performed using the DSP Analysis Suite and R packages available from Bioconductor and made available by NanoString. For CosMx™, serial FFPE sections (to those used for GeoMx) were hybridised with the CosMx Universal Cell Characterisation RNA Panel, which was customised with 20 additional targets to cover genes & pathways highlighted by the GeoMx study. We placed 235 Fields of View (FOVs) across the cross-sections in order to profile the full thickness of the artery layers, the atherosclerotic plaques and immune cell infiltrates. Data analysis was performed using the DSP Analysis Suite, AtoMx SPI, and R packages available from Bioconductor.
Results: We integrated GeoMx® and CosMx™ datasets, to perform a series of analyses, including pathway and neighbourhood analyses, and cell phenotyping.

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1709 – WS43.6

Current anti-TNF therapies do not prevent scar formation in Hidradenitis suppurativa (HS)

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Aims: To evaluate the effect of current and future therapies on HS fibroblast activation
Methods: Single cell RNA-sequencing (scRNA-seq) was performed on HS lesional and healthy control skin. Fibroblasts and myeloid cells were identified and characterised using Seurat. Myeloid-derived drivers of fibroblast activation were identified in silico using Nichenetr and validated in vitro on HS fibroblasts which were stimulated with recombinant cytokines or HS skin explant conditioned media.
Results: scRNA-seq identified inflammatory fibroblast subsets which may contribute to the highly inflammatory microenvironment of HS lesions and subsequent scarring which is characteristic of HS. Fibroblasts with high expression of podoplanin and CD90 also had elevated expression of neutrophil chemoattractants *CXCL1* and *CXCL8*, collagens and matrix metalloproteinase (MMP) genes and were unique to HS lesions. HS inflammatory fibroblasts had elevated expression of TNF and IL-1b responsive genes, suggesting that myeloid cells may promote an inflammatory phenotype in HS fibroblasts. Furthermore, myeloid cell derived IL-1b, TNF and TGFb were identified as potential regulators of inflammatory genes in HS fibroblasts. IL-1b, TNF and TGFb regulate the expression of MMPs and collagens in HS fibroblasts, suggesting that myeloid cell interactions with fibroblasts may promote fibrotic scarring in HS. Further, scRNA-seq analysis indicated that anti-TNF therapy reduced the expression of chemokines, cytokines, MMPs and anti-microbial peptides in HS fibroblasts but may not prevent HS scar formation. Finally, blocking IL-1b production in HS lesions using the NLRP3 inflammasome inibitor MCC950 failed to prevent the activation of HS fibroblasts.
Conclusion: These findings highlight an important role of myeloid cell derived TNF, IL-1b and TGFb in driving fibroblasts into an inflammatory phenotype. IL-1b, TNF and TGFb regulated the expression of the neutrophil attractants *CXCL1* and *CXCL8* which may, along with increased MMP and collagen production, contribute to HS scarring. While fibroblasts from HS patients on anti-TNF therapy had reduced expression of proinflammatory mediators, confirming the importance of TNF in the pathogenesis of HS, HS fibroblasts maintained an elevated expression of fibrotic factors. Taken together this data indicates anti-TNF therapy may not prevent the development of fibrotic scars in HS patients and highlights the complexity of HS pathogenesis.

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WS44 – COVID-19 IMMUNITY

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1240 – WS44.1

Non-classical HLA-E class I molecule and its potential role in SARS-CoV-2 infection.

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The SARS-CoV-2 pandemic has had a massive impact on global health and economies. While mRNA vaccines have effectively prevented disease, they do not guarantee long-term immunity, as neutralizing antibody titers decline. $CD8^+$ T cells recognize infected cells by binding T cell receptors to epitopes presented by human leukocyte antigen (HLA) Class Ia molecules, which are highly polymorphic. HLA-E, a low polymorphic molecule, presents limited peptides to $CD8^+$ T cells. In this study, we identified eight peptides from the 2019-nCoV/USA-WA1-A12/2020 (MT020880) complete genome SARS-CoV-2 that fit the HLA-E pocket and are recognized by $CD8^+$ T cells to provide long-term immunity against SARS-CoV-2 infection and future coronaviruses.
NetMHC-4.0 was used to predict peptide-HLA binding affinity, incorporating structural information from HLA molecules. The synthesized potentially immunogenic epitopes were validated for binding to the HLA-E molecule to generate an immunological response. We analyzed HLA-E-restricted $CD8^+$ T cells from peripheral blood mononuclear cells (PBMCs) of 20 COVID-19-recovered individuals, 20 SARS-CoV-2 seropositive hospitalized patients, and 20 people who received three doses of mRNA BNT162b2 vaccine, using flow cytometry to evaluate their phenotype and functional characteristics. To assess the functions of SARS-CoV-2-specific $CD8^+$ T cells, we used intracellular cytokine staining (ICS) to measure the expression of effector cytokines (IFN-γ, TNF-α, IL-2, IL-4) and the CD107a mobilization as a readout of cytotoxicity on isolated PBMCs, following stimulation by our designed peptide pool.
We found that a subset of $CD8^+$ T cells efficiently recognized and reacted to the pool of HLA-E-restricted peptides by producing different cytokines. The HLA-E–restricted SARS-CoV-2–specific $CD8^+$ T cell response was more frequently detected in recovered subjects than hospitalized PCR-positive patients. These $CD8^+$ T cells predominantly expressed TNF-α and IL-4 with lower expression of IFN-γ and IL-2. The detection of surface CD107a significantly differed between hospitalized and recovered individuals.

Our results suggest that HLA-E-restricted $CD8^+$ T cells may contribute to controlling SARS-CoV-2 infection, reducing disease severity, and producing long-term immunity. Hence, understanding HLA-E-restricted $CD8^+$ T cell-mediated immunity is crucial for improving therapeutic and vaccine strategies.

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2179 – WS44.2

SARS-CoV-2 virions hijack host cellular proteins to escape destruction by innate immunity

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The complement system plays a crucial role in the innate defense against invading pathogens. The complement proteolytic cascade on the microbial surface generates potent proinflammatory molecules and opsonins, resulting in lysis of the opsonized microbe or its phagocytosis by innate immune cells. Many pathogens, including viruses, have thus evolved strategies to overcome the complement-mediated destruction, e.g., by hijacking host complement regulators or encoding their own analogues. Since persistent activation of the complement is considered to play a key role in the pathogenesis of in severe coronavirus disease 2019 (COVID-19), we hypothesized that severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virions subvert normal complement recognition and/or lysis. To test that, we produced SARS-CoV-2 virions in a human cell line naturally permissive to infection and purified them by size exclusion. Analysis of the purified SARS-CoV-2 virions by liquid chromatography coupled to tandem mass spectrometry revealed that SARS-CoV-2 reproducibly incorporates a subset of host cellular proteins, including some of the known complement regulators, into its virions during budding from infected cells. We confirmed the presence of the complement regulators in SARS-CoV-2 virions by Western blotting. Subsequent functional assays using specific blocking antibodies and/or enzymatic cleavage confirmed the role of the hijacked host cellular proteins in prevention of the complement-mediated lysis of SARS-CoV-2 virions. In conclusion, our results reveal an intriguing immune escape mechanism of SARS-CoV-2 with possible implications in the immunopathology of COVID-19.

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889 – WS44.3

Individually expressed SARS-CoV-2 ORF3a disrupts cellular structure and lipid metabolism in lung epithelial cells.

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ORF3a is the largest SARS-CoV-2 accessory protein. While its role as a viroporin remains controversial, it is known that this transmembrane protein impairs immune responses and induces vesicle formation. In this study, we analysed the cellular landscape in ORF3a-transduced A549 lung epithelial cells, using a combination of imaging techniques and various omics approaches to gain insight into the molecular mechanism of ORF3a. Initially, Transmission Electron Microscopy confirmed an increase in the number of vesicles inside the cells and revealed a significant number of altered mitochondria with abnormal cristae compared to control A459 cells. Furthermore, cryo nano-tomography in the water window performed at full-field transmission X-ray microscopy (ALBA synchrotron) allowed us to grasp the effects of ORF3a in cells expressing a GFP N-terminal tagged version of this accessory protein. With the increased number of vesicles, there was almost no sign of ER or Golgi, indicating that ORF3a promotes their fragmentation. ORF3a was found to localize in some of these vesicles, which exhibited a characteristic tubular pattern inside. Transcriptomic analysis revealed a distinct transcriptome in ORF3a transduced cells, with genes involved in mitochondria morphology and function being notably downregulated. Interestingly, altered mitochondria were often found next to those ORF3a-tubular vesicles, and live-cell imaging showed an increase in the motion and speed of these organelles compared to those of control cells. Additionally, we also found an unusual increased amount of lipid drops both by tomography and lipid staining in confocal microscopy, correlating with the upregulation in lipid metabolism induced by ORF3a as observed by Genome-Scale Metabolic Models. Together, our results demonstrate that the individual expression of the SARS-CoV-2 accessory protein ORF3a is sufficient to alter cellular structure, suggesting that this protein may play an important role in viral pathogenesis.

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2008 – WS44.4

Enhanced early type I interferon signaling is associated with resistance to SARS-CoV2 infection

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An impaired type I interferon (IFN) response is associated with severe SARS-CoV2 infection, and we propose that a particularly effective early response might provide protection in individuals who do not get infected upon exposure to virus. This resistant phenotype is rarely studied as exposure is difficult to ascertain. Here, the high rates of COVID-19 infection in 3 Irish nursing homes provided a unique opportunity to study resistance to SARS-CoV2. Seven individuals out of 90 were repeatedly negative for SARS-CoV2 nucleocapsid antibody despite living in environments of high exposure. To further validate lack of prior infection, IFN-y release upon stimulation of whole blood with SARS-CoV2 antigens was completed to test for T cell memory responses. All 7 resistant individuals had low IFN-γ responses indicating a lack of T cell memory to SARS-CoV2. We then performed standardized *ex-vivo* whole blood stimulations for 4-hours with viral ligands to determine the early innate anti-viral responses of these individuals compared to 7 age and sex matched controls previously infected with SARS-CoV2 from the same nursing homes. Multiplex gene expression profiling technology was used to quantify transcripts of 785 genes involved in innate immune signaling. Digital protein quantification assays were used to measure cytokines involved in innate anti-viral signalling. We identified an increased early type I interferon signaling transcriptomic signature within the nursing home resistors in response to Poly:IC compared to controls. This included significant increases in expression of *IFNA8* and *IFNG*. Interferon stimulated genes *OAS3*, *ISG15*, *HERC5*, *IFI6* and *APOL6* were also increased in resistors, which are important in early control of SARS-CoV2 infection. This early enhanced innate immune response could contribute to resistance to SARS-CoV2 infection. Investigating the immune mechanisms of optimal control of SARS-CoV2 virus early after exposure may lead to development of new anti-viral treatments and therapeutic strategies.

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2137 – WS44.5

Longitudinal serosurveillance of severe acute respiratory syndrome coronavirus 2 in urban and rural cohorts in Malawi: characterising population exposure and protective immunity to viral variants

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Purpose: In Malawi, the extent of SARS-CoV-2 exposure and transmission is unclear due to high rates of mild/asymptomatic infections and limited diagnostic capacity. Existing SARS-CoV-2 seroprevalence studies have utilised enzyme-linked immunosorbent assays (ELISAs), lacking functional immunity estimates. Here, we employed pseudotyped virus neutralisation assays (PVNAs) to measure SARS-CoV-2 neutralisation, evaluating community exposure and protective immunity elicited by infection and vaccination.
Methods: Sera were obtained from rural (Karonga, n=968) and urban (Lilongwe, n=938) communities in Malawi, at 3-monthly intervals (February 2021-April 2022). Median age was 23 years (IQR 12-40). Human immunodeficiency virus (HIV)-based SARS-CoV-2 PVNAs assessed neutralisation of B.1 (ancestral), B.1.1.7 (Alpha), B.1.351 (Beta), B.1.617.2 (Delta), BA.1 (Omicron) and BA.2 (Omicron). Positive samples identified from fixed-dilution screening were titrated (titre at 50% reduction in infectivity). Nucleocapsid ELISAs were performed on sera from vaccinated participants to distinguish "vaccinated" from "vaccinated and infected". Findings were restricted to HIV-uninfected participants due to HIV-infected sera interfering with the pseudotypes. Ethical approval was received from the Malawi College of Medicine Research Ethics Committee (P11/20/3177) and the University of Glasgow College of Medicine, Veterinary and Life Sciences Research and Ethics Committee (200200056).
Results: Seroprevalence increased over time and varied by location (Survey 1-Karonga 6.5%, Lilongwe 11.7% ($p<0.001$); Survey 4-Karonga 45.4%, Lilongwe 68.6% ($p<0.001$)). Variant exposure also differed with time and location – novel variants emerged earlier in Lilongwe. Seroreversion (neutralisation negative following being positive) peaked at Survey 3 – the highest percentage of seroreverted individuals were Delta-exposed (Karonga – 29%, Lilongwe – 38%). Among naturally infected participants, children <15 years had the lowest B.1 titres ($p<0.05$). A single dose of ChAdOx1-S (AstraZeneca) vaccine induced stronger Beta neutralisation than one dose of Jcovden (Janssen) vaccine ($p=0.006$). Individuals with hybrid immunity (vaccinated and infected) had higher antibody titres than participants solely **vaccinated or infected ($p<0.05$).**
Conclusion: SARS-CoV-2 seroprevalence increased over time, with variants emerging earlier in Lilongwe, the international travel hub. Low neutralisation levels in children, possibly due to high asymptomatic/pauci-symptomatic infection rates, may lead to increased reinfections among this demographic. The high neutralisation responses in those with hybrid immunity reinforces the importance of vaccination in protective immunity.

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706 – WS44.6

The long Pentraxin PTX3 serves as an early predictive biomarker of co-infections in COVID-19

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Purpose: COVID-19 clinical course is highly variable and secondary infections contribute to COVID-19 complexity. Early detection of secondary infections is clinically relevant for patient outcome. Procalcitonin (PCT) and C-reactive protein (CRP) are the most used biomarkers of infections. Pentraxin 3 (PTX3) is an acute phase protein with promising performance as early biomarker in infections. In COVID-19 patients, PTX3 plasma concentrations at hospital admission are a strong independent predictor of poor outcome. In this study, we assessed whether PTX3 contributes to early identification of co-infections during the course of COVID-19.
Methods: We analyzed PTX3 levels in COVID-19 patients with (n=101) or without (n=179) community or hospital-acquired fungal or bacterial secondary infections (CAIs or HAIs), comparing it with classical biomarkers used by clinicians, i.e., PCT and CRP.
Results: PTX3 plasma concentrations at diagnosis of CAI or HAI were significantly higher than those in patients without secondary infections. In a longitudinal analysis, PTX3 peaked at admission and significantly increased again at the co-infection time-point. In patients surviving the secondary infection, the protein concentration significantly decreased after co-infection diagnosis, whereas in non-survivors, only a trend of decrease was observed. In selected patients, for whom we had serial PTX3, PCT and CRP measurements in a time period close to the HAI diagnosis, PTX3 plasma levels almost doubled few days before the diagnosis, when PCT and CRP concentration were still in the range of moderate increase or normality. Compared to PCT and CRP, the increase of PTX3 plasma levels was associated with the highest hazard ratio for CAIs and HAIs (aHR 11.68 and 24.90). In univariable and multivariable Cox regression analysis, PTX3 was also a strong predictor of 28-days mortality or intensive care unit admission of patients with potential co-infections, faring more pronounced than CRP and PCT.
Conclusions: PTX3 is a promising predictive biomarker for early identification and risk stratification of COVID-19 patients with co-infections.

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WS45 – STROMAL CELLS IN IMMUNITY AND TISSUE REPAIR

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875 – WS45.1

Enhanced long-term protective effects of CXCR4/IL10-expressing mesenchymal stromal cells in a preclinical model of inflammatory bowel disease

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Mesenchymal stromal cells (MSCs) have shown great promise as a treatment for inflammatory bowel disease (IBD), owing to their immunosuppressive and regenerative capabilities. However, their therapeutic efficacy is challenging in part due to their limited efficiency in entering the inflamed colon and their variable in vivo immunomodulatory capacity. In this study, genetically engineered adipose derived human MSCs constitutively expressing the chemokine receptor 4 (CXCR4) and interleukin-10 (IL-10) (CXCR4/IL10 MSCs) have been used to improve their migration to the inflamed colon and to enhance the immunosuppressive properties of MSCs. Compared to non-modified MSCs (native MSCs), CXCR4/IL10-MSCs exhibited enhanced trafficking to the inflamed colon in dextran sodium sulphate (DSS)-challenged colitic mice. Interestingly, the administration of the CXCR4/IL10-MSCs showed enhanced therapeutic effects when compared to native MSCs, as indicated by analyses of the pathological indices and inflammatory markers. Strikingly, upon chronic re-challenge with DSS, enhanced long-term protective effects were observed in CXCR4/IL10-MSCs treated mice when compared to native MSC-treated colitic mice and the non-MSC-treated colitic mice. Overall, these results highlight the importance of developing improved cell-based therapies with MSCs as a highly effective strategy to induce long-term protective immune memory in immune-mediated disorders in which the complete resolution of the inflammation is impaired. Overall, this strategy based on enhancing the homing and immunosuppressive abilities of MSCs may represent an optimized, with potential long-term effect, MSC-based cell product for IBD therapy.

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149 – WS45.2

Development of a 3D model of colorectal cancer to assess the stromal-mediated effects on T cells in a stromal-rich colorectal cancer tumour microenvironment.

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Purpose: Colorectal cancer (CRC) is the second leading cause of cancer related deaths worldwide. Elevated T cell levels can signify better prognosis, however activated T cells express immune checkpoint inhibitors, hindering immune-mediated responses and advancing CRC. CRC is classified into four Consensus Molecular Subtypes (CMS1-4), notably CMS4, with abundant mesenchymal stromal cells (MSCs) and an inflamed immune phenotype. Given CMS4's link to poor disease-free survival, this project explores the impact of a stromal-dense environment on T cell activation in a 3D model.
Methods: Our project describes the development of a 3D collagen embedded spheroid containing CRC cell lines, hMSCs to mimic stromal cell infiltration of CMS4 and PBMCs cultured with anti-CD3-CD28 beads. We used a variety of methodologies including flow cytometry, AlamarBlue™, real-time PCR and confocal microscopy to assess the impact of sialidase and PD1-sialidase on a multicellular 3D model in vitro.
Results: We have shown that mesenchymal stromal cells (MSCs) promote viability and outgrowth of cells from the co-culture spheroids. We have also demonstrated that MSCs increase production of fibronectin which is present in stromal rich areas *in vivo*. We have demonstrated that hMSCs induce production of exhaustion markers LAG3 and PD1 on T cells in co-cultures with HCT116 + hMSC + PBMC spheroids. Finally, we demonstrated that Sialidase and PD1-Sialidase can potentially reverse immunosuppression induced by hMSCs through decreasing the expression of PD1 on both CD4 and CD8+ T cells.
Conclusion: Overall, we have developed a multi-cellular collagen embedded spheroid, that can be used in interrogate stromal mediated immunosuppression in the TME of CRC. This model can also be used in a drug screening capacity allowing us to analyse the effects hMSCs have on the response to Sialidase treatment in a 3D CRC model. Overall, the model represents an effective screening platform to identify novel therapeutic targets and assess the impact of immunotherapies in vitro.

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1685 – WS45.3

Ehf and Fezf2 regulate thymic tissue homeostasis and thymic tuft cell development

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Thymic epithelial cells are indispensable for T cell maturation and selection and the induction of central immune tolerance. The self-peptide repertoire expressed by medullary thymic epithelial cells is in part regulated by the transcriptional regulator Aire and the transcription factor Fezf2. Due to the high complexity of mTEC maturation stages (i.e., post-Aire, Krt10+ mTECs, and Dclk1+ Tuft mTECs) and the heterogeneity in their gene expression profiles (i.e., mosaic expression patterns), it has been challenging to identify the additional factors complementing the transcriptional regulation in the past.
We aimed to identify the transcriptional regulators involved in the regulation of mTEC development and self-peptide expression in an unbiased and genome-wide manner. We employed ATAC footprinting analysis as an indirect method to identify key transcription factors influencing mTEC gene expression, further substantiated by ChIP sequencing validation and analysis of conditional knock-out mice.
We show that Fezf2 and Ehf are involved in the regulation of late developmental gene signatures implicated in cornification and keratinization in mTECs and that Fezf2 regulates Tuft-mTEC-specific gene signatures. Moreover, using conditional knockout mice, we identify Fezf2 to be essential for the development of thymic Tuft cells and Aire+-mature mTECs. Additionally, it was discovered that members of the ELF, ESE, ERF, and PEA3 subfamilies of ETS transcription factors, along with the Krüppel-like family of transcription factors, are instrumental in regulating genes crucial for advanced mTEC development.
Our findings highlight Fezf2 as a pivotal regulator of the newly characterized thymic Tuft cells. The Fezf2-dependent development of Tuft-mTECs discovered in this study identifies an alternative role of Fezf2 during mTEC development in which Fezf2 regulates the development of late developmental mTEC stages and the Tuft-mTEC stage, thereby indirectly affecting the representation of self-peptides, which are normally expressed and presented by those mTEC subsets. This newly identified role of Fezf2 in regulating late mTECs, Aire+-mTECs and Tuft-mTECs enhances our understanding of the complex cellular landscape of thymic epithelial cells.

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614 – WS45.4

Characterization of an IL-33 expressing, immune active fibroblast subpopulation

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Fibroblasts are diverse mesenchymal cells which play an important role in tissue homeostasis and repair through the production of a variety of different growth factors. In addition, they are also involved in many immune regulatory responses, where they have been implicated in both pro- and anti-inflammatory processes. Moreover, a deregulation of fibroblasts can lead to a plethora of different pathologies, such as fibrotic diseases and, in the tumor microenvironment, so-called cancer associated fibroblasts (CAFs) have been shown to support tumor growth. However, the inter- and intra-tissue heterogeneity of fibroblast populations remains unsolved. Therefore, we performed a single cell (sc) assay for transposase-accessible chromatin with sequencing (ATAC-Seq) of murine fibroblast populations isolated from the skin, lung, visceral adipose tissue, and lymph nodes and could confirm and further specify tissue-specific fibroblast subpopulations as well as fibroblast populations which we could identify across all analyzed tissues (pan-tissue fibroblasts). These data defined different prevailing footprints of transcription factor families between the populations and allowed inference of a developmental trajectory. Bulk RNA sequencing of pan-tissue and tissue-specific fibroblasts revealed that the pan-tissue fibroblast population represented a more immune-active subpopulation. Specifically, the expression of the cytokine IL-33, a member of the IL-1 family, could be attributed to the pan-tissue population. IL-33 reporter mice and conditional knock-out of IL-33 on fibroblasts revealed distinct functions. scRNA-seq of lung fibroblasts, T cells and metastatic cancer cells from the lung allowed us to identify potential ligand-receptor pairs. Our data have further characterized an immune active fibroblast subpopulation present in all tissues, a finding which helps to better understand the heterogeneity of fibroblasts and their interactions with the immune system.

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1271 – WS45.5

Stromal cells shape the immune response to mycobacteria

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Purpose: Mycobacterial tissue infections induce the formation of granulomas, which are complex immune cell structures. Macrophages (MΦ) are the key players of the granuloma. Nevertheless, a mature granuloma with a necrotic core not only leads to the accumulation of immune cells but also causes remodelling of the tissue with an impact on fibroblasts and stromal cells (SC). In this project, we aim to characterize the role of SC for granuloma formation and control of mycobacteria, as well as the interaction of MΦ and non-immune cells in mycobacterial infections.
Methods: To address these questions, we used *Mycobacterium bovis* BCG mouse infection models and *in vitro* co-cultures of SC and MΦ. We further characterized SC in mycobacterial infections by flow cytometry, confocal and live cell imaging as well as transcriptomic analyses.
Results: Mycobacterial infections lead to the accumulation of bone-marrow derived monocytes at the site of infection, which then further contribute to granuloma formation. We found high expression levels of CCL2 and CCL7 in SC up to 16 weeks post infection, indicating a regulatory role in monocyte recruitment. Transcriptome analysis further revealed an impact of SC on the cytokine environment, including IL-4, IL-13 and TNF production. Although mainly macrophages take up mycobacteria in infection, we observed by microscopy that BCG also infected SC *in vitro*. With the help of adoptive cell transfers, we were able to prove that mycobacterial infections of SC also occur *in vivo*. Additionally, killing assays suggested that the bacteria survived within SC over several days. SC accumulated lipids and showed changes in lipid metabolism during the infection, which might be beneficial for the intracellular survival of mycobacteria as those are highly lipid-dependent. Currently, we are performing single-cell-sequencing analysis to elucidate mechanisms of bacterial uptake in SC as well as MΦ-SC interactions.
Conclusion: Our data point towards an essential role of SC in mycobacterial infections. SC not only influence the MΦ immune response, but also serve as potential host cells for mycobacteria, which is of high relevance for the pathogenesis of latency and the treatment of mycobacterial infections.

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1232 – WS45.6

A circadian clock compound selectively impairs inflammation-induced antifibrinolytic activity to restore clot breakdown pathways.

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Purpose: Circadian rhythms are 24-hour cycles of human physiology that ensure processes occur at the most optimal time of day. However adverse cardiovascular events also show a time-of-day dependency. Stroke, characterised by aberrant blood clotting, has the highest incidence between 8:00 and 9:00am. Immunothrombosis is a phenomenon whereby inflammation triggers the coagulation system and vice versa. Proteins associated with immunothrombosis and inflammation including plasminogen activator inhibitor (PAI) 1, a potent inhibitor of clot breakdown (fibrinolysis), and IL-6 peak in the early morning hours. Therapeutic inhibition of immunothrombosis is challenging due to the complex network of interactions between pro-inflammatory, procoagulant, and fibrinolytic pathways. The aim of this project was to evaluate whether targeting the clock rather than the immune or haemostatic pathways can be a potential therapeutic approach to limit unwanted morning clotting events.
Methods: Bone marrow-derived macrophages (BMDMs) and primary human umbilical vein endothelial cells were stimulated with the clock modulating compound SR9009 followed by activation with the bacterial component lipopolysaccharide (LPS) or pro-inflammatory cytokine TNFα to mimic an immunothrombotic event. Following treatment, RNA from treated BMDMs was sequenced, gene and protein expression was analysed by RT-qPCR and ELISA respectively, fibrinolytic potential was analysed by plasmin generation assays.
Results: SR9009 alone did not affect plasmin generation in the presence or absence of endothelial cells or macrophages. However, under inflammatory conditions, SR9009 potently inhibited inflammation-induced expression of anti-fibrinolytic proteins PAI-1 (Serpine1) and PAI-2 (Serpinb2) in both macrophages and endothelial cells. Promoter analysis shows both Serpine1 and Serpinb2 possess binding sites for circadian transcriptional regulators. In an RNAseq screen in macrophages, SR9009 + LPS suppressed genes involved in leukocyte migration as well as fibrinolysis, with the most significantly suppressed gene being Serpinb2 when compared to LPS-treated cells. Consequently, inflammation-induced suppression of both macrophage and endothelial cell fibrinolytic activity was restored by SR9009 in a plasmin generation assay.
Conclusion: These data suggest SR9009 selectively restores fibrinolysis via inhibition of inflammation-induced anti-fibrinolytic protein generation. These therapeutic properties may have potential application in restoring normal plasmin generation and thus normal clot lysis in individuals with dysregulated fibrinolytic activity in the morning hours.

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WS46 – DENDRITIC CELL DIFFERENTIATION AND FUNCTION

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1131 – WS46.1

Antigen-specific dendritic cell immunotherapy to halt autoimmunity in Type 1 Diabetes

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In Type 1 diabetes (T1D) insulin-producing b cells are destroyed by autoreactive T cells. Dendritic cells (DC) engineered with a lentiviral vector (LV) encoding for IL-10 and an insulin peptide ($InsB_{9-23}$) modulate Ins-specific T cells *in vitro* and *in vivo*. Our goal is to develop tolerogenic (tol)DC-based therapy to restore tolerance in T1D, we i) compared HLA-DQ8/A2-restricted epitopes to select the best autoantigen (autoAg) for DC engineering, ii) assessed the ability of T1D monocytes to differentiate in engineered tolDC, and iii) implemented a humanized mouse model (hu-mice) to test *in vivo* tolDC immunotherapy.
$CD14^+$ cells from HLA-$DQ8/A2^+$ T1D patients were transduced during DC differentiation with a LV encoding for the autoAg-derived peptide fused to the invariant chain and IL-10 (LV-Ag/IL-10). LV encoding for autoAg without IL-10 or invariant chain alone were used as controls. DC were characterized by flow cytometry and cytokine profiling. *In vitro* function was tested by stimulating autologous T cells and responses were evaluated by IFNg ELISA/ELISPOT and by the induction of type 1 T regulatory (Tr1) cells. To implement hu-mice, HLA-$DQ8^+$ cord blood (CB) cells were transplanted into newborn HLA-DQ8-trangenic NSG mice. Reconstituted mice were immunized. T cell response was monitored by flow cytometry.
We selected Hybrid-insulin-peptide 11 (HIP11), Neuropeptide Y_{60-75}, and a mimotope of $InsB_{9-23}$ (InsMIM) as the most HLA-DQ8 cross-reactive peptides. DC from T1D patients engineered with LV-autoAg/IL-10 ($DC^{Ag/IL-10}$) acquired tolDC phenotype ($CD14^+CD16^+CD163^+CD141^+ILT\text{-}4^+$), secreted high levels of IL-10, inhibited Ag-specific IFNg release by autologous $CD4^+$ T cells. Moreover, the frequency of Tr1 cells in culture with $DC^{Ag/IL-10}$ was increased as compared to control DC in T1D pts with detectable Ag-specific reactive T cells. $DC^{Ins/IL-10}$ dampened Ins-specific $CD8^+$ T cell response in HLA-$A2^+$ T1D pts. In hu-mice immunization with CB-derived Ins-pulsed B cells promoted the proliferation of autologous $CD4^+$ T cells *in vivo*. Preliminary data indicate that injection of $DC^{Ins/IL-10}$ modulate $CD4^+$ T cell response in this model.
We identified candidate autoAg-derived epitopes to generate tolDC in T1D patients, demonstrated that Ag-specific tolDC from T1D patients modulate Ag-specific $CD4^+$ and $CD8^+$ T cell responses *in vitro*, and developed a hu-mice model for assessing tolDC-based immunotherapy.

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1569 – WS46.2

Distinct ontogenetic lineages dictate cDC2 heterogeneity

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Conventional dendritic cells (cDCs) include functionally and phenotypically diverse populations, such as cDC1s and cDC2s. The latter population has been variously subdivided into Notch-dependent cDC2s, KLF4-dependent cDC2s, T-bet$^+$ cDC2As and T-bet$^-$ cDC2Bs, but it is unclear how all these subtypes are interrelated and to what degree they represent cell states or cell subsets. All cDCs are derived from bone marrow progenitors called pre-cDCs, which circulate through the blood to colonize peripheral tissues. Here, we identified distinct mouse pre-cDC2 subsets biased to give rise to cDC2As or cDC2Bs. We showed that a Siglec-H$^+$ pre-cDC2A population in the bone marrow preferentially gave rise to Siglec-H$^-$ CD8α$^+$ pre-cDC2As in tissues, which differentiated into T-bet$^+$ cDC2As. In contrast, a Siglec-H$^-$ fraction of pre-cDCs in the bone marrow and periphery mostly generated T-bet$^-$ cDC2Bs, a lineage marked by the expression of LysM. Our results showed that cDC2A versus cDC2B fate specification starts in the bone marrow and suggest that cDC2 subsets are ontogenetically determined lineages, rather than cell states imposed by the peripheral tissue environment.

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966 – WS46.3

Skin DCs display circadian (24 hour) rhythms in metabolic function which may be harnessed by dissolvable microneedle patch to improve vaccine efficacy

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Several vaccines have demonstrated time-of-day dependent responses. Dendritic cells (DCs) are the main target as they process and present vaccine antigens to naïve T cells to elicit protective adaptive immunity. Peripherals DCs display a circadian rhythm in antigen processing and lymph node migration which is dependent on their metabolism. The skin is an ideal target for circadian vaccination because of its large network of DCs, which can be effectively reached using dissolvable microneedle (dMN) drug delivery technology. Thus, we aimed to create the first 'circadian-informed' skin DC vaccine using dMN.
Nanoparticles (NPs) were prepared using an oil-in-water emulsion method. Bone marrow derived DCs were used for *in vitro* work and assessed by flow cytometry (FC). Fluorescent-NP uptake by DC2.4 cells was studied by confocal microscopy. Skin circadian rhythmicity was assessed by Lumicycle using the circadian reporter Per2::Luciferase. Circadian whole skin metabolism was analysed by qPCR/Seahorse, while skin DCs metabolism was studied by spectral FC.
Fabricated NPs were ~200nm in diameter, and we obtained 30% encapsulation efficiency of the model antigen OVA. NP uptake by DCs caused upregulation of activation markers, such as CD40. Lumicycle analysis showed a strong circadian rhythm of core clock components. Seahorse analysis revealed a circadian rhythm in mitochondrial metabolism with a peak in the active phase. Spectral FC on skin DCs revealed circadian rhythmicity in glycolysis with a peak during resting phase.
We have identified that skin DCs are an ideal organ for circadian vaccination using dMN. Our dMN with encapsulated NPs promote DC activation and maturation. Within the skin, maximal basal respiration, which is associated with increased antigen processing and an immature state, occurs during the active phase. However, skin DCs show highest glycolysis, which is associated with maturation and migration, during resting phase. In future work, the circadian transcriptome of skin DCs will be assessed by single-cell RNA-seq to elucidate potential adjuvants to be incorporated into the dMN. We then hope to be able to formulate a dMN incorporating all these features and vaccinate mice at the optimal time of day in terms of skin DC metabolism, to achieve maximal protective immunity.

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2027 – WS46.4

Claudin 1-mediated maturation of thymic dendritic cells type 1 promotes clonal deletion and Treg selection via antigen transfer

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The presentation of self-antigens in the thymus by medullary thymic epithelial cells (mTECs) and dendritic cells (DCs) is crucial for the establishment of central tolerance. While mTECs produce and present self-antigens in an autonomous manner, DCs acquire them from mTECs by cooperative antigen transfer (CAT). Although CAT represents a key process of central tolerance, molecular determinants which define CAT's effectivity as well as the the capacity of DCs to subsequently present acquired antigens are largely unknown. To reveal the nature of these determinants, we compared the transcriptomes of CAT-experienced and inexperienced DCs via scRNAseq, which allowed us to redefine their heterogeneity and maturation. We focused on the candidate gene determinant, *Cldn1*, encoding the tight junction protein Claudin 1, which was found to be highly expressed among the CAT-experienced type 1 lineage DCs (DC1). Using DC1-specific Claudin 1 knockout, we found that mature DC1, which are the most effective in antigen presentation, are impaired. This translates to insufficient clonal deletion and Treg selection of self-reactive clones. A similar phenotype is observed when MHCII expression is specifically ablated in DC1 advocating that, in general, the deficiency of antigen presentation by mature DC1 leads to the impairment of Treg selection. In addition, we mapped the expression of the Claudin 1 ligand, Claudin 3, to mTECs which express the highest levels of self-antigens, suggesting the existence of a molecular bridge between mTECs and DC1. Hence, we propose a dual role for Claudin 1 in DC1: (i) mediating DC1, becoming part of the mTEC network (ii) having a critical role in maturation of DC1. Ultimately, Claudin 1 is a regulatory molecule which coordinates CAT and T cell selection processes. The comprehension of these processes will aid in the resolution of the current controversy concerning the role of DC1 in central tolerance.

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1639 – WS46.5

Click chemistry allows the visualization of an immunogenic HLA-A2-restricted epitope on human monocyte-derived dendritic cells

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Antigen processing and presentation by dendritic cells is essential for the induction of effective T-cell responses. Conventional T-cell based methods to study antigen processing and presentation do not provide information about the underlying processing processes. Having antigens that can be tracked throughout the processing pathway *and* activate a cognate T-cell would be highly insightful in correlating the mechanics of the antigen presentation pathway to the ability of a DC to activate a T-cell. However, the introduction of fluorophores into epitopes blocks T-cell activation and even prevents normal loading of the peptide onto an MHC.
We here show that click chemistry is a powerful solution to this problem. Click reactions are chemical reactions that can be performed selectively without interference of complex cellular environments. They can thus be used to introduce fluorophores selectively onto the specific sites in the epitope *after* processing and MHC-loading. In addition, the click handles used are so small that the clickable epitopes can still activate a T-cell.
Here, we generated clickable variants of the immunogenic, HLA-A2-restricted hepatitis B virus-derived epitope HBc18-27 by replacing each position with the clickable amino acid homopropargylglycine to study the cross-presentation of this epitope. HLA-A2 binding was assessed with the T2 cell HLA stabilization assay. Preservation of immunogenicity was determined with an HBc18-27-specific T cell assay. The accessibility of the handle in HLA-complex was determined on the surface of human monocyte-derived dendritic cells (moDCs) by flow cytometry. Moreover, the presence of the epitope on the surface of and within moDCs was visualized by confocal and superresolution microscopy. Furthermore, including the modified epitope within a synthetic long peptide allowed us to image the uptake, intracellular distribution and surface appearance of the epitope in moDCs.
This is, to our knowledge, the first study to visualize an epitope peptide within an MHC on the surface of an APC without loss of T-cell recognition during cross-presentation. The herein presented clickable epitopes provide a promising prospect for the study of epitope routing and presentation throughout the antigen presentation pathways and may enable direct and quantitative epitope studies in a T-cell-independent manner.

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1120 – WS46.6

Factors determining dendritic cell identity in peripheral tissues

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Tissue-resident dendritic cells (DCs) are central to the maintenance of tolerance within peripheral tissues such as the skin, lung, and intestine. These DC populations exist in immature and migratory states, maintained and fine-tuned by microenvironmental signals. While several master transcriptional regulators of DC states have been identified, the complex interplay of these proteins and how tissue-derived signals are relayed remain poorly understood in both steady-state and inflammatory conditions. Transforming growth factor-β (TGF-β) family members are such tissue-derived signals that can alter DC subset specification and tolerogenicity. Following a microarray screen, we identified several TGF-β1-dependent transcription factors that were rapidly induced or repressed during the early stages of DC/epidermal Langerhans cell commitment. Lentiviral-mediated gain and loss-of-function experiments of these transcription factors reveal their role in DC subset identity. In the context of the skin, a picture emerges whereby monocyte and cDC2-lineage blood precursors are programmed by these factors to follow epithelial versus subepithelial DC fate decisions and that repression of TGF-β1-driven factors is required for maturation. Our data provides further insight into the complex transcriptional networks governing DC subset specification.

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WS47 – CAR-T DEVELOPMENT AND DESIGN

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865 – WS47.1

Regnase-1 KO B7-H3 CAR T cells for osteosarcoma: improved antitumor activity and potency to reprogram endogenous immune cells

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CAR T cell therapy has been successful for hematological malignancies leading to their FDA approval. However, CAR T cell therapy has been less effective in early-phase clinical studies for solid tumors, including osteosarcoma (OS), the most common bone cancer in children. We and others have demonstrated that Regnase-1 (Reg-1) knock-out (KO) CAR or TCR T cells have improved antitumor activity. However, the effects of Reg-1-KO on OS-specific CAR T cells is unknown. Thus, the goal of this project was to determine if Reg-1-KO CAR T cells have improved effector function and induce an immunostimulatory lung microenvironment in an immune-competent OS model with lung tumors, the most common site of metastases.
To target OS, we focused on B7-H3, an antigen that is reliably expressed in OS. Murine Reg-1-KO and Ctrl-KO B7-H3-CAR T cells recognized and killed murine OS cell lines in an antigen-specific manner as judged by cytokine (IFN-γ, GM-CSF, IL-2) production and cytotoxicity assays. To determine if Reg-1-KO improves the effector function of B7-H3-CAR T cells *in vivo*, mice received an intravenous (i.v.) injection of F331 cells to induce lung tumors, and on day 7 an i.v. dose of $1x10^6$ Reg-1-KO or Ctrl-KO B7-H3-CAR T cells, Reg-1-KO Sp6-CAR T cells or PBS. Reg-1-KO B7-H3-CAR T cells had improved antitumor activity and significant survival advantage. Flow cytometry and scRNAseq revealed that Reg-1-KO enhanced the expansion of B7-H3-CAR T cells on day 7 post-infusion and induced the expression of pro-inflammatory cytokines (IFN-γ, IL-13), cytokine receptors (IL-12R), increased OXPHOS and mitochondrial fitness. Endogenous immune cells revealed that Reg-1-KO B7-H3-CAR T cells induced immunostimulatory lung microenvironment with an influx of IFN-γ producing CD4 and CD8 T cells, and NK cells that was paired with a reduction of inhibitory cells, M2 macrophages. These effects lasted for at least 21 days post-CAR T cell infusion.
In conclusion, we demonstrate that Reg-1-KO improves the antitumor activity of B7-H3-CAR T cells and that Reg-1-KO CAR T cells reprogram endogenous immune cells without systemic toxicity. These promising results warrant further active exploration and if confirmed, early-phase clinical testing of Reg-1-KO B7-H3-CAR T cells in patients with recurrent/refractory OS.

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1329 – WS47.2

FOXO1 enhances CAR T stemness, metabolic fitness and efficacy

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Chimeric antigen receptor (CAR) T cell therapy has transformed the treatment of hematological malignancies such as ALL, B cell lymphoma and multiple myeloma but the efficacy of CAR T cell therapy in solid tumors has thus far been limited. This is due to a number of factors including the immunosuppressive tumor microenvironment that gives rise to poorly persisting and metabolically dysfunctional T cells. Analysis of anti-CD19 CAR T cells used clinically have shown that positive treatment outcomes are associated with a more "stem-like" phenotype and increased mitochondrial mass. We therefore sought to identify transcription factors that could enhance CAR T cell fitness and efficacy against solid tumors. Here we show that overexpression of FOXO1 significantly promotes a "stem-like" phenotype in CAR T cells derived from either healthy human donors or patients, which correlates with improved mitochondrial fitness, persistence and therapeutic efficacy in vivo. This work thus reveals an engineering approach to genetically enforce a favorable metabolic phenotype that has high translational potential to improve the efficacy of CAR T cells against solid tumors. The study has recently been accepted for publication in Nature.

#Chan, #Scheffler, #Munoz, Sek……*Lai, *Beavis and *Darcy. FOXO1 enhances CAR T cell stemness, metabolic fitness and efficacy. **Nature** (accepted Feb, 2024).
#Contributed equally
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1748 – WS47.3

Generating CRISPR/CAS9 armoured CAR- and TCR-T cells for the treatment of solid tumours

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Purpose: CAR T cell therapy has exhibited remarkable clinical success in the treatment of haematological malignancies, however, their efficacy in solid tumours is limited by antigen heterogeneity and immunosuppression imposed by the tumour microenvironment. In order to overcome these barriers, 'armoured' CAR T cells which secrete pro-inflammatory cytokines have been developed. However, toxicities related to the unrestricted expression of the armouring transgene has limited the application of these CAR T cells in the clinic. Our lab has previously demonstrated enhanced CAR T cell efficacy upon CRISPR/Cas9 mediate deletions of A2A (Giuffrida et al. *Nature communications* May. 2021), and here, our lab has developed a novel CRISPR knock-in strategy whereby we aimed to engage endogenous gene regulatory mechanisms drive transgene expression in a tumour-localised manner (*in review, Cell)*.
Methods: Genome-wide RNA-sequencing was used to identify genes in CAR T cells with tumour-specific expression. Promotors *NR4A2* and *RGS16* were identified as key candidates due to their inhibitory role in T cells. A novel CRISPR/Cas9-mediated homology directed repair (CRISPR HDR) strategy was then employed to knock in (KI) proinflammatory cytokines into these gene loci. This enabled the simultaneous deletion of an inhibitory gene while achieving tumour-specific control of cytokine expression.
Results: CRISPR HDR knock in of pro-inflammatory cytokines such as IL-12 and IL-2 into *NR4A2* and *RGS16* promoters supported the delivery of cytokines directly to the tumour site, leading to enhanced anti-tumour efficacy and long-term survival of mice in both syngeneic and xenogeneic models. This was concomitant with improved CAR T cell polyfunctionality, activation of endogenous anti-tumour immunity, a favourable safety profile and was applicable using CAR T cells from patients. Furthermore, with the aim to provide an even greater safety profile for this technology, this CRISPR HDR KI method was applied to tumour neoantigen specific TCR-T cells. These TCR-T cells induce T cell activation, produce pro-inflammatory cytokines upon T cell activation, and eliminate HLA-matched KRAS G12D expressing tumour cells.
Conclusion: CRISPR HDR enables the generation of armoured CAR T and TCR-T cells with tumour-restricted cytokine secretion. This is superior to analogous strategies currently used clinically such as artificial NFAT-based promoter systems.

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2051 – WS47.4

MALT1-induced Roquin cleavage in T cells and CAR T cells is critical for anti-tumor immunity

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Roquin is an RNA-binding protein that regulates the decay of mRNAs, many of which are proinflammatory targets. Expression of Roquin-1 and Roquin-2 proteins is crucial for T cell homeostasis and for the prevention of autoimmunity. Inactivation of Roquin-1/2 leads to spontaneous T cell activation and augments adoptive T cell functions in preclinical tumor models. During T cell activation, Roquin-1 and Roquin-2 undergo cleavage by the paracaspase MALT1, resulting in transient loss of function and subsequent derepression of various targets. Yet, the functional repercussions of Roquin cleavage on CD8+ T cells and CAR T cells remain inadequately understood.
To investigate the importance of Roquin cleavage, we engineered mice harboring MALT1 insensitive alleles of Roquin-1 and Roquin-2, termed "Double Mins" or “dMins”, to prevent cleavage events. Additionally, we developed a novel monoclonal antibody specific for the amino-terminal fragment of MALT1-cleaved human and mouse Roquin proteins.
CD8+ T cells from dMins/dMins mice displayed substantial impairments in T cell receptor (TCR) and co-stimulation induced activation, proliferation, and mTOR/S6 phosphorylation, alongside a markedly reduced production of Tumor Necrosis Factor-alpha (TNF-alpha). Investigation of Roquin cleavage in human and mouse T cells and CAR T cells revealed a correlation between Roquin cleavage and TCR strength, as well as enhanced effector functions. Notably, we demonstrate for the first time that Roquin cleavage regulates T cell-mediated killing of tumor cells *in vitro* and *in vivo*.
Taken together, this study unveils the significance of Roquin cleavage as a critical checkpoint in effectively controlling tumor growth and broadens our understanding of how to improve adoptive T cell therapy.

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1477 – WS47.5

Learning the effect of CAR T cell design on single cell distributions

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Introduction: Chimeric antigen receptor (CAR) T cells are a promising approach in cancer immunotherapy. Their safety, efficacy and functionality depend on the design of the CAR, which is a synthetic receptor that contains up to three intracellular signalling domains derived from a range of cellular signalling pathways. Due to its modular design and the multitude of possible domains, there is a vast combinatorial space of CAR designs. There are ongoing efforts to improve CAR T cell safety and efficacy based on CAR designs. However, testing the effect of each CAR design experimentally is resource and labour intensive, and not feasible beyond a few hundred combinations. Preliminary efforts on leveraging machine learning for CAR design have emerged, which focus on predicting bulk readouts. However, the underlying cell-to-cell heterogeneity is not considered.
Purpose: We aim to use single-cell multi-omic profiling and machine learning to model the effect and associated heterogeneity of CAR design on stimulating T cell states.
Methods: We developed a library of 30 CAR designs using combinations of five different intracellular domains. We also include two control designs, one that only contains the CD3zeta domain, and one without any signalling domains. CAR T cells were generated from two donors and characterised by single-cell CITE-sequencing at three timepoints in a 12-day repeated antigen stimulation (RAS). We used machine learning to map a single cell population of control cells to a population of cells expressing a CAR variant.
Results: We found that the major sources of heterogeneity are the donor, the time spent in RAS, and the cell state. CAR design affected the relative abundances of T cell states but CAR-related gene expression patterns where too subtle to identify. We show preliminary encouraging results on mapping single-cell profiles from the control to the CAR design populations, capturing the cell state distribution of the CAR population. Additionally, we predict the T cell state composition for unseen combinations of domains.
Conclusion: In the future, we aim to extend our method to unseen domains, which would give insights into the cell states associated with new CAR designs and guide CAR T cell therapy.

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790 – WS47.6

Microfluidic approach to study T and CAR-T confined and 3D cell migration

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Purpose: The immune system plays a decisive role in the defence against pathogens and aberrant cells, as tumoral cells. Nevertheless, the collective immune surveillance against solid tumours appears to be rather ineffective overall (Gonzalez et al., 2018). This lack of effectiveness is comprised by the physical barriers to infiltration and the active suppression by the tumour, resulting in poor migration (White et al., 2022). To overcome this limitation, it is essential to characterise this mechanism in physio-pathologically relevant scenarios to decipher the immune response.
Methods: In this context, we have developed novel microfluidic-based approaches that recreate the biomechanical aspects of solid tumours (Juste-Lanas et al., 2022; Movilla et al., 2022). Two distinct microfluidic geometries were used: one of them based on a central chamber which allowed hydrogel polymerization for 3D-migration (Hervas-Raluy et al., 2023), while the other one on microstructures of confined channels with varying widths (Paul et al., 2016). The microfluidic platforms were fabricated with polydimethylsiloxane (PDMS) due to its many benefits, including biocompatibility, transparency, flexibility and gas permeability. Then, same-donor T or CAR-T cells were seeded on the microchips, visualised via time-lapse microscopy and the obtained images were processed using ImageJ and Matlab to quantify cell migration.
Results: We found that T cells confined velocity is microchannel width-independent, while CAR-T cells show a great reduction in the highest confinement degree. Additionally, both types of immune cells display higher velocity under confinement compared to 3D migration.
Conclusion: These results highlight that CAR-T cell migration behaviour differs from T cells under confinement and that biomechanical cues, such as confinement, affect the correct infiltration of immune cells in solid tumours. As potential applications, our methodology will not only enhance the characterisation of immune cell migration but also enable the prediction of infiltration efficacy in patients. Additionally, it will facilitate the study of gene expression implicated in the decrement observed in CAR-T migration within confinement microchannels, paving the way to enhance immunotherapy targeting solid tumours. This work was supported by the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (ICoMICS grant agreement No 101018587).

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WS48 – CYTOKINES AND CHEMOKINES IN CANCER

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683 – WS48.1

IL-1R8 acts as an immune checkpoint in CD8+ T cells, limiting cytokine-induced anti-tumor immune responses

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Purpose: IL-1R8 is an atypical Interleukin-1 receptor (ILR) family member that controls inflammation by dampening ILR and Toll-like receptor (TLR) downstream pathways. Studies conducted on IL-1R8-deficient NK cells highlighted its pivotal role as immune checkpoint and negative regulator of IL-18-dependent activation. In particular, IL-1R8-deficiency has been shown to trigger NK cell-mediated immune surveillance against hematogenous metastasis, liver cancer and viral dissemination. Currently, genetic and pharmacological targeting of ILR and IL-18 are under investigation to improve $CD8^+$ T cell-based immunotherapy. The relevance of IL-1R8 in mitigating IL-18-driven activation and, conversely, the pathways instigated by the IL-1R8 loss-of-function in T lymphocytes are still elusive.
Methods: Combing transcriptomic analysis, high dimension flow cytometry, genetic silencing/inhibition, and *in vivo* models, we investigated IL-1R8 expression by $CD8^+$ T cells and its function in Tumor Infiltrating Lymphocytes (TILs).
Results: IL-1R8-deficiency led to anti-tumor immune resistance against colon carcinoma and fibrosarcoma by enhancing $CD8^+$ TIL fitness, synergizing with the immune checkpoint inhibitor anti-PD-1. TILs exhibited accelerated maturation\proliferation kinetic, induction of IL-2R complex and increased tumoricidal potential independently of antigen presenting cells (APCs). Mechanistically, we found that IL-1R8 controlled IL-18-mediated Type-1 polarization by affecting the Transcription Factor (TF) T-bet and, in turn, the effector molecules Interferon-γ (IFNγ) and Granzyme B (GZMB) in both antigen-specific and polyclonal T cell responses. Unexpectedly, IL-1R8 further curtailed the IL-2/EOMES/IL-2R signaling axis, tuning cell-autonomous mechanisms sustained by autocrine IL-2 signaling. In human, we demonstrated that IL-1R8 is rapidly induced in early-mature precursors and the central memory stage of $CD8^+$ T cells in healthy donors and cancer patients. Finally, an original anti-human IL-1R8 inhibitor and CRISPR-Cas9 mediated IL-1R8 ablation potentiated both primary human T lymphocytes and CAR-T cells against B-cell lymphoma.
Conclusions: Overall, the study revealed a profound impact of IL-1R8 deficiency or blockade on $CD8^+$ T lymphocyte immunobiology, which promoted cytokine-induced responses and conferred superior anti-tumor potential. Thus, IL-1R8 is a promising and targetable immune checkpoint of both NK and $CD8^+$ T cells to be exploited for implementing cancer immunotherapy.

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312 – WS48.2

Investigating regulators of CXCL9 and CXCL10 expression to improve T cell infiltration and immunotherapy responses in solid tumors.

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Purpose: Immune checkpoint blockade (ICB) has revolutionised the treatment of numerous cancer types, including melanoma and non-small cell lung carcinoma. ICB targets immune-inhibitory molecules on the surface of T cells, unleashing their anti-tumour potential. Despite ICB's success, a high frequency of patients fail to respond to this therapy. A key limiting factor to ICB responses is the number of T cells that infiltrate the tumour microenvironment. T cell infiltration in the context of ICB has been shown to be dependent on chemoattractant molecules CXCL9 and CXCL10. The expression of these chemokines is also predictive of a positive response to ICB across multiple cancer types, highlighting their importance in ICB efficacy. We have previously demonstrated that these chemokines are predominantly produced by intratumoral macrophages. Therefore, we aimed to identify genes that we could target to enhance CXCL9/10 production in macrophages as a strategy to improve T cell infiltration and ICB responses in solid tumors.
Methods: We performed whole-genome CRISPR/Cas9 screening on a macrophage cell line to identify regulators of CXCL9 and CXCL10. To screen for secreted factors, we utilised a CRISPR-HDR technique we have previously validated to generate a macrophage cell line that expressed GFP and BFP as a bona fide readout of CXCL9/10 production. This screen identified PTPN2 as a key negative regulator of both CXCL9 and CXCL10 production.
Results: PTPN2 deletion enhanced CXCL9/10 production by primary murine/human macrophages *in vitro*, confirming our screen results. With PTPN2 inhibitors currently in clinical trials, we sought to define how PTPN2 depletion in macrophages plays a role in anti-tumor immunity. Myeloid-specific depletion of PTPN2 *in vivo* improved ICB efficacy and delayed tumor growth in a murine breast cancer model. CXCL9 expression was elevated in both intratumoral macrophages and dendritic cells, and improved T cell infiltration was observed without an increase in T_{reg} numbers when treated with ICB.
Conclusion: This work has uncovered that PTPN2 has a role in limiting CXCL9/10 expression in myeloid cells, which subsequently limits anti-tumor immunity. Myeloid-specific PTPN2 depletion led to improved responses to ICB, providing rationale to combine PTPN2 inhibitors with ICB for patients that are refractory to treatment.

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218 – WS48.3

Interferon epsilon limits ovarian cancer metastasis via regulation of peritoneal immune cells

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Interferon epsilon (IFNε) is a unique type I interferon which is constitutively expressed by the female reproductive tract (FRT) epithelium. High grade serous ovarian cancer (HGSOC) is a cancer of the FRT which frequently presents with extensive metastasis throughout the peritoneal cavity, and carries a 5-year survival rate of <40%. We have determined that IFNε is highly expressed by healthy Fallopian tube epithelial cells (the cell of origin of HGSOC), but is lost in HGSOC tumours. In the ID8 mouse model of ovarian cancer, IFNε$^{-/-}$ mice developed a greater number of metastases compared to controls. Moreover, treatment of mice with exogenous IFNε, but not IFNβ, significantly reduced peritoneal metastasis and disease scores [1].
Mechanistically, IFNε demonstrated intrinsic anti-tumour activities when applied to HGSOC tumour cells, including increased apoptosis and reduced proliferation. The role of extrinsic anti-tumour activity by IFNε was investigated using an IFN-insensitive IFNAR1$^{-/-}$ ID8 cell line. Mice were injected intraperitoneally with wild-type (WT) or IFNAR1$^{-/-}$ ID8 cells, and treated with PBS or IFNε for six weeks. Analysis of disease scores revealed that IFNε treatment effectively limited HGSOC metastasis and progression, with no loss of efficacy observed in mice bearing IFNAR1$^{-/-}$ tumours versus WT. Furthermore, IFNε treatment was associated with marked alterations in peritoneal immune cells. IFNε treatment reversed the infiltration of immunosuppressive immune cells and suppression of immune cell proliferation observed in PBS-treated mice. Moreover, IFNε-treated mice displayed a greater frequency of activated immune cells such as CD8^{+} T cells [1].
To further elucidate the immunological mechanism of action of IFNε in the peritoneal cavity, single-cell RNA sequencing was performed on peritoneal cells from PBS- and IFNε- treated tumour-bearing mice. Significant remodelling of the peritoneal macrophage populations by IFNε was observed, suggesting these cells may regulate the immune response to HGSOC in the peritoneal cavity. Together, these results demonstrate that IFNε effectively inhibits metastasis of HGSOC, mediated primarily through its activity on immune cells, and may have potential as a novel cancer immunotherapy.

1. Marks, Z.R.C., Campbell, N.K., Mangan, N.E. et al. Interferon-ε is a tumour suppressor and restricts ovarian cancer. *Nature* 620, 1063–1070 (2023).

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671 – WS48.4

Targeting IL1R2+ tumor-infiltrating regulatory T cells with an anti-IL1R2 nanobody construct induces a strong anti-tumor effect in combination with anti-PD1 therapy

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Immune checkpoint blockade (ICB) has revolutionized cancer therapy. However, ICB still suffers from low response rates and disease hyper progression. Several pieces of evidence point towards the involvement of a strongly immune-suppressive cell population called tumor-infiltrating regulatory T cells (tiTregs) in these phenomena. Hence, targeting Tregs has high potential in cancer therapy, specifically in combination with ICB. However, peripheral Tregs are essential to maintain tolerance and immune homeostasis, therefore, the therapy needs to specifically target the tiTregs. This can be achieved by targeting a tiTreg-specific marker. Interleukin 1 receptor 2 (*IL1R2*) gene has been shown to be specifically upregulated by tiTreg. Hence, we verified that IL1R2 protein is absent from peripheral Tregs and from anti-tumoral immune cells infiltrating mouse tumor models. We also showed that IL1R2 marks a highly activated and suppressive population of tiTregs. However, a deficiency of this receptor, either in the whole body or specifically within Treg, did not affect tumor characteristics nor tiTreg infiltration or phenotype, suggesting that this receptor is not functionally important on these cells in a cancer setting. Next, we generated nanobodies (Nbs), the smallest naturally occurring antigen-binding fragments, against IL1R2 and functionalized them with the Fc region of mouse IgG2a, known for its capacity to mediate antibody-dependent cell-mediated cytotoxicity (ADCC). This construct could indeed successfully deplete a proportion of the tiTregs which, in combination with ICB, caused a significant delay in mammary tumor growth. Furthermore, we enhanced the efficacy of the Nb construct by introducing point mutations in the Fc region known to potentiate the ADCC activity. We showed that the combination of ICB with this potentiated construct was superior to the combination with the original construct, causing full regression of 60% of the mammary carcinoma tumors with maintenance of immunological memory against a later challenge with the same cancer cells.

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378 – WS48.5

Role of the atypical chemokine receptor ACKR2 in the immune response to tumors

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ACKR2 is an atypical chemokine receptor that negatively controls inflammation being able to bind and lead to degradation most inflammatory CC chemokines. Previous results indicate that genetic deletion of ACKR2 resulted in increased tumor growth in inflammation-driven models. On the contrary, ACKR2 KO mice are protected in metastatic models of melanoma and breast cancer. To better understand the relative contribution of the cell types expressing ACKR2 in metastasis protection, an inducible and conditional reporter knockout mouse was generated.
ACKR2 expressed by hematopoietic progenitor cells regulates their metabolism and genetic deletion of ACKR2 induces long-term functional reprogramming of neutrophil differentiation that protects against cancer.
ACKR2 is also selectively expressed by capillary endothelial cells in the lung and deletion of ACKR2 from these cells resulted in protection from melanoma metastasis with increased lung extravasation of T lymphocytes.
These results indicate that targeting ACKR2 may be an innovative therapeutic strategy to unleash long-term functional reprogramming of neutrophil differentiation toward an antitumoral state. In addition, targeting ACKR2 could improve lung metastasis immunotherapy by improving lung lymphocyte extravasation.

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723 – WS48.6

Targeting IL-38 triggers γδ T cell-dependent anti-tumor immunity

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Purpose: The IL-1 family receptor antagonist IL-38 acts as a suppressor of auto-inflammation. Given the connection between auto-immunity and anti-tumor immunity, we wondered if targeting IL-38 would affect the anti-tumor immune response.
Methods: The transgenic PyMT mammary carcinoma model, which is suitable for identifying strong immunomodulators due to being immunologically cold, was employed to target IL-38 genetically or with a self-generated neutralizing antibody. Immune cell profile and localization were determined by flow cytometry and immunohistochemistry, respectively. Whole transcriptome RNA sequencing, gene expression analysis and *in vitro* functional assays were used for mechanistic studies.
Results: IL-38-deficient (KO) PyMT mice presented a markedly delayed tumor growth compared to WT mice, accompanied by increased cytotoxic T cell infiltrates, including CD8+ T cells and γδ T cells. This phenotype was recapitulated upon the treatment with IL-38-neutralizing antibodies, where antibody-treated mice showed a major decrease in tumor growth compared to IgG-control treated animals, accompanied by increased CD8+ T cell and γδ T cell infiltrates. Particularly, the IFN-γ-producing Vγ1 γδ T cell subset was increased by targeting IL-38. In a therapeutic model of chemoresistance, anti-IL-38 antibodies in combination with doxorubicin increased tumor control followed by increase in CD8+ T cells and γδ T cells as well. Importantly, the delayed growth of IL-38 tumors was abolished upon γδ-TCR blockade with antibodies, accompanied by a strong decrease in both, CD8+ T cells and the Vγ1 γδ T cell subset, indicating a crosstalk between both populations. While mechanistic studies revealed no direct cross-talk between CD8+ T cells and γδ T cells, we found that γδ T cells recruit conventional dendritic cells (cDC1) into IL-38 targeted tumor via the specific chemokine Xcl1, which subsequently activate CD8+ T cells via the Notch pathway. Accordingly, in human mammary carcinoma, IL-38 expression negatively correlates with patient survival as well as CD8+ T cell and γδ T cell infiltrates.
Conclusion: Taken together, these results provide evidence for a therapeutic potential of anti-IL-38 antibodies to activate anti-tumor immunity in mammary carcinoma.

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WS49 – REGULATION OF ADAPTIVE IMMUNITY

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223 – WS49.1

T-cell receptor triggering requires inactivation of Lim kinase-1 by Slingshot-1 phosphatase

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Purpose: Actin dynamics control early T-cell receptor (TCR) signalling during T-cell activation. However, the precise regulation of initial actin rearrangements is not completely understood. Here, we have investigated the regulatory role of the phosphatase Slingshot-1 (SSH1) in the regulatory signaling pathways driving this process.
Methods: The distribution of SSH1 at the immunological synapse was studied in human CD4 T lymphocytes using live-cell imaging. Knockdown of SSH1 expression by CRISPR/Cas9-mediated genome editing in Jurkat T-cells or small interfering RNA (siRNA) in primary human CD4 T lymphocytes were used to analyse the TCR early signalling and CD3ε conformational change by western blot and pull-down, and the distribution of F-actin and integrins towards the immunological synapse by confocal microscopy. Total Internal Reflection Fluorescence Microscopy was used to study the dynamics of actin cytoskeleton during synaptic contacts and T-cell effector function was determined by CD69 and CD25 expression by flow cytometry and IL-2 secretion by ELISA.
Results: Our data show that SSH1 rapidly polarises to nascent cognate synaptic contacts and later relocalises to peripheral F-actin networks organised at the mature synapses. Knockdown of SSH1 expression revealed a regulatory role for SSH1 in CD3ε conformational change, enabling Nck binding and proper downstream signalling and immunological synapse organisation. TCR triggering induced the SSH1-mediated activation of actin dynamics through a mechanism mediated by Limk1 inactivation.
Conclusion: These data suggest that during early TCR activation, Limk1-dependent early F-actin rearrangements are regulated by SSH1 phosphatase. This pathway fine-tunes the initial conformational changes of the TCR, integrin organization, and proximal signaling events to properly organize the immunological synapse and T-cell effector function.

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1304 – WS49.2

Smoking changes adaptive immunity with persistent effects

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Human immune responses differ across individuals, with age, sex and genetics playing major roles in this diversity. However, the variables that drive such differences remain poorly defined. We quantified 13 cytokines after 12 immune stimulations (bacterial, fungal, viral), and tested their associations with 136 variables from the Case Report Forms of the healthy donors of the "Milieu Intérieur" cohort. Through multi-variate data analysis and integration, we identified three novel variables associated with cytokine secretion variability - Smoking, Body Mass Index (BMI) and Cytomegalovirus (CMV) latent infection - with effects comparable to those of age, sex and genetics. We observed short-term effects of smoking on innate immunity and a previously unappreciated long-term effect of smoking on adaptative immunity[1]. The short-term effects of smoking were associated with increased CXCL5 cytokine induction and CEACAM6, a soluble blood protein that is involved in inflammatory processes and has been proposed as a clinical biomarker for multiple cancers. The long-term effects of smoking were increased induction of IL2 and IL13 in smokers and past smokers, compared to non-smokers, that were associated with long-lived B and T cells and DNA methylation levels of specific genes that encode signal trans-activators or metabolism regulators.
To extend upon these findings, we are now reconstructing the transcriptional networks involved in immune response differences from analysis of RNA-seq data of 200 donors after immune stimulations. We are also performing a longitudinal analysis of cytokine induction for 413 of the original 1,000 individuals of the cohort, sampled 10 years later. The study of the evolution of immune responses over time, depending on changes and pathologies experienced by the donors should help us refine the effects of aging, smoking, BMI, CMV infection and their interactions, and has potential clinical implications for the risk of developing infections, cancers or autoimmune diseases.
This programme is managed by the Agence Nationale de la Recherche and benefit from support of the French government's Invest in the Future programme ANR-10-LABX-69-01.

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1163 – WS49.3

IFN-γ Suppresses T Follicular Helper Cell Differentiation and Antibody Responses

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Humoral and cellular immune responses typically co-exist during viral infections. However, there are instances where one response predominates, determining the primary antiviral activity. For example, lymphocytic choriomeningitis virus (LCMV) infection elicits a pronounced cellular response, yet exhibits a suboptimal neutralizing antibody (nAb) response. This deficiency in nAb response hinders its clearance and facilitates persistent infection. Recent findings indicate that this preference for cellular immunity over humoral immunity is significantly regulated at the CD4+ T cell differentiation stage. Specifically, subcutaneous LCMV infection predominantly induces T helper 1 (TH1) differentiation, which augments cellular immunity, while largely neglecting T follicular helper (TFH) differentiation, a key driver of humoral immunity. Here, we investigated the mechanisms responsible for this inhibited TFH differentiation. We found that the TH1 cells induced by subcutaneous LCMV infection are heterogeneous. They encompass a terminally differentiated TH1 subset expressing Granzyme-B (GzmB) and a Tcf-1+ subset that retains the potential for TFH differentiation. While IL-12 appeared to be non-essential for this differentiation, T cell-derived IFN-γ facilitated the proliferation of the GzmB+ subset and inhibited the Tcf-1+ cells' progression into TFH. Consistently, inhibition of IFN-γ enabled robust TFH differentiation, leading to the formation of germinal centers and increased antibody production. Our study provides novel insights into the mechanisms inhibiting nAb production in response to specific viruses and offers a foundation for the development of advanced vaccine strategies.

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1774 – WS49.4

Tlr7 bi-allelism defines a transcriptionally and functionally distinct B cell subset

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Evidence of sexual dimorphism in the immune response has been clearly established. Women develop a stronger immune response than men, giving them better protection against bacterial and viral infections, but this increased immunity makes them more susceptible to developing autoimmune pathologies such as systemic lupus erythematosus (SLE). While in women, one of the two X chromosomes is inactivated (XCI), some genes can escape XCI, leading to increased expression of these genes in female cells. Among them, the Toll-7 receptor (TLR7) encoding an endosomal receptor recognizing ssRNA from self and non-self, can escape X chromosome inactivation in human and murine female B cells. To address the phenotypic profile of B cells where Tlr7 evades X silencing, we generated an original dual reporter mouse model in order to track *Tlr7* biallelic cells referred as BiA7 B cells in vivo. We will present evidence that BiA7 B cells represent a functionally distinct subset of B cells. BiA7 B cell overexpressed various FO B cell markers, and are poised for immediate differentiation into antibody secreting cells and PC upon in vitro stimulation. To address the contribution of BCR- and TLR-signaling in the development of this distinct B cell populations, we generated MD4 dual reporter mouse model, expressing a monoclonal BCR against hen egg lysozyme. We also generated Tlr7 dual reporter mice on a Myd88-deficient background. Our data demonstrate that BCR-signaling is dispensable for BiA7 B cells development and function. The contribution of Myd88-signaling is under investigation. BiA7 B cells can be easily generated using an in vitro iGB cell expansion culture system. We will present data regarding the contribution of parental imprinting effects and epigenetic chromatin marks on the development of BiA7 B cells as well as the role of cytokines. Together our results provide evidence for the existence of a sub-population of B lymphocytes with biallelic expression of Tlr7 in female cells, poised for a rapid activation and differentiation into ASC and PC. The existence of BiA7 B cells could be an advantage by being preferentially selected during antiviral responses, but could also contribute to the development of B cell systemic autoimmunity.

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2238 – WS49.5

Centromeric AA haplotype and -21 dimorphism in HLA-B T/T as powerful predictors for recurrent pregnancy loss

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The role of NK cell alloreactivity in pregnancy outcomes remains uncertain. It is well-established that NK cells become activated through interactions with inhibitory KIR (iKIR) and NKG2A receptors, which recognize specific ligands on fetal cells. NKG2A specifically recognizes HLA-E bound by recipient HLA class I leader peptides, a process requiring methionine (M) at position 21 of the leader sequence. A rs1050458C/T dimorphism results in approximately 40% of individuals expressing at least one copy of 21 HLA-B (M/M or M/T), enabling ligand expression. We evaluated the impact of recipient HLA-B leader genotype (M+ versus M- (T/T)) and HLA-C group iKIR missing ligand (ML, C1C1/C2C2) on patients with recurrent pregnancy loss and compared them with a control group.
We conducted our study in a cohort of 77 patients with RPL and 75 healthy women, from whom we performed analysis of KIR, HLA-B, and HLA-C genes using peripheral blood samples and luminex technology. Our results showed that, as previously observed, the centromeric haplotype AA is increased in the RPL group compared to the control group, with a difference of 24%, with a p-value of 0.0056. Furthermore, upon analyzing the results through the iKIR missing ligand hypothesis, we observed that patients had significantly fewer KIR2DL1 ligand absences, with a p-value of 0.018. Lastly, we analyzed the dimorphism, and the results show that the -21 (T/T) dimorphism is significantly increased in the RPL group compared to controls, with a p-value of 0.0031. Additionally, when combining the Cen AA haplotype with the B dimorphism, we see that CenAA-T/T is much more prevalent in the RPL group with a p-value of 0.0005. Therefore, we confirm that the CenAA haplotype is a good marker for the diagnosis of patients with RPL, and furthermore, HLA-B could be a promising biomarker, as the results are conclusive, but we would need to increase the number of patients to confirm it.

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1007 – WS49.6

Effect of rs2204985 polymorphism on human thymic T cell differenciation at the single cell level

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Purpose: We previously described an association between human thymopoiesis and a common variant located within the TCRA/D T-cell receptor locus, the rs2204985 SNP, with the G allele associated with higher thymic output. This variant has recently been shown to be linked to the clinical outcome of hematopoietic stem cell transplantation, as well as to response to COVID-19. Our aim is therefore to understand how this genetic variation influences the thymocyte differentiation process and has a significant impact on human health.
Methods: In order to capture the transcriptional changes through thymopoiesis, we perfomed scRNA-Seq analysis on 8 postnatal thymi ranging from 1 week to 10 months, including 4 samples of each homozygous genotype (AA or GG). To analyze the entire thymic population, we included total thymic samples as well as sorted cells from the earliest and rarest subpopulations that were obtained by flow-sorting using CD34, CD38 and CD1a markers. From these populations, 16 scRNA-seq libraries for gene expression profiling were generated using the 10X Genomics platform.
Results: We obtained a total of 37936 cells that we visualized using the uniform manifold approximation and projection. Cell clusters were annotated into 19 different cell types representing the whole range of human thymocyte differentiation from thymus seeding progenitors to single positive cells.
CD34+ cells mapped to distinct clusters depending on their polymorphism status highlighting transcriptional differences. By differential analysis of gene expression according to the variant within CD34+ cells, we observed differences in expression of genes involved in Notch signaling (*NOTCH1, NOTCH3, RBJP*), in Notch target genes (*HES4*, *PTCRA*, *IL7R*) as well as in genes involved in stemness maintenance (*HES1, CD44, MEFC2*), cell proliferation and metabolism. These results suggest that progenitors initiate T cell specification differently depending on their genotype. To investigate these results further, SNP-dependant analysis of proliferation and recombination rate, β-selection and trajectories toward αβ or γδ lineages will be performed.
Conclusion: In summary, our findings support a SNP rs2204985-related "fine tuning" of thymopoiesis in the human postnatal thymus. Further exploration of the transcriptional networks and the epigenetics marks linked to the polymorphism will provide new insights in human thymopoiesis.

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WS50 – INNATE IMMUNITY IN CANCER I

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263 – WS50.1

The memory phenotypic immunity of tumor-draining lymph nodes in ovarian carcinoma patients

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The lymphatic system is strategically positioned at dedicated sites throughout the body to facilitate a rapid and efficient immune response. However, their involvement by solid tumors in the peripheral tissues is a hallmark of cancer and a strong indicator of poor prognosis. Emerging evidence suggests that tumor-specific humoral immune responses occur within the tumor tissue. Nonetheless, the contribution of tumor-draining lymph nodes (TDLNs) to this process in humans is not yet clear. Here, we studied TDLNs derived from high-grade serous ovarian carcinoma (HGSOC) patients. Comparatively, we included inflamed lymph nodes from IBD patients to underscore the TDLNs' unique state in cancer. We discovered that TDLNs are disrupted and inactive, hosting a deposit of memory lymphocytes that show tumor-binding potential. Through single-cell sequencing and multiplex imaging techniques, we observed that TDLNs in HGSOC patients lack germinal center structures and demonstrate limited T and B cell clonal expansion. Yet, the B cell phylogeny has shown extensive class-switch recombination and somatic hypermutation, indicating past tumor immune responses. Additionally, our research identified a specific population of macrophages that appear to hinder the formation of germinal centers. The lack of activity in TDLNs may explain the ineffectiveness of immune checkpoint blockade therapies in HGSOC. These results point to a novel population of TDLN-resident tumor-specific memory B cells, suggesting that their reactivation in the clinic could benefit HGSOC patients.

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1394 – WS50.2

Prior Acute Influenza Infection Drives a Transient Pro-Tumoural State in the Lungs

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Following acute infection, an anti-viral state exists in the immune system that can impact the course of subsequent infections. Since this anti-viral state is broad, we hypothesised that prior infections could alter immune responses to tumours.
Mice were infected with influenza A virus (IAV) and, at 28-days post-infection with viral clearance, the lung and draining lymph node showed persistent changes in composition and organisation. Surprisingly at this point mice developed more lung tumours in models of both primary and metastatic disease. The same result was observed after infection with influenza B virus or intranasal challenge with lipopolysaccharide suggesting that ongoing post- viral inflammation may generate a pro-tumoural state.
To address this, mice were treated with anti-inflammatories during IAV infection, which not only prevented the subsequent increase in tumour burden but led to a reduction. This decrease suggests an underlying anti-tumoural mechanism in addition to the pro-tumoural inflammation. We therefore looked longer term following flu to where inflammation had fully resolved and found that when tumours were induced at 90-days post IAV infection, tumour burden was reduced.
At this later time point there was an increase in type 1 conventional dendritic cells (cDC1). To determine whether this increase was sufficient to replicate the protection from tumour induction mice were treated with FLT3L prior to tumour challenge. This treatment increased cDC1 numbers and led to a highly significant decrease in metastatic seeding.
Acute IAV infection drastically reshapes the lung and lymph nodes, driving a temporary post-viral inflammation which is pro-tumoural. Later this resolves, unmasking an anti-tumoural effect potentially related to persistently increased cDC1 and T-cell priming. We now aim to investigate the importance of cDC and T cell immunity in protecting from subsequent cancer development and to explore FLT3L as an anti-metastatic therapeutic.

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295 – WS50.3

Dissecting macrophage diversity in cancer to unveil distinct contributions to therapeutic resistance

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Background and rationale: The efficacy of immune checkpoint inhibitors (ICIs) in cancer relies on the presence of an effective T cell-mediated immune response. However, multiple features of the tumor microenvironment constrain T cells activation thus contributing to therapy resistance. Importantly, emerging studies have shown that ICIs efficacy is in part dependent on tumor associated macrophages (TAMs). On this line, we recently discovered that lipid-laden macrophages (LLMs) infiltrate tumors, including prostate cancer, ovarian cancer and melanoma.
Methods: Here we applied single cell RNA sequencing to the immune infiltrate of prostate cancer and melanoma. We then implemented a multiparametric flow cytometry strategy to confirm transcriptional findings. In vivo, we set up transgenic and transplantable models of prostate cancer and melanoma to investigate the immune composition of tumors. Finally, we isolated tumor infiltrating lipid-loaded macrophages and we performed bulk RNA sequencing and mass spectrometry to dissect the transcriptional features and the proteome profile of LLMs.
Results: We demonstrated that the abundance of LLMs correlates with tumor size and we discovered that LLMs promote cancer progression in association with immune evasion and poor response to chemotherapy. Lipid intake in TAMs is partially dependent on scavenging by MARCO and MARCO neutralization in vivo deplete LLMs both in prostate cancer and melanoma models. Mechanistically, we also found that TAMs display a dysfunctional autophagy provoked by a deregulation of the TFEB-dependent CLEAR signalling pathway that is responsible of lipid accumulation. Finally, we demonstrated that LLMs infiltrate melanoma and ovarian tumors in patients that are refractory to ICIs. Importantly, this enrichment is even observed prior to treatment initiation, suggesting that LLMs may be predictive of ICIs response.
Conclusions: Together, our findings identify a heterotypic crosstalk involving LLMs and cancer cells that drives tumor aggressiveness and is implicated in resistance to immunotherapies.

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1272 – WS50.4

Role of JNK in chronic intestinal inflammation and its relationship with tumor development

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Purpose: The c-Jun N-terminal kinases (JNKs) are conserved proteins which regulate various cellular responses, including cell proliferation, differentiation or survival. Hyperactivation of the JNK signaling is observed in many inflammatory diseases, contributing to pathology through the regulation of multiple inflammation-related genes. The objective of this work was to identify specific mechanisms of the JNK signaling pathway involved in intestinal chronic inflammation and its relationship with tumor development.
Methods: Mice with conditional deletion of *Jnk1* and *Jnk2* in the intestinal epithelial cells ($JNK^{\Delta IEC}$) were generated by crossing $Jnk1/2^{flox/flox}$-mice with VillinCre-mice. We analyzed small and large intestine samples using histological techniques, flow cytometry, RNAseq and Western Blot. Intestinal permeability was assesed *in vivo* using FITC-Dextran. Moreover, we induced colitis-associated colorectal carcinoma (CRC) using AOM/DSS method.
Results: Samples from 10, 52, and 72 weeks old $JNK^{\Delta IEC}$ mice were analysed. Young $JNK^{\Delta IEC}$ mice developed mild spontaneous inflammation in the duodenum and colon, associated with an increase in the percentage of intraepithelial γδT lymphocytes and NKG2D-expressing γδT lymphocytes, NK cells, and NKT cells. Intestinal permeability and microbiota-epithelium contact were also increased. In 52-week-old mice, inflammation progressed to high-grade epithelial dysplasia with cellular infiltrates in the duodenum, culminating in spontaneous duodenal tumor formation by 72 weeks. Transcriptomic analysis revealed enrichment of the complement pathway in duodenal samples. C3-protein expression was restricted to intestinal crypts in healthy tissues, while it was expressed throughout tumor tissues. Colon samples exhibited a dramatic downregulation of defensins genes. Additionally, increased apoptosis, unchanged proliferation, and elevated levels of non-phosphorylated RIPK1, implicated in cell survival, were observed in the duodenum of $JNK^{\Delta IEC}$ mice. Furthermore, $JNK^{\Delta IEC}$ mice were more susceptible to CRC than control mice, developing a greater number of tumors with a more aggressive histopathology.
Conclusion: The deletion of *Jnk1* and *Jnk2* in IECs is associated with a clear pro-inflammatory and pro-tumor phenotype. The complement system, highly expressed in tumor tissues, may play a crucial role in this process, alongside dysregulated death-survival mechanisms.

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827 – WS50.5

Deciphering the myeloid cell landscape of neuroblastoma

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Neuroblastoma (NB), a neoplasm of the sympathetic nervous system, is the most common solid extra-cranial tumor of infancy being located mostly in the adrenal glands. Its prognosis is extremely variable depending on disease features. High risk disease is associated with age (>18 months), metastatic stage and *MYC* amplification. Even though chemo and immune therapy are used as treatment, long-term survival is still poor and around half of all patients will relapse and die. Hence, a better understanding of its biology is urgently needed to develop new drugs that will improve long-term survival. Over the last decade, the presence of myeloid cells has been correlated with adverse outcome in several cancers, largely attributed to their suppression of T- and NK- cell mediated immune response. However, most of the immune profiling studies in NB have been performed by techniques that do not allow a comprehensive analysis of myeloid cell heterogeneity. Here, we integrated 9 single cell RNAseq dataset of biopsies from more than 50 patients and 10 fetal adrenal glands, and compared with in-house spatial and flow cytometry data. We observed the recruitment of monocytes and their differentiation to *TREM2-SPP1+* macrophages as it happens in non-fetal adult cancers. *TREM2+* macrophages as well as *IL1B+* monocytes were more abundant in patients of high-risk NB. Moreover, we observed the recruitment of conventional dendritic cells, which are less abundant in high-risk disease. Together this data suggests that high-risk NB are characterized by the inability to mount an efficient immune response against the tumor. Our study provides a deep cartography of the immune composition of NB environment with putative underlying mechanisms of immune escape.

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421 – WS50.6

Tissue-resident macrophages and circulating monocytes might contribute to bacterial infection susceptibility of lung cancer patients

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Background: Lung cancer (LC) remains a significant global health challenge, with a high mortality rate and limited treatment options. LC patients are more susceptible to infections due to their immune system, lung damage, comorbidities, and potential immunosuppressive treatments. *Staphylococcus aureus* (*SA*), *Streptococcus pneumoniae* (*SP*), and *Pseudomonas aeruginosa* (*PA*) are just some of the pathogens involved in pneumonia development and in infectious complications in cancer patients. Considering that human lung macrophages (HLMs) are the most abundant immune cells in the lung and that hypoxia is the most common microenvironment feature of LC tissue, the aim of this study was to investigate the effect of *SA*, *PA*, and *SP* infection on HLMs under hypoxic and normoxic conditions. Considering the impossibility to isolate HLMs from healthy donors (HDs), we also evaluated the effect of *SA*, *PA*, and *SP* on monocytes, circulating progenitors of macrophages, of patients with LC compared to HDs.
Methods: HLMs were isolated from tissue of patients undergoing lung resection and were incubated at different times and with different concentrations of heat-killed *SA*, *PA*, and *SP* under hypoxic and normoxic conditions. At the end of the experiments, we evaluated cytokine and ROS production and chemiotaxis. In addition, monocytes of LC patients and HDs were stimulated with HK-bacteria and cytokine release was measured in supernatants.
Results: HK-bacteria induced IL-6, IL-1β, TNF-α, and ROS production from HLMs after 6 h of incubation. Furthermore, HK-bacteria promoted chemiotaxis of HLMs. The effect of HK-bacteria on cytokine release from HLMs was stronger under hypoxic condition. Monocytes of LC patients displayed a higher pro-inflammatory response to HK-bacteria compared to HD monocytes.
Conclusion: HLMs and monocytes seems to contribute to the susceptibility and outcomes of infections in LC patients. The hyperactivation of these immune cells in response to pathogens underscores the intricate interplay between the immune system and LC progression. Further research is essential to elucidate the mechanisms driving the dysregulated immune responses in LC patients.

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WS51 – VIRAL IMMUNITY I

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442 – WS51.1

Prevotella timonensis enhances HIV-1 transmission by primary human blood dendritic cells

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HIV-1 remains a serious global health problem with many newly acquired infections affecting young women and girls in sub-Saharan Africa. The main route of HIV-1 infection is via mucosal surfaces in the genital tract during sexual intercourse. Alterations in the vaginal microbiota enhance susceptibility to HIV-1 infection however, underlying molecular mechanisms remain largely unknown. Here, we investigated the role of vaginal dysbiosis associated bacteria in altering HIV-1 uptake and transmission by dendritic cell (DC) subsets. *Prevotella timonensis,* but not other microbiota, enhanced uptake of different HIV-1 strains in monocyte-derived DCs. Notably, *P. timonensis*-enhanced uptake seems specific for viruses as *P. timonensis* enhanced cellular uptake of different viruses, but not other antigens or pathogens, such as fungi or bacteria. Furthermore, *P. timonensis* enhanced HIV-1 uptake over time and this process is independent of cellular HIV-1 capture and entry (co-)receptors. In addition, HIV-1 localises to tetraspanin-positive intracellular compartments, that are thought to be involved in HIV-1 transmission. Clathrin but not caveolin specific inhibitors blocked HIV-1 uptake in *P. timonensis* treated cells. Strikingly, analysis of primary blood DC subsets showed that *P. timonensis* not only enhanced HIV-1 uptake, but this also resulted in increased HIV-1 transmission to T cells. Besides increasing HIV-1 uptake and transmission, we observed that *P. timonensis* enhanced clustering of DCs with CD4 T cells, providing an additional mechanism enhancing HIV-1 transmission. *P. timonensis*-induced transmission could contribute to the enhanced HIV-1 susceptibility observed in women with vaginal dysbiosis. To conclude, our study provides new insights into the role DCs play in HIV-1 uptake and transmission after bacterial exposure, how bacteria modulate DCs to efficiently transmit HIV-1, and underscores the importance of examining the role of the microbiome in viral pathogenesis. Identification of underlying mechanisms and potential targets could facilitate the design of therapies to reduce the risk of HIV-1 acquisition and AIDS.

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1108 – WS51.2

Cross-Reactive Immune Responses Following Concomitant Immunization Against Flaviviruses

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Flaviviruses are distributed all over the world and pose a significant global health burden. Infection with heterologous flaviviruses generate cross-reactive immune response but the clinical outcome of pre-existing immunity to subsequent flavivirus infection is still not fully understood. To prevent disease from flaviviruses, there are commercially available vaccines that work effectively against several flaviviruses, including against yellow fever virus (YFV), Japanese encephalitis virus (JEV) and tick-borne encephalitis virus (TBEV). Exposure to multiple flaviviruses is common, and co-administration of different vaccines against flaviviruses could be a useful strategy to generate broad, cross-reactive, immunity. However, safety and immunogenicity have until recently not been appropriately evaluated upon concomitant delivery of flavivirus vaccines.

To describe safety and immunogenicity upon co-vaccination with different combinations of flaviviruses at the same time, we studied adaptive memory B and T cell responses in a cohort of healthy individuals vaccinated with single or multiple immunizations against flaviviruses, as part of an open label, non-randomized clinical trial (EudraCT number: 2017-002137-32, Sandberg et al., 2023).

We assessed the extent of the cross-reactive responses from antigen-specific memory B-cells, CD4+ T-cells and CD8+ T-cells in both single and co-vaccinated participants against YFV, TBEV, JEV, in both temporal and qualitative measurements.

Our results from our clinical trial demonstrated that inactivated TBEV or JEV vaccines could be co-administered with the live attenuated YFV vaccine without an increased risk of adverse events. Furthermore, the development of neutralizing antibodies to the respective viruses was not diminished by co-vaccination. Regarding cross-reactivity, we observed a high degree of cross-reactive antibody responses across single and co-vaccinated participants. Furthermore, extensive cross-reactivity between different structural and non-structural antigens of flaviviruses was observed among antigen-specific T cells.

In conclusion, this study characterized cross-reactivity of adaptive immunity against multiple flaviviruses and the impact of heterologous immunization.

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624 – WS51.3

Longitudinal antibody and T cell response to Ebolavirus disease amongst a cohort of survivors in Guinea

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Purpose: 10 years ago, an epidemic of *Ebolavirus* disease in West Africa resulted in over 28,600 cases and 11,325 deaths. Since the conclusion of this outbreak in 2016 we have been working with survivor groups from the affected regions to better understand immunological memory following natural infection. Working with the local community, we recruited 117 survivors and received blood donations at regular intervals between 2015-2024.
Methods: Using processed serum, we measured the live virus neutralisation response to Zaire Ebolavirus as well as the antibody response to recombinant glycoprotein (GP) and inactivated virus. Using peripheral blood mononuclear cells (PBMC) we performed IFNγ ELISpot and in-depth B and T cell phenotyping assays.
Results: We found that the mean anti-GP and T cell responses have remained remarkably stable over time, although individual results varied greatly. Modelling of the longitudinal antibody data suggested a half-life of ~10years. The mean T cell response, as measured by ELISpot, was 305 spot-forming units, this again remained stable over time. We found the dominant antigen specific CD8+ polyfunctional T cells showed a stem cell memory like phenotype whereas the CD4+ T cells showed a central memory phenotype and that Ebola virus glycoprotein specific memory B cell are detectable in the blood in the years following infection.
Conclusion: The continuous high titre of neutralising antibodies and T cell response supports the concept of long-term protective immunity in survivors.

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1681 – WS51.4

Single-cell profiling reveals signatures of protective immunity to human SARS-CoV-2 challenge

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Human SARS-CoV-2 infection induces widespread cellular and molecular changes in the immune landscapes of exposed individuals. However, the role of pre-exposure immune variation and temporal dynamics of immune responses following SARS-CoV-2 infection remain insufficiently understood. To systematically map immune responses to a defined exposure of SARS-CoV-2, we performed longitudinal single-cell sequencing of nasopharyngeal swabs and blood samples, collected in the context of a highly controlled human infection model (CHIM). We expand upon previous single cell analysis of SARS-CoV-2 challenge in seronegative individuals by including a cohort of seropositive volunteers, with prior history of community-acquired infections and vaccinations. This integrated single cell resource allowed us to resolve primary immune responses, immunological memory and recall responses at unprecedented detail. Longitudinal analysis revealed highly diverse response dynamics, with several temporally and tissue-restricted cell states generated during abortive, transient and sustained SARS-CoV-2 infections. We track the clonal dynamics of adaptive immune cells and showcase evidence for mucosal germinal centre reactions, reflecting a potential mechanism of regulating B cell immunity at the local site of infection. In addition, we introduce Cell2TCR, a computational tool for adaptive immune receptor profiling, which identified public SARS-CoV-2 specific TCR motifs in subsets of activated T cells. Notably, baseline immune profiling revealed adaptive immune signatures in seropositive individuals, who resisted SARS-CoV-2 infection despite exposure to a high viral challenge dose. Taken together, we leverage single cell multi-omics and analyse unique samples from SARS-CoV-2 CHIMs to delineate immune signatures and cellular dynamics associated with protective antiviral immunity.

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267 – WS51.5

NK cell derived IFN-γ mobilizes free fatty acids from adipose tissue to promote B cell activation during viral infection

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Viral infection typically causes us to lose fat, but how and why this happens is unclear. The immune system plays a major role in the regulation of adipose tissue biology during homeostasis and in context of metabolic disease. Here, we investigated whether immune cells also modulate adipocyte metabolism during viral infection. We find that visceral adipose tissue transiently decreases adiposity following viral infection. Upon pathogen encounter, adipocytes upregulate surface expression of ligands for the receptor NCR1 on NK cells, which drives their secretion of IFNγ. This cytokine directly stimulates adipocytes to downregulate PPARγ, which leads to their release of lipids in circulation, most notably of free fatty acids (FFAs). The FFA oleic acid stimulates the early activation of B cells by promoting oxidative phosphorylation. Oleic acid induced their expression of costimulatory B7 molecules and promoted their ability to prime CD8 T cells. Prevention of lipid release by adipocytes during infection impaired B cell activation, leading to reduced CD8 T cell responses and increased viral replication. Our findings uncover a new mechanism of metabolic adaptation to infection and provide a physiological background for the activation of immune cells in adipose tissue in context of metabolic disease.

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642 – WS51.6

β-glucan immunometabolic rewiring enhances anti-viral immunity via Interferon-dependant anti-viral training (IDAT)

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Background: Early viral containment through a proper, balanced immune response is critical to drive successful immunity and avoid disease. It is now emerging that yeast β-glucans can improve innate immune responses to bacterial infections and cancer through a process termed "Trained Immunity". However, little is known about impact on anti-viral innate immunity.
Results/Methods: Using a yeast-derived β-glucan whole glucan particle (WGP), we first confirmed the ability to drive classical training responses in bone-marrow derived macrophages (BMDMs) with enhanced pro-inflammatory cytokine production (TNF, IL-6). However, the production of anti-viral cytokines (IFN-β, CXCL10) was also enhanced, particularly after restimulation with viral ligands. RNA-sequencing of BMDMs from WGP-supplemented mice revealed upregulated Oxphos and TCA cycling pathways. Heightened rates of oxidative metabolism was confirmed by Extracellular-flux analysis of similar cells, while in-vitro inhibition of Oxphos was shown to impede the training effects induced by WGP, highlighting the importance of Oxphos for maintaining a trained phenotype. These metabolic changes coincided with increases in mitochondrial membrane potential and IFN-β production after WGP-stimulation. These changes coincided with increased expression of interferon stimulated genes (ISGs) in macrophages from WGP-treated mice, with notable increases in two key anti-viral transcription factors, Irf7 and Ikßkε. BMDMs from mice-fed WGP also increased type-I interferon responses in response to viral ligand stimulation, including IFN-β and CXCL-10. We also saw enhanced expression of the immune-response gene 1 (IRG1) as well as a reduction in the expression of fumarate hydratase (Fh). These results suggest that enhanced responsiveness of WGP-trained cells was driven by immunometabolic IFN-β signalling. Using IFNAR-deficient cells to investigate this, we observed that while IFN-β signalling did not affect immunometabolic responses or alter TNF production induced by LPS or PAM, it was vital for enhanced responsiveness to viral ligand stimulation. Similarly, IFNAR-deficient mice supplemented with WGP lost their ability to drive enhanced myelopoiesis showing the importance of this immunometabolic phenotype both in-vitro and in-vivo.
Conclusions: Therefore, we posit a novel anti-fungal signalling pathway that confers cross protection against viral infections, termed interferon-signalling dependant anti-viral training (IDAT). This novel training pathway coupled with classical trained immunity may hold potential to tackle future viral pandemics.

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WS52 – REGULATORY T CELLS IN HOMEOSTASIS AND DISEASE

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400 – WS52.1

Homeostatic balance of GUT resident pTregs and tTregs plays a pivotal role in maintaining bone health under post-menopausal osteoporotic conditions

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Introduction: Osteoporosis is a skeletal disease that leads to the deterioration of bone tissue. Recent research has shed light on the potential involvement of the intestinal immune system in regulating bone-health throughout the body. Gut-Tregs play an essential role in the modulation of immune responses and prevent various inflammatory manifestations. Understanding how Gut-Tregs contribute to bone homeostasis in post-menopausal osteoporosis (PMO) could pave way for novel therapeutic strategies targeting "immune-gut-bone" axis.
Aims and Objective: To investigate the role of gut-resident Tregs in regulating bone-health in preclinical model of post-menopausal osteoporosis (ovx).
Material Methods: Female C57BL/6 mice were divided into two groups: Sham (control) and ovx (bilateral removal of ovaries). At day 45, mice were euthanized, feces, intestine and bone-marrow were harvested for various analyses using SEM, μCT, FACS, HPLC and ELISA/CBA. *in vitro*, assays were conducted to investigate the role of SCFAs on the differentiation of gut-Treg cells and their effect on osteoclasts and osteoblasts.
Results: We observed a notable shift in the composition of gut-resident-Treg subsets in PMO, characterized by a decrease in peripherally-derived Tregs (pTregs) alongside an increase in thymus-derived Tregs (tTregs). Expanding on these findings, we delved into the potential role of pTregs in modulating bone remodeling using *in vitro* assays. Our results demonstrated that gut-resident pTregs exert a significant positive-influence on osteoblastogenesis while concurrently inhibiting-osteoclastogenesis, highlighting their regulatory impact on bone-remodeling. HPLC data further confirmed that ovx mice had significantly lower levels of SCFAs (acetate, propionate, and butyrate) in the feces as compared to the sham-group. Intriguingly, we observed that SCFAs play a crucial role in replenishing gut-resident Tregs via promoting the differentiation of pTregs. Interestingly, pTregs primed/treated with SCFAs exhibited significantly enhanced potency in suppressing osteoclastogenesis compared to unprimed pTregs, underscoring the therapeutic relevance of SCFAs in bolstering Treg-mediated regulation of bone metabolism.
Conclusion: Our findings altogether represent a groundbreaking revelation regarding the critical role played by the pivotal balance of pTregs and tTregs in the pathophysiology of osteoporosis. This discovery marks a significant advancement in highlighting a previously unrecognized frontier centered around the "pTreg-tTreg" cell axis, with huge clinical implications.

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309 – WS52.2

A novel aryl hydrocarbon receptor ligand inhibits the development of Type 1 diabetes by enhancing the function of tolerogenic dendritic cells and regulatory T cells

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Type 1 diabetes (T1D) is a chronic autoimmune condition characterized by an imbalance between pathogenic CD4+ and CD8+ lymphocytes on one side and regulatory T cells (Treg) on the other. Activating Treg and/or tolerogenic dendritic cells (tolDC) through various methods has shown benefits in animal models of T1D. Both cell types express high levels of the aryl hydrocarbon receptor (AHR), and AHR activation is typically associated with enhanced tolDC and Treg functionality. In this study, the novel fluorescent indole-containing compound AGT-5 was investigated for its potential to act as an AHR ligand and modulate immune cells both in vitro and in vivo. Through in silico docking analysis, AGT-5 demonstrated the ability to bind AHR, and its agonistic effects were confirmed using the reporter Caco-2 cell line. Additionally, due to its fluorescent properties, AGT-5 was visualized in macrophages in vitro and in the small intestine lamina propria ex vivo after oral administration using confocal microscopy. AGT-5 exhibited no toxicity towards murine macrophages and human tonsillar cells. Moreover, tests on zebrafish embryos revealed no nephrotoxicity, hepatotoxicity, or cardiotoxicity. Utilizing an ADME virtual platform it was confirmed that AGT-5 possesses drug-like characteristics. Subsequently, AGT-5 was orally administered to C57BL/6 mice that had received low doses of streptozotocin to induce T1D. Treatment with AGT-5 commencing from the first day of T1D induction and lasting for 20 days effectively prevented immune cell infiltration into the pancreas, preserved insulin production, and halted the progression of T1D. AGT-5 achieved this by promoting tolDC and Treg activity along the gut-pancreatic lymph node-pancreas axis. Mechanistically, AGT-5 upregulated indoleamine 2,3-dioxygenase 1 in tolDC and enhanced ATP-degrading enzyme expression on Treg, thereby promoting immunosuppressive action. The positive outcomes seen in T1D animals suggest that AGT-5 holds promise for a potential treatment for inflammatory conditions that can benefit from stimulation of the regulatory arm of the immune response.

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1399 – WS52.3

The role of regulatory T cells in thymic regeneration

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Purpose: T cell production is essential for patients undergoing stem cell transplantation and for cancer immunotherapy. A functional thymus is needed for the maturation of T cells, but unfortunately the thymus is highly sensitive to injury. While the thymus does harbor an endogenous capacity to regenerate itself, the role of regulatory T cells (Tregs) in this process is unknown.
Methods and results: In our murine models of thymic injury (SL-TBI, cyclophosphamide, dexamethasone, LPS and MCMV), we observed numeric and fractional expansion of Tregs within the thymus, peaking at the thymic cellularity nadir after injury. Depletion of Tregs before injury using the Foxp3^{DTR} model impaired thymic regeneration, whereas adoptive transfer of sorted thymic Tregs improved thymic regeneration. Using RAG2^{GFP} mice, where GFP is expressed under the promotion of RAG2, we observed that the increase in Tregs was mainly due to mature RAG2^{GFP}- Tregs but not de-novo produced RAG2^{GFP}+ Tregs after injury. Brdu administration after injury indicated active proliferation of these Tregs, and a RAG2^{GFP}parabiotic system, where a RAG2^{GFP}.CD45.1 mouse is surgically joined to a RAG2^{GFP}.CD45.2 mouse, indicated active recirculation of these Tregs after injury. Single-cell sequencing of CD45+ RAG2- cells in RAG2^{GFP} mice before and at day 1, 4, and 7 after sublethal irradiation revealed a unique transcriptional signature in RAG2^{GFP}- Tregs, including the regenerative factor amphiregulin. Its cognate receptor was seen to be upregulated on thymic epithelial cells and spatial transcriptomics of the thymus revealed increased EGFR signaling after injury. Conditional depletion of amphiregulin in Tregs using Foxp3^{Cre}Areg$^{fl/fl}$ mice resulted furthermore in impaired thymic regeneration after injury. Applying machine learning derived transfer of murine gene orthologs of recirculating Tregs to single cell sequencing of human thymi from children undergoing open heart surgeries enabled us to identify an analogous population of Tregs in the human thymus. These human recirculating Tregs where clonally expanded, expressed high levels of CD39 and ICOS, and secreted amphiregulin after stimulation.
Conclusion: Altogether, our data indicates that 1) recirculating thymic Tregs mediate thymic regeneration, 2) their regenerative capacity is dependent on amphiregulin, and 3) the presence of an analogous population of Tregs exists in the human thymus.

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88 – WS52.4

Conventional type 2 dendritic cells license FoxP3+ regulatory T cells in a Ccr4-dependent manner to suppress allergic inflammation

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FoxP3+ regulatory T cells (Tregs) play an indispensable role in restraining aberrant immune responses, including allergen-specific Th2 cell immunity that drives allergic disease. Treg suppression of adaptive immune responses requires co-localization with antigen presenting cells (APCs) and/or effector T cells. Ccr4 is a chemokine receptor expressed by both Th2 cells and Tregs and implicated in promoting cell trafficking into non-lymphoid tissues. While APCs have been shown to express the Ccr4 ligands Ccl17 and Ccl22, the lung APC subsets expressing the Ccr4 ligands and the cell-intrinsic role of Ccr4 in Tregs during allergic inflammation remain unclear. Here, using a murine model of allergic asthma and single cell RNA-sequencing analysis of lung APCs, we demonstrate that a population of activated, type 2 conventional dendritic cells (cDC2s) are the dominant source of Ccl17 and Ccl22 during allergic inflammation. We show that the type 2 cytokines IL-4 and IL-13 promote the development of activated cDC2s expressing the Ccr4 ligands. Using a novel, conditional Ccr4 knockout mouse, we show that specific and inducible deletion of Ccr4 in Tregs during allergic inflammation does not impair Treg trafficking into the lungs, but impairs their suppressive function, leading to a dramatic increase in allergic inflammation, including increased numbers of activated cDC2s, Th2 cells, and eosinophils. Our results suggest that the type 2 cytokines promote the development of a cDC2-dependent hub to license Ccr4+ Tregs to suppress allergic inflammation. Thus, we define a critical circuit between cDC2s and Tregs in a barrier tissue with implications for developing novel therapeutic approaches to suppress chronic allergic disease.

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367 – WS52.5

Regulatory T cells drive OPC differentiation independent of MHC-II

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Background: Multiple sclerosis (MS) is an immune-mediated inflammatory disease of the central nervous system (CNS), characterised by the loss of myelin and oligodendroglia (demyelination). Although myelin regeneration (remyelination) can occur in MS, it often fails, leading to disease progression and subsequent disability. While there are no treatments targeting myelin repair, our lab has shown that regulatory T cells (Treg) can drive oligodendrocyte progenitor cell (OPC) differentiation and remyelination. The mechanisms underlying this effect remain mostly unknown but have been linked to the T cell activator MHC-II. In MS, MHC-II is highly expressed in de- and remyelinating lesions and can be upregulated by glial cells important to myelin regeneration (microglia, astrocytes, and oligodendrocytes). Thus, we hypothesised that MHC-II is required for efficient OPC differentiation and remyelination.
Methods: To test this hypothesis, we used *in vitro* OPC-T cell co-cultures and an *in vivo* model of lysolecithin-induced demyelination in WT and MHC-II-deficient mice.
Results: *In vitro*, we found that non-activated Treg significantly drive OPC differentiation independent of MHC-II. This pro-regenerative effect was impaired when Treg were not exposed to MHC-II during T cell development. In a transwell assay, Treg directly co-cultured with OPCs significantly enhanced OPC differentiation and not when physically separated. Immunofluorescence staining of lesioned spinal cord sections *in vivo* also revealed the absence of MHC-II does not significantly affect the number of oligodendrocyte lineage cells, proliferating OPCs and differentiated oligodendrocytes, but may impair CNS remyelination.
Conclusion: Together, these findings suggest a novel MHC-II-independent mechanism for Treg-driven OPC differentiation. Ongoing work is investigating whether MHC-II is necessary for remyelination *in vivo* and the mechanism(s) by which Treg function beyond what is classically known in regeneration.

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621 – WS52.6

Revisiting the role of the transcription factor Foxo1 in the biology of thymic and peripheral regulatory T cell subsets

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Regulatory CD4 T (Treg) cells are the main mediators of peripheral tolerance under physiological conditions. In the periphery, Treg cells include cells that have been naturally produced in the thymus, called thymic Treg (tTreg) cells, and cells that have differentiated from naive CD4 T cells after activation in secondary lymphoid organs (SLOs), called peripheral Treg (pTregs) cells. In addition to and within this ontogenic heterogeneity, Treg cell activation level, location in SLOs or tissues or the age at which they were produced further drives the diversity of this cell subset. Although they share equivalent functions, all these Treg cell subsets have been shown to play complementary roles in the prevention of autoimmune diseases. Importantly, the signals driving the generation of these subsets of Treg cells could differ. For instance, while TCR and IL-2 signaling pathways dictate the generation of tTreg cells, TGFβ signaling (through Smads activation and binding to Foxp3 CNS1 enhancer) is crucial for pTreg cell differentiation. Many other factors have been identified as controlling the homeostasis of Tregs in general, but only few of them have been shown to regulate specific Treg cell subsets.
We recently identified Foxo1 as a putative specific regulator of the perinatal tTreg cell subset homeostasis. Indeed, analysis of Treg cells from mice invalidated for Foxo1 specifically in T cells showed a major and specific alteration of this Treg cell subset. We have shown that in perinatal Foxo1-deficient mice, the most activated Treg cells die by apoptosis and fail to proliferate due to an intrinsic defect of $Foxo1^{KO}$ tTreg cells to sense IL-2 results. Interestingly, our results strongly suggest in $Foxo1^{TKO}$ neonates, an intrinsic defect of $Foxo1^{KO}$ tTreg cells to sense IL-2 results in the apoptosis of this Treg cell subset and ultimately leads to an inversion of the balance between tTreg and pTreg cell subsets. This hypothesis was further confirmed by our latest data showing that whereas $Foxo1^{TKO}$ mice only develop with age a mild autoimmune disease, limiting pTreg cell generation in $Foxo1^{TKO}$ $CNS1^{KO}$ mice leads to a severe multi-organ autoimmunity in this mouse model.

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WS53 – T CELLS IN AUTOIMMUNE AND INFLAMMATORY DISEASE

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1653 – WS53.1

The interplay between innate Immunity mechanisms in advancing neurodegeneration

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Alzheimer's disease (AD) is a chronic, progressive neurodegenerative disorder and is the most common form of dementia in elderly people worldwide. The main pathological hallmarks of AD consist of the accumulation of extracellular senile plaques of amyloid-beta, intracellular neurofibrillary tangles, synaptic degeneration, and neuronal injury in areas of the brain that control cognition. In addition to the established role of the brain's resident glial cells, infiltrating peripheral leukocytes have been recently discovered to play a pivotal role in brain physiology and contribute to disease pathogenesis. Recent studies on AD patients and mouse models with AD-like pathology have suggested a role for T cell accumulation in the brain.
Our study aimed to characterize the interplay between innate immunity mechanisms in disease development in 3xTg-AD mice, a transgenic mouse model developing both amyloid and tau pathologies. The non-canonical population of T lymphocytes harboring γδ TCR chains represents a unique lymphocyte population with innate immunity characteristics. We first evaluated the extent of γδ T cell infiltration in the meninges and brain, and observed an increased number of γδ T cells in 3xTg-AD mice compared to wild-type control animals. Moreover, flow cytometry data revealed an activated, IL-17–producing phenotype of meningeal and brain infiltrating γδ T cells in 3xTg-AD mice. These data were confirmed by single-cell RNA sequencing showing γδ T-cell pathogenic signatures, including genes involved in T activation and IL-17 production. Then, to demonstrate a role for γδ T cells in disease induction, we generated an AD mouse model deficient in the TCR δ chain (3xTg-ADxTcrd$^{-/-}$). In 3xTg-ADxTcrd$^{-/-}$ mice, memory and neuroinflammation were improved, and the typical signs of AD were reduced. In addition, when γδ T cells were genetically ablated in 3xTg-AD mice, peripheral and central neutrophil dysregulation was reversed, suggesting that these IL-17+ γδ T cells lead to an inflammatory condition that relied on circulating neutrophils.
Our findings show a new role for IL-17-producing γδ T cells in AD development, suggesting that targeting the interaction between γδ T cells and neutrophils could be a promising approach for AD treatment, potentially leading to new therapies.

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116 – WS53.2

Post-translational modification of LL37, the autoantigen of psoriasis, polarize responder CD4-T-cells towards an alternative T helper phenotype

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Psoriasis is a chronic skin disease evolving to psoriatic arthritis (PsA) in 30% of cases. LL37 is a psoriasis T-cell autoantigen, recognized by both CD4 and CD8 T-cells and, in complex with self-DNA/RNA, is a trigger of type I interferon (IFN-I) in plasmacytoid dendritic cells (pDCs) and of pro-inflammatory factors in myeloid dendritic cells (mDCs). LL37 can undergo irreversible post-translational modifications, namely citrullination and carbamylation, favored by a neutrophil-dominated inflammation. Of note, carbamylated and citrullinated LL37 (carb-LL37 and cit-LL37) are antibody targets in PsA
Purpose: Our goal was to understand whether cit-LL37 and carb-LL37 are detectable in psoriatic skin biopsies and to address whether T-cells of psoriasis patients react not only to LL37, but also to cit-LL37 and carb-LL37.
Methods: We used laser scan confocal microscopy (LSCM) to analyze skin biopsies of psoriasis patients and control healthy donors. We used Ki67 expression proliferation assays and intracellular staining to analyze CD4 T-cell responses in psoriasis and cytokine production by flow cytometry
Results: We detected carb-LL37 and cit-LL37 in lesional psoriasis skin. Of interest, both modified peptides co-localized with neutrophil infiltrates. Neutrophil extracellular traps (NETs) were visible in psoriatic dermis where carb-LL37 and cit-LL37 were present. Psoriatic CD4 T-cells proliferated and produced Th1/Th17 cytokines in response to LL37 and carb-LL37. Interestingly, only cit-LL37-specific CD4 T-cells tend more often to up-regulate CXCR5 and Bcl-6, markers of T helper follicular cells (Thf).
Conclusion: These data, obtained using LL37 as a model autoantigen, reveal for the first time in psoriasis that post-translational modifications of an autoantigen, which also exert its effect also on innate immune cells, can influence autoreactive T helper-cell-polarization and, possibly, their effector functions. Citrullination and carbamylation, occurring when neutrophil infiltrate the skin, may favor or exacerbate autoreactive responses and possibly play a role in PsA development.

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1167 – WS53.3

Characterising Effector T-cell Responses to Novel Autoantigens in Rheumatoid Arthritis

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Purpose: Antigen specific CD4+ T-cells play a key role in the early pathogenesis of rheumatoid arthritis (RA). Challenges to our understanding of these key subsets are underpinned by limited knowledge of putative autoantigens as well as a lack of assays to capture the full phenotypic and functional heterogenicity of the responding population. Here, we describe a multi-modal approach combining proliferation, surface marker expression and cytokine production to characterise CD4+ T-cells responding to novel RA peptides.
Methods: Peptides presented by HLA-DR in RA synovial tissue were identified by mass spectrometry (see abstract by Steel et al) and screened for reactivity in RA (n=11) and PsA (n=5) PBMC by 48hr IFNγ production measured by FluoroSpot. Peptides with the greatest reactivity were tested in PBMC from RA patients (n=12) by labelling with CFSE and culturing for 7 days. A pathogen-derived peptide pool (CEFT) was used as positive stimulation control. For positive responses (stimulation index >2) phenotypes of divided cells was assessed by multi-parameter flow cytometry and FlowSOM clustering. Cytokine secretion was assessed in Luminex assays.
Results: Following 48hr stimulation with synovial derived peptides, IFNγ production was higher in RA PBMC compared to PsA PBMC. Peptides derived from Desmoglein-2 (DSG2) and Immunoglobulin heavy constant gamma 1 (IGHG1) showed robust IFNγ responses. Stimulation with DSG2 and IGHG1 derived synovial peptides resulted in a higher proportion of either CD25/ICOS/OX40^{high} CCR4^{+} or CD25/OX40/ICOS$^{low/med}$ CXCR3^{+} populations at 7 days, dependent on the individual. 41BB^{+}PD1^{+} CD4 T-cells were significantly more abundant in the CEFT responding population compared to synovial peptide responding cells (p=<0.0001). A small population (1-2%) of ICOS^{high} CCR4^{+} cells was present in 3 individuals responding to DSG2 and IGHG1 derived peptides. Following 7-day stimulation with DSG2 and IGHG1, a substantial proportion of CD4+ T-cells responded with production of TNFα (65%), GM-CSF (85%) and IFNγ (40%).
Conclusions: Circulating CD4+ T-cells responding to novel autoantigenic peptides, have a pro-inflammatory CD25^{+}ICOS^{+}OX40^{+} phenotype and produce pro-inflammatory cytokines. Phenotypic signatures of antigen responding populations are heterogeneous, likely reflecting distinct TCR signals generated by each peptide, and the unique state of the host immune response.

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43 – WS53.4

Caveolin-1/PPARα axis restrains pathogenic T follicular helper cell response in primary Sjögren's syndrome

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Purpose: Primary Sjögren's syndrome (pSS) is a common autoimmune disease characterized by exocrinopathy involving salivary and lacrimal glands. Our accumulated works have demonstrated the critical roles of Th17 and T follicular helper (Tfh) cells in the pSS pathogenesis. In this study, we investigated the role of a scaffold protein caveolin-1 (Cav-1) in disease development and effector T cell dysregulation.
Methods: pSS patients were recruited and circulating T cells were analyzed. Wildtype (WT) or Cav-1-/- mice were induced for experimental SS (ESS). Disease phenotype and immune responses were assessed. WT and Cav-1-/- CD4+ T cells were subjected to RNA-seq and metabolic analysis. WT and Cav-1-/- Tfh cells migration was assessed by two-photon intravital images *in vivo*. Humanized SS mice were established by engrafting PBMCs from pSS patients.
Results: Cav-1 deficiency in hematopoietic origin exacerbated ESS pathology and humoral autoimmunity, together with enhanced Tfh cell responses, while Th17 cells were unaffected. Cav-1-/- Tfh cells exhibited increased motility and follicular localization. RNA-seq revealed impaired peroxisome proliferator-activated receptor alpha (PPARa) pathway in the absence of Cav-1. PPARa served as a downstream transcription factor of Cav-1, which rapidly repressed *Icos* transcription upon Tfh polarization, interestingly, independent of lipid metabolism. Thus, PPARa-/- CD4+ T cells also showed elevated ICOS expression, which can not be restored by overexpression of the *Cpt1a*, the downstream metabolic factor of PPARa. Prevention of PPARa translocation into nucleus could abolish this transrepressive effect. Phenotypic analyses suggested that Cav-1 and PPARa expressions were decreased in CD4+ T cells from pSS patients and ESS mice, and thus negatively correlated with Tfh cell frequencies. Notably, pharmaceutical activation of PPARa with fenofibrate could suppress human and murine Tfh cells in culture, in ESS mice and in humanized SS mice. In ESS mice, oral administration of fenofibrate effectively ameliorated disease pathology at both acute or chronic stages.
Conclusion: These results revealed an unrecognized role of Cav-1/PPARa axis in Tfh cell tolerance and pSS pathogenesis, suggesting PPARa as a promising target in the treatment of humoral autoimmunity.

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1125 – WS53.5

Rapamycin rescues loss-of-function in blood-brain barrier-interacting regulatory T cells

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In autoimmunity, it has been established that FOXP3+ regulatory T cells (Tregs) skew towards a pro-inflammatory, non-suppressive phenotype, making them unable to control the exaggerated autoimmune response. This largely impacts the success of autologous Treg therapy which is currently under investigation for autoimmune diseases, including multiple sclerosis (MS). There is a need to ensure in vivo Treg stability before successful application of Treg therapy. Using genetic fate-mapping mice, we demonstrate Tregs which have lost FOXP3 expression (exFOXP3 T cells) accumulate in the central nervous system during experimental autoimmune encephalomyelitis. In a human in vitro model, we discovered that interaction with inflamed blood-brain barrier endothelial cells (BBB-ECs) induces a loss of suppressive function in Tregs. Transcriptome and cytokine analysis revealed that in vitro migrated Tregs have a disrupted regenerative potential, a pro-inflammatory Th1/17 signature and upregulate the mTORC1 signaling pathway. In vitro treatment of migrated human Tregs with the clinically-approved mTORC1 inhibitor rapamycin restored their suppressive capacity. Finally, flow cytometric analysis identified an enrichment of inflammatory, less suppressive CD49d+ Tregs in the cerebrospinal fluid of people with MS. In sum, interaction with BBB-ECs is sufficient to affect Treg function, and BBB transmigration triggers an additive pro-inflammatory phenotype switch. These insights will help to improve the efficacy of autologous Treg therapy of MS.

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964 – WS53.6

The Effects of CXCR5, PD-1 and ICOS Molecules of Helper T Cells on B Cell Responses and the Implications in Myasthenia Gravis

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Follicular helper T (Tfh, CXCR5$^+$) cells regulate B cell responses mainly at the germinal centers by interacting with B cells via PD-1 and ICOS molecules as well as cytokines. Similarly, peripheral helper T (Tph) cells lacking CXCR5 provide help for B cell responses mainly in the periphery. Both cell groups are implicated in the pathogenesis of myasthenia gravis (MG). This study examined molecular interaction of T and B cell populations by comparing T cell subsets according to these molecules and aimed to determine therapeutic target molecules in this interaction for MG patients.
The study included 10 healthy controls (HCs) and 6 untreated acetylcholine receptor-antibody-positive MG (AChR-MG) patients. CD4$^+$ helper T cells were sorted by combinations of CXCR5, PD-1 or ICOS (using FACSAria), stimulated with CD3/CD28 antibodies and then co-cultured with sorted CD19$^+$ B cells of the same donors. The impact of separated T cell subgroups on the development of plasmablasts (CD27$^+$CD38$^+$ cells) in CD19$^+$ B cells, on the production of total IgG as well as anti-AChR IgG, IL-4, IL-21 and IL-17 in culture supernatants (ELISA and cytometric bead assay) were assessed.
In HCs, not CXCR5 alone, but the presence of PD-1 or ICOS molecules with CXCR5 (Tfh) on T cells induced higher plasmablasts and higher IgG production compared to co-cultures without these molecules. The effects of these molecules did not differ from each other. Similarly, T cells without CXCR5 (Tph) were effective in inducing B cell responses with PD-1 or ICOS and PD-1 was superior to ICOS in plasmablast induction.
In AChR-MG MG patients, CXCR5$^{+/-}$ PD-1$^+$ T cells also exhibited higher plasmablasts, IgG, as well as AChR-IgG, IL-4 and IL-17 compared to CXCR5$^{+/-}$ PD-1$^-$ cell cultures. This effect was predominant in the CXCR5$^+$PD-1$^+$ co-culture compared to the CXCR5$^-$PD-1$^+$ co-cultures.
Both PD-1-expressing CXCR5$^+$(Tfh) or CXCR5$^-$(Tph) cells exhibited a higher potential to enhance B cell responses compared to cells without PD-1. This effect is particularly evident in antigen-specific antibody production in AChR-MG patients. Modulation of PD-1 or CXCR5 in MG patients should be explored aiming to reduce AChR-IgG production.

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921 – WS54.1

iNKT cell immunomodulation and mucosal healing by microbiota-derived lactate

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Purpose: Invariant natural killer T (iNKT) cells play a critical role in mucosal immune homeostasis. Although iNKT cells release proinflammatory cytokines in response to the altered gut microbiota of IBD patients, exposure to microbiota-derived metabolites can promote homeostatic IL10-mediated iNKT cell responses resulting in better clinical outcomes in Crohn's disease (CD) patients. Thus, understanding the mechanisms leading to iNKT cells' functional shaping by microbiota-derived metabolites is important to design novel therapies for IBD patients.
We hypothesised that iNKT cells, serving as sentinels of tissue integrity, are the primary immune cells sensing microbiota-derived metabolic signals promoting the resolution of inflammation in IBD. In particular, we show that microbiota-derived lactate can control iNKT cell functions promoting iNKT cell-mediated mucosal tolerance while preventing T-cell-mediated overt inflammation and tissue injury.
Methods: We performed immunophenotyping of iNKT cells by multiparametric flow-cytometry from surgical specimens of CD patients and correlated NKT10 responses with intra-colonic levels of lactate, gut microbiome composition and tissue injury/healing. We studied the molecular pathway determining the NKT10 phenotype in a lactate-enriched microenvironment by RNA-seq and CHIP-seq. We validated our results by using in-vivo models and spatial transcriptomics on patient specimens.
Results: Our data suggest that microbiota-derived lactate plays a role in the epigenetic control of the immunoregulatory phenotype of iNKT cells through histone lactylation, thereby protecting animals from intestinal inflammation
Conclusion: On overall our data show i) how microbiota-derived lactate modulates the immunophenotype of iNKT cells, ii) the mechanisms underlying sensing of microbiota-derived lactate by iNKT cells, iii) iNKT cells role in inflammation resolution.

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920 – WS54.2

Microbial-derived metabolites promote NKT22 responses in colitis associated colorectal cancer patients

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Purpose: Patients suffering from inflammatory bowel diseases (IBD) manifest an increased risk of developing colitis-associated colon cancer (CAC). Interleukin 22 (IL22) is a cytokine involved in the proliferation and survival of epithelial cells. However, it also correlates with the development of tumoral lesions. IL22 is secreted by different immune cell types, including iNKT cells, tissue resident cells possessing cytotoxic properties in colorectal cancer (CRC). In IBD patients, though, iNKT cells perform both pro-inflammatory or tolerogenic functions in a microbiota-dependent fashion, opening questions on their functional role in CAC development.
Methods: Immune cell composition and iNKT cell phenotype of inflamed and cancerous tissue lesions of CAC (n=49), IBD (n=20) and sporadic CRC (n=38) patients were analysed in samples obtained from Policlinico Hospital, Milan, and IOV-IRCCS, Padua. High-dimensional single-cell flow cytometry, metagenomics, RNAseq, spatial immunophenotyping and trascriptomic (Cosmx, Nanostring), ex vivo and in vitro experiments were performed to evaluate the phenotype and function of human iNKT cells. Metabolomics was performed to identify microbial-derived molecules involved in iNKT cells activation. Mechanisms were dissected in CAC murine models, either iNKT proficient or deficient.
Results: An increased expression of IL22 and a higher NKT22 infiltration was observed in CAC tissues, as well as an enrichment of *Odoribacter*, a gram-negative bacteria implicated in tryptophan metabolism. Stimulation of iNKT cells with *Odoribacter*-derived metabolites induced Ahr-dependent IL22 production by iNKT cells. Metabolomic analyses revealed an increased presence of tryptophan metabolites in the *Odoribacter* supernatant, promoting NKT22 differentiation both in vitro and in vivo models. Moreover, the administration of *Odoribacter* Supernatant or of the single purified metabolites were sufficient to increase tumorigenesis in CAC mouse models, but not in iNKT-deficient mice. Spatial proteomics and trascriptomic analyses of human CAC tissues confirms the microbiota-dependent functional skewing of iNKT cells residing closely to cancer cells.
Conclusion: iNKT cells in CAC patients are the major IL22 producing cells and are induced by the recognition of microbiota-derived metabolites implicated in tryptophan metabolism and Aryl hydrocarbon receptor (AhR) stimulation. NKT22-microbiota interaction in CAC patients plays a potential crucial role in tumor development.

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709 – WS54.3

Neutrophils mediate protection against colitis and carcinogenesis by controlling bacterial invasion and driving IL-22 production by γδT cells.

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Purpose: Neutrophils are the most abundant leukocytes in human blood and play a primary role in resistance against invading pathogens and in the acute inflammatory response. However, their role in colitis and colitis-associated colorectal cancer is still under debate. This study aims to dissect the role of neutrophils in these pathological contexts by using a rigorous genetic approach.
Methods: Neutrophil-deficient mice (*Csf3r*$^{-/-}$ mice) were used in classic models of colitis and colitis-associated colorectal cancer (CAC) and the role of neutrophils was assessed by histological, cellular and molecular analyses coupled with adoptive cell transfer. We also performed correlative analyses using human datasets.
Results: *Csf3r*$^{-/-}$ mice showed increased susceptibility to colitis and colitis-associated colorectal cancer compared to control *Csf3r*$^{+/+}$ mice and adoptive transfer of neutrophils in *Csf3r*$^{-/-}$ mice reverted the phenotype. In colitis, *Csf3r*$^{-/-}$ mice showed increased bacterial invasion and a reduced number of healing ulcers in the colon, with lower tissue levels of IL-22, indicating a compromised regenerative capacity of epithelial cells. The activation state of γδT cells from *Csf3r*$^{-/-}$ mice was altered towards decreased production of IL-22 and increased production of IL-17. Both adoptive transfer of neutrophils and ABX treatment were sufficient to rescue the polarization and activation state of γδ T cells toward high expression of AhR and IL-22 and low expression of IL-17. In patients with ulcerative colitis, expression of *CSF3R* was positively correlated with *IL22* and *IL23* expression. Moreover, gene signatures associated with epithelial-cell development, proliferation, and antimicrobial response were enriched in *CSF3R*^{high} patients.
Conclusion: The findings reported here highlight the importance of neutrophils in maintaining intestinal homeostasis in response to inflammatory insults and describe a model where neutrophils control the susceptibility to intestinal inflammation and CAC by shaping the intestinal microbiota composition and the activation of an IL-22-dependent tissue repair pathway.

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221 – WS54.4

Differential effects on fecal microbiota, gut immunity and vaccine response conveyed by a natural environment vs. co-housing with wild or pet store mice

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Purpose: Laboratory rodents may fail to develop a diverse microbiome and immune training, risking poor translatability to humans. We have established a housing system exposing C57BL/6 (B6) mice to soil and domestic animal excrements, representing a realistic living habitat for house mice. These "feralized" mice shift their gut microbiota, obtain a matured immunophenotype and are more resilient to colorectal cancer development. In earlier studies we exerted microbial pressure by cohousing B6 mice with wild (WildCoH) or pet store mice (PetCoH) as microbial donors. The present study compared immunological performance following several such conditions. We also compared the burden of pathobiont exposures in pet store-bred and free-living wild mice, and B6 mice co-housed with the donors, and measured their ability to respond to an influenza vaccine.
Methods: B6 mice were exposed to various microbially rich conditions; a naturalistic habitat, and/or co-housing with wild-caught or pet store mice. Analyzed parameters included microbiota and virome sequencing, pathobiont immunity, gene expression in colon, flow cytometry, immunohistochemistry and serological response to parenteral influenza vaccine.
Results: A variety of environmental exposures revealed effects on the intestinal barrier, microbiota, gut immunity and vaccine response. Feralization moved the B6 fecal microbiota towards that of wild mice, while WildCoH brought the microbiota even closer to the "wild" type. Expression of genes for barrier integrity and antimicrobial functions increased accordingly. Feralization, and to a greater extent WildCoH, increased resident memory CD8+ T-cells in the colon mucosa. Antigen experience markers increased along similar trends in NK and T-cells, while T-reg numbers were unchanged. In a separate experiment, PetCoH mice showed a stronger exposure to pathobionts than feralized or WildCoH mice. Following influenza vaccination, PetCoh mice showed a dampened specific antibody production, not detected in feralized or WildCoH mice.
Conclusion: Exposure of model mice to soil and domestic animal excrements conveyed a shift of the gut-associated microbiota, antigen experience markers and colonic barrier function. Exposure to wild mice potentiated these changes. Finally, cohousing model mice with pet store animals, shown to carry a higher pathogenic burden, made the strongest impact on systemic immunity and compromised a vaccine response.

(Authors 1-3 contributed equally.)

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1278 – WS54.5

Integrating functional metagenomics to decipher microbiome-immune interactions

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Purpose: Recent studies have linked the composition of the gut microbiota to various aspects of human health, including host metabolism, immunity and infection susceptibility. With shotgun metagenomic sequencing methods, inventories of the microbial residents of the gut have become more detailed and precise, but primary investigation of molecular mechanisms involved in key microbiome-immune interactions has remained challenging. Our research group recently built important biological and bioinformatic resources that facilitate functional and mechanistic studies of the gut microbiome and translation of findings between mice and humans. We have now leveraged these tools to understand how microbiota metabolites act as dynamic messengers to the host epithelium and underlying immune system in homeostasis and disease.
Methods: Shotgun metagenomic sequencing datasets generated in our lab and public datasets were functionally annotated using our functional metagenomics pipelines, UHGG and MGBC database and ToolKit to provide functional profiles of human patient and mouse microbiomes. Immune impacts of differentially abundant microbial functions and metabolic pathways were further explored *in vitro* and in mouse models using reporter assays, flow cytometry, bulk- and single-cell RNA-seq, proteomics and mass spectrometry-based metabolomics.
Results: Moving from humans to mice, we uncovered a taxonomy-independent association in melanoma patients between gut microbiota acylation of lipopolysaccharide (LPS) and response to the immune checkpoint inhibitor anti-PD-1. In *in vitro* assays, we went on to show that the balance of LPS acylation states determines the capacity of gut LPS to stimulate immune activation. In tumor-bearing mouse models, oral administration of immunostimulatory LPS significantly enhanced anti-PD-1 efficacy. Moving from mice to human patients, by pairing functional metagenomics, proteomics, metabolomics and transcriptomics with gut immune cell profiling, we revealed a role for a microbiota-derived fatty acid in driving epithelial antigen presentation and downstream anti-inflammatory intraepithelial lymphocyte abundance in mice. Corresponding microbiome and host transcriptomic signatures were then uncovered in Crohn's disease patients with lower disease severity, confirming an association with decreased inflammation.
Conclusion: Leveraging our generated resources and multi-omic functional approaches to microbiome research, we have utilized observations from both mice and human patients to mechanistically investigate how the microbiota-derived metabolite milieu impacts multiple aspects of host immunity.

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1280 – WS54.6

Gut microbiota and maternal immune transfer at birth precede pre-allergic clinical outcome.

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Purpose: The gut microbiota of 2–3-month-old infants is associated with later pre-allergic signs, while the microbiota at the time of allergic manifestation is not. We hypothesized that the infant gut microbiota and immune system are primed shortly after birth, and that this is influenced by maternal transfer of humoral immunity.
Methods: Using biological samples from 187 mother-child dyads from a French-Spanish birth cohort we investigated the association between allergic outcomes and composition and humoral immunity to gut microbiota at birth, 2 months, and 2 years-of-age. Experimentally we employed flow cytometry to monitor the proportion of antibody-bound gut microbiota and 16S rRNA gene sequencing to evaluate the gut microbial composition. Finally, we sorted IgA+ and IgA- gut microbiota and identified microbes bound to IgA by 16S rRNA gene sequencing (IgAseq).
Results: Meconium microbiota clustered into three groups dominated by Escherichia, Enterococcus, and mixed genera, respectively. The Escherichia cluster was associated with protection against later allergic manifestations. We moreover studied the proportion and specificity of humoral immunity to gut microbiota. Breastmilk hugely affected the proportion of antibody-bound microbiota in early-life. The diversity of IgA-binding increased with age in concordance with general microbiota diversification.
Conclusion: Gut microbiota composition at birth was associated with future allergies. Future studies should evaluate whether interventions to alter gut microbiota and/or humoral immunity in early-life could protect against allergy.

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WS55 – ADOPTIVE CELL THERAPY FOR CANCER AND INFECTIOUS DISEASE

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83 – WS55.1

Identification of serial killing natural killer cells

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Natural killer (NK) cells are important for early and effective immune responses through cytokine production and by killing transformed and virally infected cells. NK cell cytotoxicity is regulated by different germline encoded activating and inhibitory receptors and executed by the release of cytotoxic granules or the engagement of death receptors. Human NK cells have been shown to be heterogeneous and differ in their ability to kill target cells. NK cells that are able to sequentially kill multiple target cells are called serial killers. The serial killing activity of NK cells is essential for their cytotoxic function since the majority of kills can be performed by a minority of cells. Consequently, serial killing NK cells are of particular interest for therapeutic applications. However, while it is known that only a fraction of NK cells perform serial killing, it is currently not possible to predict which NK cells will engage in serial killing.
To this end, we established a staining protocol that can differentiate between the timing and the count of multiple NK cell degranulation events occurring during target cell co-culture. NK cells are analyzed via flow cytometry which enables us to combine degranulation data with other phenotypic readouts. Additionally, cell sorting can be used to open up possibilities like RNA-sequencing. Donors with high proportions of serial degranulating NK cells can be reproducibly identified by our method. Loss of CD16 was associated with the number of degranulation events, whereas the upregulation of CD69 correlated with the timing of degranulation. Our method will be an important tool to identify NK cell populations with large proportions of serial killers and to potentially identify phenotypic markers specific for serial killing NK cells.

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2141 – WS55.2

A novel CD5-based adoptive cell transfer therapy for systemic fungal infection

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Invasive fungal infections have become a global concern, driven primarily by the widespread use of aggressive immunosuppressive and surgical interventions, and the emergence of antimicrobial-resistant (AMR) strains. The paucity of the antifungal pipeline and the important side effects reported for the available ones, place the need of developing alternative strategies. Immunotherapeutic approaches involving adoptive transfer of immune cells engineered with chimeric antigen receptors (CARs) are gaining interest. Pre-clinical studies have already demonstrated the efficacy of human T cells redirected against fungi (e.g., *Aspergillus fumigatus* and *Cryptococcus neoformans*) and viruses (e.g., Human Immunodeficiency Virus, Hepatitis B Virus, Hepatitis C Virus, Human Cytomegalovirus, and Epstein-Barr Virus) through the expression of specific CARs. Our previous research highlighted CD5, a scavenger-like lymphocyte surface receptor, as a promising candidate for broad anti-fungal CAR designs due to its high binding affinity to β-glucans, a conserved constituent of fungal cell walls. Thus, a CD5-based second generation CAR has been expressed by lentiviral transduction on cord blood-derived human NK cells. Subsequent *in vitro* assessments revealed enhanced cytokine and chemokine production as well as surface activation markers up-regulation by CD5CARCBNK cells when co-cultured with a broad panel of fungal species. Moreover, under these co-culture conditions enhanced fungal killing and reduced fungal metabolic activity was also observed. Furthermore, *in vivo* experiments involving infusion of CD5CARCBNK cells into immunocompromised NSG mice undergoing systemic fungal infection induced by *Candida albicans* and *C. neoformans* demonstrated significantly higher survival rates and lower fungal load count compared to untreated controls. In summary, the antifungal properties exhibited by immune cells expressing our CD5-based CAR open new avenues for development of novel off-the-shelf adoptive cellular immunotherapies against invasive fungal infections. These approaches could be pursued independently or in combination with traditional antifungals, offering a promising strategy addressing the challenges posed by invasive fungal infections independently of their AMR status.

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1417 – WS55.3

Dual modulation of cytotoxic and checkpoint receptors tunes the efficacy of Delta One T cells against colorectal cancer

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Colorectal cancer (CRC) remains a major unmet medical need. Notably, most microsatellite stable (MSS) advanced CRC tumors employ mechanisms to avoid neoantigen generation and/or HLA class I presentation, rendering them inconspicuous to αβ T cells. Thus, only around 10-15% of CRC with microsatellite instability (MSI), presenting high mutational burden due to deficient DNA mismatch repair, are responsive to current immunotherapies. In this context, Vδ1$^+$ γδ T cells, with their robust HLA-I-independent and neoantigen-independent cytotoxic functions, coupled with natural tropism for the gut and tumors, emerge as promising candidates for a broader CRC immunotherapy. We have recently developed a Vδ1$^+$ γδ T cell-based adoptive cell therapy, named Delta One T (DOT) cells, which has been translated to the clinic for hematological malignancies, but has never been applied in the challenging solid tumor space. Here we optimize and establish the pre-clinical proof-of-concept for using DOT cells for CRC treatment. First, we demonstrate the capacity of DOT cells to target a panel of both MSI and MSS CRC cell lines and patient-derived biopsies *in vitro*. Second, using an orthotopic (intercaecal) xenograft model of human CRC with MSS SW620 cells, we show that blood-infused DOT cells infiltrate the tumor lesions, controlling tumor growth *in vivo*. Importantly, tumor-infiltrating DOT cells exhibited a dysregulated balance of cytotoxic and inhibitory receptors, limiting their cytotoxic potential. We identified a dominant role of the innate receptor NKG2D in mediating CRC targeting, as blockade or knockout of this receptor in DOT cells impairs CRC killing. Enhancement of the NKG2D axis or inhibition of immune checkpoint receptors potentiated DOT-cell tumoricidal functions against CRC *in vitro* and *in vivo*. This study demonstrates the strong potential of DOT cells as a novel immunotherapy for CRC, while emphasizing avenues for improved efficacy through combinatorial approaches.

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1612 – WS55.4

Engineering NK cell immunotherapy to optimise liver homing and T cell regulation

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Purpose: NK cells are being developed for adoptive cell therapy of cancer with the advantages of intrinsic tumour-killing capacity, lack of MHC restriction and low toxicity. Cytokine activation (IL2/12/15/18) is widely used to promote memory-like NK cells with enhanced anti-tumour functionality. However, we recently showed that cytokine activation induces PD-L1 on human and murine NK cells, resulting in inhibition of hepatitis B (HBV)-specific T cells (*Diniz, SciTranslMed 2022*). Using monoclonal antibodies, we found that PD-L1-blockade reverted NK cell negative role and enhanced their capacity to help T cell responses. We hypothesised that targeting PD-L1-mediated regulation could generate more efficient NK cells for adoptive cell therapy in cancer.
Methods: The aims of this study were therefore to engineer primary NK cells to be retained within Hepatocellular carcinoma (HCC), exerting anti-tumour function without impeding the activity of neighbouring anti-tumour T cells through PD-L1. To do this, we transduced NK cells to express the chemokine receptor CXCR6 (which retains liver-resident NK cells) and to secrete anti-PD-L1 antibodies to counteract their checkpoint inhibition of T cells.
Results: Using VSV-G pseudotyped lentivirus we achieved transduction of primary human NK cells and confirmed induction of CXCR6 expression at similar levels of a reporter protein (GFP). Higher numbers of $CXCR6^{+}$NK-cells were recovered from matrigel containing CXCL16 or HepG2 supernatant compared to untransduced cytokine-activated NK-cells, indicating that the encoded CXCR6 receptor was functional. Production and secretion of anti-PD-L1 was confirmed by its capacity to block PD-L1 on NK or hepatoma cells *in vitro,* preventing staining of this ligand by flow cytometry. Their secretion of anti-PD-L1 antibodies converted cytokine-activated transduced NK cells into predominant 'helpers', able to boost $CD8^{+}$T cell responses to HBV peptide stimulation or hepatoma cells.
Conclusion: In summary, we genetically engineered cytokine-activated NK cells to have enhanced homing/retention within liver tumours and to mediate anti-tumour activity whilst releasing their helper function to boost antigen-specific T cells. This approach should promote the dual anti-tumour functionality of NK cells and T cells, to work in tandem rather than in opposition, against HCC.

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590 – WS55.5

Development of γδ T cell immunotherapy against Glioblastoma

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Background: γδ T cells are unconventional T cells harboring features of innate and adaptive response. Indeed, they express both TCR receptors (although described interacting with their target in a mostly MHC independent manner) and NK receptors, conferring them cytotoxic characteristics. Actually, clinical scale *in-vitro* expanded γδ T cells (DOT cell procedure) are currently in clinical trial in Acute Myeloid Leukemia (AML). This sub-population is besides, specifically enriched in tissues and their number in tumors associates with better survival in different solid cancers. These characteristics make them of great interest to develop as therapeutic agent particularly in tumors with low mutational burden or low HLA-I expression, such as Glioblastomas (GBM).
GBM are the most frequent and aggressive primary brain malignancies, with very limited therapeutic options and a dismal prognosis. So far, immunotherapies evaluated in clinical trials have failed to provide significant clinical benefit for GBM patients, notably due to the high genetic heterogeneity and immunosuppressive environment of the tumor.
Here, we aimed at developing a new cell therapy strategy in GBM, using *in-vitro* expanded γδ T cells (DOT cells).
Results: Characterization of γδ T cells isolated from GBM patient samples highlighted Vδ1 and Vδ2 recruitment within tumors. In parallel, using commercially available GBM cell lines and patient-derived glioblastoma stem cells (GSCs), we showed that amplified γδ T cells, used in combination with interleukin (IL)-15, could efficiently recognize and kill GBM cells and spheroids *in-vitro*. Target cell killing relied on the granule exocytosis pathway and was impacted upon CRISPR-mediated knock-out of γδ TCR and NKG2D molecules suggesting their implication in GBM sensing by amplified γδ T cells. Finally, patient's γδ T cell amplification was also possible, and patient derived cells were still able to kill autologous and allogeneic targets. Altogether, our results are providing encouraging results to develop allogeneic use of γδ T cell therapies in GBM patients.
Perspectives: These data provide an *in vitro* proof of concept for off-the-shelf use of γδ T cells to target GBM and γδ T cell adoptive transfer is now being evaluated *in-vivo* in patient-derived xenograft mouse models.

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2146 – WS55.6

Ethanol-treated human fibroblasts and their extracellular vesicles modulate TCD4+ lymphocytes

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Purpose: The processes involved in carcinogenesis depend on changes in tumor cells and the dynamic communication in the microenvironment. Studies report smoking and alcohol consumption as risk factors for oral squamous cell carcinoma (OSCC). Still, the mechanism of alcohol on the OSCC initiation needs to be elucidated.
Methods: Peripheric blood mononuclear cells (PBMC) from healthy donor buffy coats were co-cultured with primary fibroblasts, previously treated with ethanol (EtOH), and their extracellular vesicles (EVs). T-cell activation was analyzed by CD25 and CD69 modulation at mRNA and protein expression using RT-qPCR (n=5) and flow cytometry (anti-CD25, n=8, and anti-CD69, n=5), respectively. To further explore the signaling effects, mass spectrometry-based proteomics of fibroblasts and their released EVs (n=6) was performed and biological processes were enriched by Panther tool.
Results: PBMC co-cultured for 72 hours with EtOH-treated fibroblasts or EVs showed lower mRNA expression of *CD25* and *CD69* (treated *vs.* control; $p \leq 0.05$; paired Student´s t-test) and a decrease in the percentage of $TCD3^+$ and $TCD4^+$ cells expressing CD25 (treated *vs.* control; $p \leq 0.05$; Wilcoxon test) and $TCD4^+$ cells expressing CD69 (treated *vs.* control; $p \leq 0.05$; paired Student´s t-test). Moreover, from 4,609 and 162 proteins identified and quantified in fibroblasts and their EVs, respectively, 236 proteins from fibroblasts and 10 from EVs were statistically modulated (treatment *vs.* control; $p \leq 0.05$; unpaired Student's t-test or proteins identified exclusively in one group). Proteins deregulated after ethanol treatment of fibroblasts were involved in cellular metabolism gene ontology (GO) processes such as cellular metabolic process, metabolic process, organic substance metabolic process, primary metabolic process, and proteasome-mediated ubiquitin-dependent protein catabolic process (FDR ≤ 0.05; Fisher exact test followed by FDR correction). Twenty-two out of the 236 EtOH-associated fibroblast proteins are mainly involved in cell metabolism, cell respiration, are part of the mitochondrial complexes I or IV, or have evidence of mitochondrial localization.
Conclusion: The results indicate that alcohol-treated fibroblasts negatively and indirectly modulate the activation of T cells, and this signaling may result from proteins involved in the cell metabolism.

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WS56 – REGULATION OF AUTOIMMUNE AND INFLAMMATORY DISEASE

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1148 – WS56.1

Tolerogenic presentation of an alpha-synuclein antigen on a chimeric major histocampatibility complex class Ib molecule induces regulatory T cells and confers neuroprotection in an animal model for Parkinson's disease

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Purpose: In patients with Parkinson's disease (PD), alpha-synuclein (aSyn) specific autoreactive T cells become detectable long before neuronal pathology. Cohort studies have also shown immunosuppressants to prevent the onset of PD. However, due to the severe side effects, such treatments are unsuitable to treat patients with prodromal PD.
Methods: New model systems like our aSynA53T-AAV-driven mouse model of PD recapitulate involvement of T cells, behavioral symptoms, and histopathology with high face validity. This provides the opportunity to explore possible therapeutic modalities for this incurable disease. To inhibit neurodegeneration and prevent development PD in the absence of systemic immunosuppression, we have developed novel antigen-specific tolerance-inducing biomolecules that present peptide antigens on MHC class Ib-derived molecules. We have thus fused (i) the alpha3 domain of the T cell inhibitory human MHC class Ib molecule HLA-G to (ii) species-adapted MHC class I alpha1-alpha2 antigen-presenting domains, and covalently linked (iii) disease-related or unrelated antigenic peptides and (iv) beta2-microglobulin. We named the resulting single-chain proteins AutoImmunity Modifying Biologicals (AIM Bios).
Results: AIM Bios can polarize cognate $CD8^+$ T cells towards an antigen-specific $CD8^+CD122^+$ IL-10 secreting phenotype, and thereby induce antigen-specific tolerogenic $CD8^+$ T reg cells in human cells *in vitro* and in mice *in vivo*. Molecules built according to this pattern were tested for their ability to prevent tissue damage in the aSynA53T-AAV driven model for PD. While presentation of a virus-derived gp34 control antigen showed no effect, an AIM Bio molecule presenting a disease-associated aSyn $CD8^+$ epitope ameliorated mobility impairments in aSynA53T-AAV PD mice. *Post mortem* histopathological assessment confirmed the induction a favorable *in situ* immune cell composition and the rescue of dopaminergic neurons in the *substantia nigra*, and of dopaminergic termini in the *striatum*.
Conclusion: These molecules point towards a new treatment modality for patients with prodromal PD. Candidate molecules for use in humans are being developed.

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692 – WS56.2

Coenzyme A fueling with pantethine limits autoreactive T cell pathogenicity in experimental neuroinflammation

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Immune cell metabolism governs the outcome of immune responses and contributes to the development of autoimmunity by controlling lymphocyte pathogenic potential. In this study, we evaluated the metabolic profile of myelin-specific murine encephalitogenic T cells, to identify novel therapeutic targets for autoimmune neuroinflammation. By performing unbiased metabolomics analysis, we detected a potential break in the coenzyme A (CoA) synthesis pathway in actively-proliferating encephalitogenic T cells, compared to resting T cells. CoA fueling with the CoA precursor pantethine affected essential immune-related processes of autoreactive T cells, such as antigen-specific proliferation, cytokine production, and integrin-mediated cell adhesion, both *in vitro* and *in vivo*. Mechanistically, pantethine exerted its immunomodulatory effects in encephalitogenic T cells by linking metabolic reprogramming to alteration of intracellular signaling pathways. We then evaluated the impact of pantethine treatment on the development of experimental autoimmune encephalomyelitis (EAE), a mouse model of human multiple sclerosis (MS). Our data show that pre-clinical treatment with pantethine inhibited EAE development in two different mouse strains. Importantly, pantethine also significantly ameliorated the disease course when administered after disease onset in a therapeutic setting. Finally, pantethine limited pro-inflammatory cytokine production by human T helper 1 (Th1) and Th17 cells *in vitro*, as well as by T cells from MS patients, confirming its translational potential. In conclusion, we demonstrated that CoA fueling with pantethine in pro-inflammatory and autoreactive T cells may represent a novel therapeutic approach for the treatment of autoimmune neuroinflammation.

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1246 – WS56.3

Role of the combination of serum Neurofilaments light chains and IgM bands in the prediction of progression in Multiple Sclerosis

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Background: Multiple sclerosis (MS) is a chronic immune-mediated disease of the central nervous system. To find reliable prognostic biomarkers in MS is of the utmost clinical importance since it allows taking early accurate clinical decisions. In this line, high serum levels of neurofilament light chains (sNfL) have demonstrated to associate with a highly inflammatory MS and predict a higher probability of EDSS progression. On the other hand it has been widely proven that intrathecal IgM synthesis identified by the presence of oligoclonal IgM bands (OCMB) predicts an aggressive MS course. We explored the added value of OCMB to predict MS outcome.
Methods: This is a retrospective analysis performed in 366 patients with a clinically isolated syndrome suggestive of MS and positive oligoclonal IgG bands followed prospectively for five years, with available serum samples and OCMB data. We quantified serum light chain neurofilaments (sNfL) by a single molecule array (SIMOA) method using the NF-LIGHT™ Assay (Quanterix), and studied the prognostic value of OCMB, sNfL levels and the combination of both variables by quantifying time to didability progression measured using the Expanded Disability Status Scale (EDSS). Statistical analyses were performed using survival curves in Prism GraphPad version 10 software.
Results: We established the cut-off for sNfL values by exploring a cohort of 100 age and sex matched healthy controls. Values below 10 pg/ml were considered as normal. We the classified MS patients according bto showing high or normal sNfL values at disease onset. Having high sNfL values predicted an increased risk of disability progression (HR=1.82; 95% CI:1.12-3.11; p=0.016). Showing OCMB further increased the risk of progression in patients with high sNfL values (HR=2.33; 95% CI 1.148 to 4.730; p=0.034). Accordingly, the combination that more accurately predicted disability worsening was the combination of OCMB and high sNfL values. Patients with this condition showed a clearly higher risk of progression compared to the remaining ones (HR=2.28; 95% CI 1.23- 4.25; p=0.0024).
Conclusions: These results strongly suggest that the combination of sNfL and OCMB at disease onset may improve the ability of sNfL alone to early predict disease outcome in MS patients.

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284 – WS56.4

Size Matters; Particle size regulates the induction of anti-inflammatory responses and immune tolerance via αvβ3 mechano-sensor engagement

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The increased incidence of auto-immune and inflammatory diseases demands the identification of immune-therapies modulating tolerance and resolution without the risk of systemic immune-suppression. Here we propose the use of biodegradable micro-particles as adjuvants; with the propensity to drive anti-inflammatory responses and antigen-specific tolerance.

The Lavelle lab has focused on the importance of biomaterial-based adjuvant physico-chemical properties in the regulation of innate and adaptive immunity. Notably, particle size was found to be a critical factor that modulates dendritic cell activation and the subsequent activation and polarisation of T cell responses. We have identified that biodegradable poly (lactide-co-glycolide) (PLGA) particles within the narrow size window of 1-2 μm in diameter, drive potent secretion of IL1ra and IL-10 from key antigen presenting cells (APC's). Consequently, these anti-inflammatory APC's were proficient in priming and expanding a CD4+ regulatory T-cell (T-reg) population both in an *in-vitro* co-culture model and *in-vivo* studies.

Investigations into the mechanism by which these particles enhance anti-inflammatory responses revealed a key role for the integrin; αvβ3. Activation of this mechano-sensory integrin is hypothesised to be driven by changes in cell morphology and membrane tension induced by particles of the 1-2 μm range. Engagement of αvβ3 increased downstream anti-inflammatory signalling, initiating a positive feedback loop, promoting increased surface expression of αvβ3 and the TGFβ cell-membrane anchor; Glycoprotein A repetitions predominant (GARP).

These findings demonstrate the therapeutic potential of biodegradable micro-particles of this size as an adjuvant to promote immunological tolerance in a manner independent of biologic agents or potent immunosuppressants.

Furthermore these findings exhibit the importance of mechanical cues in driving immune responses, and how these can be harnessed in order to improve existing immune-therapies.

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581 – WS56.5

Single cell sequencing of antigen-specific autoreactive B cells in MuSK myasthenia gravis

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Recently a new group of autoimmune diseases hallmarked by predominant pathogenic IgG4 autoantibodies (IgG4-AID) was recognized. Why IgG4 predominates these disorders is unknown. Muscle-specific kinase (MuSK) myasthenia gravis (MG) is one of these IgG4-AIDs, in which autoantibodies block MuSK at the neuromuscular junction, causing fatigable muscle weakness. We hypothesize that targeted removal of $IgG4^+$ pathogenic B cells in these diseases could have therapeutic value. However, the unique phenotypical characteristics of both $IgG4^+$ B cells in general and autoreactive $IgG4^+$ B cells are poorly understood.
To characterize autoreactive ($IgG4^+$) B cells, we isolated PBMCs from MuSK MG patients and enrichment-sorted MuSK-reactive B cells using fluorescently-labeled, DNA-barcoded recombinant MuSK tetramers. As controls, we sorted equal numbers of IgM, IgG1-4 and IgA1-2 B cells from the non-MuSK-reactive population and from age-matched healthy donors. Next, libraries for 5' gene expression, B cell receptor (BCR) V(D)J mapping and CITE-seq scores were constructed on the 10x Genomics V2 platform. We recovered a median of 1.091 genes per cell and 80,6% of the cells had a complete V-J spanning pair. Subsequent quality control yielded 83% of all sequenced B cells suitable for further analysis.
Using CITE-seq scores, we identified 246 cells with >75 antigen tetramer binding events/cell. BCR sequences from several strongly MuSK binding cells were recombinantly produced to confirm reactivity in a MuSK ELISA. Unsupervised clustering shows highly MuSK-reactive patient B cells cluster separately from B cells of healthy controls with increased tetramer binding events, which likely arose from aspecific binding. The origin and clonality of the autoimmune response is currently being investigated by phylogeny reconstruction of BCR sequences. Potential therapeutic targets were selected from genes and associated pathways that are uniquely activated in MuSK-reactive $IgG4^+$ B cells. Furthermore, we identified generalized $IgG4^+$ B cell-specific differentially activated pathways by gene ontology analysis.
In conclusion, we successfully developed a multimodal labeling strategy to identify antigen-specific autoreactive B cells in single cell transcriptomics experiments. This modular strategy can be readily translated to any antigen. Autoreactivity- and isotype-specific gene expression profiles are currently being confirmed with *in vitro* experiments.

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138 – WS56.6

Microbiota-dependent T-cell response to α-synuclein-derived antigens triggers motor and non-motor symptoms associated to Parkinson's Disease

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Purpose: Previous evidence has shown that both the T-cell response and the microbiota are fundamental on the development of Parkinson's Disease (PD), which is characterized by the accumulation and post-translational modification of α-synuclein (αSyn). Modified αSyn constitutes a source of neo-antigens able to trigger an autoreactive T-cell response. Nevertheless, the relationship between the microbiota and the development of this autoreactive T-cell response in PD remains unexplored. Here we studied whether the dysbiosis of the gut microbiota and the T-cell response to αSyn-derived antigens associated to PD are functionally connected.
Methods: We used transgenic *SNCA* mice, which involve the overexpression of human αSyn (hαSyn). To deplete the microbiota, we used a wide-spectrum antibiotic cocktail. To deplete lymphocytes we generated *SNCA* mice deficient on recombination-activating gen 1 or deficient on membrane-bound IgM. Microbiome was analyzed by sequencing the variable V4 region of the 16S rRNA gene. Bioinformatics tools were used to analyse the sequence homology between bacteria and hαSyn.
Results: We observed that the depletion of either gut microbiota or T-cells abrogated the development of motor deficits, sensory threshold impairment, neuroinflammation and gut inflammation. Moreover, our results reveal the development of a T-cell response specific to neo-antigens derived from hαSyn in cervical and mesenteric lymph nodes. Of note, *SNCA* mice presented higher intestinal barrier permeability, which was associated with a significant gut inflammation. Furthermore, we found that particular bacterial components from intestinal microflora display some structures with sequence homology with hαSyn and significant potential binding to MHC molecules, thus suggesting molecular mimicry.
Conclusion: Our findings indicate that the development of both motor and non-motor manifestations as well as neuroinflammation in PD involves a T-cell mediated autoimmune response, which is triggered by changes in the gut microbiota that induce increased intestinal barrier permeability. Moreover, our findings suggest that dysbiosis in this PD model involves bacterial components with molecular mimicry with hαSyn.

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WS57 – RESPIRATORY INFLAMMATION

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825 – WS57.1

Recruited atypical Ly6G+ macrophages license alveolar regeneration after lung injury

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Purpose: The lung is constantly exposed to airborne pathogens and particles that can cause alveolar damage and appropriate repair post-injury is essential for gas exchanges and life. Severe respiratory viral infections represent a global health issue and are typically associated with excessive inflammation and damage and abnormal tissue repair that can lead to acute respiratory distress syndrome, pneumonia and death, thus representing a serious unmet medical need. If the role on recruited blood monocytes and macrophages during the acute inflammatory phase is known to contribute to host innate defense mechanisms, the function of these cells in the induction of the lung functional repair remain underinvestigated. In this study, we deciphered the spatiotemporal trajectory and function of macrophages after virus-triggered lung injury.
Methods: By employing an *in vivo* model of influenza A virus-induced lung injury in combination with single-cell and spatial transcriptomic analyses, bone marrow chimeras, monocyte fate-mapping and gene targeting, we investigated the dynamic behavior and function of a distinct subset of monocyte-derived Ly6G-expressing macrophages ($Ly6G^+$ Macs).
Results: In the early recovery phase post-influenza A virus infection, $Ly6G^+$ Macs were transiently recruited in the alveolar spaces of lung perilesional areas. $Ly6G^+$ Macs exhibited a high metabolic potential, engulfed immune cells locally and clustered with alveolar type 2 (AT2) epithelial cells in zones of active epithelial regeneration. *In vivo* gene targeting and *ex vivo* wound healing assays showed that $Ly6G^+$ Macs were dependent on interleukin-4 receptor signaling and crucially contributed to euplastic alveolar regeneration by directly interacting with AT2 cells. Notably, similar macrophages were recruited in other models of injury and in the alveolar spaces of human injured lungs.
Conclusion: Our study identifies perilesional alveolar $Ly6G^+$ Macs as a spatially-restricted, short-lived macrophage subset engaging in a crosstalk with AT2 to promote epithelial regeneration and host recovery post-injury, thus representing an attractive therapeutic target.

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391 – WS57.2

Targeting neutrophil extracellular traps to reduce immunopathology in tuberculosis

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Purpose: *Mycobacterium tuberculosis (Mtb)*, the causative agent of Tuberculosis (TB), is responsible for 1.6 million deaths annually. Up to 94% of survivors suffer from clinically significant decreased lung function due to immunopathological changes. Neutrophils, key immune cells in the immune response to TB, can form extracellular traps (NETs) composed of DNA, citrullinated histone H3 (citH3) and myeloperoxidase (MPO) to encapsulate *Mtb*. They may regulate lung tissue remodelling by acting on fibroblasts, key cells in wound healing and fibrotic processes, which is in part regulated by secretion of matrix metalloproteinases (MMPs) and their inhibitors (TIMPs). We hypothesise, that NETs induced during *Mtb* infection drive lung immunopathology in TB.
Methods: Human peripheral neutrophils were infected with *Mtb* or stimulated with 100 µg/ml *Mtb*-antigen, ESAT-6, to induce NETs, which were quantified by PicoGreen dsDNA staining or an ELISA against citH3 and MPO. For confocal microscopy, NETs were stained for citH3, MPO and DNA. Primary human lung fibroblasts (PHLF) were stimulated with NETs or co-cultured with peripheral neutrophils and *Mtb* in a novel cellular model. PHLF gene expression and secreted mediators were measured by qRT-PCR and ELISA.
Results: Infection of neutrophils with *Mtb* at a multiplicity of infection of 10 induced NETs compared to resting neutrophils (238.9 vs. 112.7 ng/ml dsDNA, $p<0.001$; and OD_{450} 0.16 vs 0.07, $p=0.0197$, respectively). Confocal microscopy of ESAT-6 stimulated neutrophils confirmed co-localisation of extracellular citH3, MPO and DNA, key components of NETs. Stimulation of PHLF with exogenous NETs significantly increased secretion of the collagenase MMP-1 (2.2 vs 88.2 ng/ml $p<0.0001$), but not TIMP-1. In a neutrophil-PHLF co-culture system, *Mtb*-induced NETs lead to significantly increased MMP-1, MMP-3, IL-6, and IL-1β secretion and gene expression in PHLF. Pre-treatment with DNase, that degrades NETs, or with an inhibitor of NET formation, GSK484 significantly reversed the inflammatory response.
Conclusions: *Mtb* drives formation of NETs which act on lung fibroblasts to induce proinflammatory cytokine and MMP secretion which induce tissue remodelling in TB. Degrading NETs or preventing NET formation may represent a potential novel host-directed therapy to decrease fibroblast-dependent impaired lung function in TB patients.

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106 – WS57.3

Elevated matrix-metalloproteinase and pro-inflammatory cytokine activity due to helminth co-infection drives pulmonary tissue destruction in tuberculosis

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Purpose: Tuberculosis (TB) and soil-transmitted helminth (STH) infections are widespread, infecting approximately 1.8 and 1.5 billion people respectively worldwide. TB-STH co-infected individuals appear to suffer increased lung damage compared to those with TB alone, however the mechanisms behind this remain unclear. We explored the impact of STH and TB infection on the innate human immune response, investigating pro-inflammatory cytokines and matrix metalloproteinases (MMPs), the drivers of lung tissue destruction.
Methods: We recruited adults with culture-confirmed pulmonary TB and healthy controls in Iquitos, a city in the Peruvian Amazon only accessible by boat or plane. Three stool samples were obtained for parasitological examination, sputum for TB confirmation, and blood for analysis of cytokines, MMPs and *Strongyloides* serology via ELISA or Luminex multiplex assay. CXRs performed as part of routine care were used to determine lung damage severity score.
Results: Forty out of 61 TB-positive participants (65.6%) had at least one STH diagnosed compared with 43.1% of TB-negative participants (P=0.02). The adjusted odds of TB-positivity in individuals with >=1 STH were 2.69 times higher than in STH uninfected (95%CI 1.14-6.59).
MMPs: Multivariable analysis showed STH infection was positively associated with MMP-1 (beta 1.3, P=0.025), MMP-9 (beta 1.5, P=0.022), MMP-10 (beta 1.3, P<0.001), and MMP-13 (beta 1.2, P<0.001). TB infection was also significantly positively associated with all MMPs measured. Furthermore, MMPs positively correlated with CXR severity score, with a significant association in MMP-1 (beta 2.06, P=0.03), MMP-8 (beta 1.83, P=0.01) and MMP-9 (beta 1.82, P=0.05).
Cytokines: Multivariable analysis found STH infection was positively associated with IL-1beta (beta 1.17, P=0.021), IL-6 (beta 1.3, P=0.029), IL-12 (beta 1.15, P=0.012) and IL-17 (beta 1.16, P=0.002). TB infection was also significantly positively associated with all cytokines measured.
Conclusion: STH infection was associated with an overall increase in matrix-metalloproteinase and pro-inflammatory cytokine activity. In addition, MMPs positively correlated with CXR severity score indicating increased lung damage in these individuals. TB co-infection with STHs may drive an increased type I immune response leading to increased pulmonary inflammation and tissue destruction. These data have future implications on screening and treatment of STHs in TB.

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801 – WS57.4

Single cell RNA sequencing reveals endothelial cell killing and resolution pathways in experimental malaria-associated acute respiratory distress syndrome

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Plasmodium parasites cause malaria, a global health disease that is responsible for more than 200 million clinical cases and 600 000 deaths each year. Most deaths are caused by various complications, including malaria-associated acute respiratory distress syndrome (MA-ARDS). Despite the very rapid and efficient killing of parasites with antimalarial drugs, 15% of patients with complicated malaria succumb. This stresses the importance of investigating resolution mechanisms that are involved in the recovery from these complications once the parasite is killed. To study the resolution of MA-ARDS, *P. berghei* NK65-infected C57BL/6 mice were treated with antimalarial drugs after onset of symptoms, resulting in 80% survival. Micro-computed tomography revealed alterations of the lungs upon infection, with an increase in total and non-aerated lung volume due to edema. Whole body plethysmography confirmed a drastically altered lung ventilation, which was restored during resolution. Single-cell RNA sequencing indicated an increased inflammatory state in the lungs upon infection, which was accompanied by a drastic decrease in endothelial cells, consistent with CD8+ T cell-mediated killing. During resolution, anti-inflammatory pathways were upregulated and proliferation of endothelial cells was observed. MultiNicheNet interactome analysis identified important changes in the ligand-receptor interactions during disease resolution that warrant further exploration in order to develop new therapeutic strategies. In conclusion, our study provides insights in pro-resolving pathways that limit inflammation and promote endothelial cell proliferation in experimental MA-ARDS. This information may be useful for the design of adjunctive treatments to enhance resolution after *Plasmodium* parasite killing by antimalarial drugs.

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109 – WS57.5

Identification of specific monocyte epigenetic signatures in sarcoidosis and tuberculosis patients

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Purpose: Sarcoidosis (SARC) is a multisystemic inflammatory and granulomatous disease that most commonly affects the lungs. Causes and mechanisms underlying granuloma formation and maintenance are still unknown, but SARC shares many similarities with tuberculosis (TB). We hypothesized that monocytes from patients with SARC and TB may retain a specific epigenetic signature that drives maladaptive innate immune training and disease.
Methods: To test this, we performed genome-wide epigenetic profiling of monocytes from newly diagnosed SARC or TB patients using CUT&Tag technology, and assessed the acetylation of lysine 27 of histone 3 (H3K27Ac) to identify enhancers of expressed genes. From this we identified super-enhancers (SE) and predicted the autoregulatory transcription factors (TF) that are part of the core regulatory circuitries (CRC) in each disease.
Results: Through differential analysis of genome-wide H3K27Ac profiles we identified genomic regions that specifically distinguished SARC patients from TB patients and from Healthy Controls (HC). There were 9 H3K27Ac enriched regions and 84 H3K27Ac depleted regions in SARC patients compared to HC. One region was specifically H3K27Ac depleted in SARC compared with TB (shrunken$\log_2$FC > | 0.1|, adj-p < 0.1). SE were identified in all samples and among SE-associated genes, we identified 8 auto-regulatory TF specific to SARC (i.e., TCF7, FOXJ2, PRDM1, FOXK1, FOXO3, USF1, ELF2, and NR4A1), 3 specific to TB (i.e., ZNF219, EGR3, IRF7) and 5 shared by both diseases (KLF11, MNT, E2F2, SREBF1, and IRF2). Functional analysis suggested a role played by type I interferon, glucose metabolism and apoptosis pathways. This confirmed our hypothesis with evidence of specific CRCs in either disease.
Conclusion: Our findings provide evidence that there are distinct monocyte epigenetic signatures associated with SARC and TB, and raise new and challenging perspectives on the role played by trained immunity in inflammatory diseases. Ongoing work will incorporate transcriptomic data in the identification of CRCs and integrate additional immune phenotyping and functional assays for a more comprehensive understanding of granulomatous diseases.

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1897 – WS57.6

Clinically severe chronic obstructive pulmonary disease patients are characterised by a distinct immune lung cell signature

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Rationale: COPD is a severe, progressive and heterogeneous condition with several clinical phenotypes. However, the composition of the local immune environment in COPD patients has rarely been investigated and may assist patient stratification.
Methods: Comprehensive immune cell and cytokine profiles were created from lung explants from end-stage COPD patients (n = 23 & 40) and healthy donor lungs (n = 20 & 31) following transplantation using computational flow cytometry and multiplex assays, respectively.
Results: COPD lungs presented with a distinct immune cell signature. Application of machine learning algorithms revealed an enrichment of lymphocytic populations (including cytotoxic and T helper cells) and decreased neutrophils in end-stage COPD patient lung compared with donor lungs. COPD patients presented with strongly divergent cytokine profiles in the lung and circulation, with cytokines being co-regulated or differentially regulated in between both compartments e.g. IL-6, CCL2 and CCL5. Plasma cytokines were most strongly affected by disease status, however, smoking history did not alter the circulating cytokine profile. In contrast lung cell counts correlated strongly with smoking pack years and decreased oxygenation, and decreased lung function correlated strongest with circulating proinflammatory cytokine levels. Sub-clustering the COPD cohort revealed the presence of severe adaptive-immune patient subtype.
Conclusions: End-stage COPD patients display unique inflammatory cellular and cytokine signatures that can be stratified in a novel, clinically meaningful, endotypes. Our approach may provide a future rationale for targeted personalized treatments.

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WS58 – T CELL REGULATION AND FUNCTION II

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1576 – WS58.1

The RNA-binding proteins TIA1 and TIAL1 control T-cell activation and germinal centre formation.

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Purpose: Effective germinal centre responses rely on the dynamic regulation of genetic programs for ample activation and production of immunomodulator molecules by T cells. Emerging evidence show the prevalence role of post-transcriptional mechanisms regulated by RNA-binding proteins (RBPs) in the control of T cell function. Our recent studies uncovered the RBPs T-cell antigen 1 (TIA1) and like 1 (TIAL1) as essential players for B-cell expansion and selection in germinal centres, but their roles in T cells remains unknown. Our hypothesis is that TIA1 and TIAL1 are part of a post-transcriptional regulatory program that shapes the transcriptome of T cells during activation for mounting effective immune responses.
Methods: We used conditional knock out (KO) mice to assess the intrinsic role of TIA1 and TIAL1 in T cells in different models of vaccination. To identify the molecular mechanisms regulated by TIA1 and TIAL1 in T cells, we performed individual crosslinking immunoprecipitation (iCLIP) and RNA sequencing in TCR-activated CD4 T cells.
Results: Phenotypical characterization of T cell subsets in different experimental models revealed an important role for TIA1 and TIAL1 in T cell activation both in-vivo and in-vitro. TIA1 and TIAL1 deletion in T cells impaired both acute and chronic germinal centre responses. TIA1 and TIAL1 were found associated mainly with introns and 3'UTR of thousands of mRNA targets. Molecular characterization of TIA1 and TIAL1 double KO CD4 T cells showed that these RBPs shaped quantitatively and qualitatively the transcriptome of T cells by controlling the expression of essential transcription factors like Myc required for T-cell metabolic reprograming and proliferation upon TCR activation.
Conclusion: TIA1 and TIAL1 are essential components of a post-transcriptional network that control antigen-mediated T cell activation of T lymphocytes. Understanding the functions of TIA1 and TIAL1 in T cells will uncover novel post-transcriptional mechanisms controlling immunity which could be exploited in the development of innovative immunotherapies against disease.

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1505 – WS58.2

CD4-Lck signalling activates CDK1 to enhance nuclear transport in T cells

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Engaging the TCR at the immune synapse of CD4 T cells activates Lck that is complexed with CD4 to initiate downstream signalling. Here, we sought to examine CD4-Lck signalling in isolation from the canonical pMHC-TCR pathway. We first turned to a naturally occurring example of such antigen-independent CD4 engagement that occurs when HIV-1 envelope protein (Env) binds to CD4 on T cells to initiate viral infection. In this assay, the HIV-1 Env expressed on a virus-infected T cell binds CD4 on the surface of an uninfected cell, forming a so-called virological synapse that we and others have shown shares similarity with the immune synapse. Using resting primary CD4 T cells we found that Env-CD4 engagement resulted in a rapid and transient increase in phosphorylation of Lck, Zap70, Erk, p38 and Akt, which was similar in kinetics, but smaller in amplitude compared to CD3-crosslinking. We confirmed that CD4 engagement alone was sufficient for this signalling using 5 μm beads coupled with anti-CD4 antibody and observed that this reductionist approach similarly phosphorylated Lck and other downstream kinases. Next, we investigated the functional consequences of this CD4-Lck signalling. Measuring classical activation markers, we found no evidence for cell activation or cell cycle progression. Instead, we observed an increased rate of nuclear transport in CD4-stimulated T cells that was evident for both karyopherin-independent transport of HIV-1 capsid through the nuclear pore complex, and more globally for karyopherin-dependent transport measured by increased nuclear localisation of importin β1. Strikingly, this correlated with altered localisation of nuclear pore proteins Nup62 and TPR, suggesting that CD4-Lck signalling triggers nuclear pore remodelling to facilitate increased nuclear transport kinetics. Consistent with these changes, we found that CD4-Lck signalling activated CDK1 which is known to phosphorylate nuclear pore proteins. Moreover, inhibitors of Lck or CDK1 abolished the changes in nuclear transport kinetics and nuclear pore architecture initiated by CD4-Lck signalling. We conclude that CD4-Lck signalling at the synapse activates changes in nuclear pore architecture and transport dynamics, revealing a role for CDK1 beyond cell cycle progression and providing new insights in signalling propagation in T cells.

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2148 – WS58.3

NRK1-dependent cytoplasmic NAD/H synthesis determines CD4+ T cell inflammatory function and survival

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$CD4^+$ T cell function is underpinned by metabolic reprogramming upon activation. Increased glycolysis provides biosynthetic precursors for clonal expansion and promotes cytokine expression. In parallel, elevated mitochondrial oxidative phosphorylation (OXPHOS) generates heightened ATP and reactive oxygen species (ROS). ROS disseminate and signal, promoting T cell differentiation, but must be mitigated to prevent oxidative damage. Nicotinamide adenine dinucleotide (NAD/H) is an essential redox cofactor for glycolysis and mitochondrial OXPHOS. It is also phosphorylated to NADP/H, which regulates ROS levels. NAD/H abundance increases in line with $CD4^+$ T cell metabolism upon activation, but synthetic pathways are not fully characterised.
In this study, we interrogated expression and activity of nicotinamide riboside kinase 1 (NRK1) in $CD4^+$ T cells, which phosphorylates nicotinamide riboside (NR), directing it into the NAD salvage pathway. We identified this increases upon cell stimulation, driven by TCR and CD28 signalling. NRK1 non-redundantly contributes to NAD/H abundance in these cells but suppresses their activation and function. Consistently, NRK1-deficient $CD4^+$ T cells have a hyper-inflammatory phenotype, expressing high levels of effector cytokines, which occurs alongside impaired viability.
Mechanistically, this is linked to NRK1 redistribution to the cytoplasm upon $CD4^+$ T cell activation, where it locally elevates NAD/H levels. This supports glycolysis, but more profoundly impacts cytoplasmic NADP/H generation, thereby determining ROS abundance and nuclear NFAT translocation. During invasive fungal infection, NRK1 activity critically maintains effector $CD4^+$ T cell frequencies within affected tissues, confirming that regulation of immune cell metabolism at the subcellular level determines whole organism immune responses.

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955 – WS58.4

Extracellular ATP Induces Aging-Associated Traits in CD4 T Cells

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Purpose: The cellular aging process leads to immune function alterations, rendering aged individuals more vulnerable to various diseases. $CD4^+$ T cells, vital in adaptive immunity, undergo functional changes with age, including senescence-induced dysregulation. Beyond cellular changes, aging also affects cellular metabolism, including nucleotide metabolism. Additionally, tissue damage provoked by for example inflammaging can boost the release of nucleotides, such as adenosine-tri-phosphate (ATP). This study aims to explore the impact of extracellular ATP (eATP) on $CD4^+$ T cell characteristics reminiscent of aged immune systems, shedding light on potential mechanisms underlying immune aging.
Methods: Peripheral blood mononuclear cells (PBMCs) from young (<30 years) and aged (>55 years) donors were isolated, and T cell subsets were characterized using flow cytometry. A comprehensive panel assessed T cell activation markers, calcium signaling, proliferation, migration, mitochondrial activity, ROS production, and cytokine/granzyme B secretion upon extracellular ATP (eATP) stimulation. Additionally, subset-specific expression of ATP-binding P2Y receptors was evaluated.
Results: ATP-binding P2Y receptors, particularly P2Y2 and P2Y11, were significantly elevated in $CD4^+$ T cells from aged individuals, especially in memory subsets. Senescent T cell populations lost receptor expression, potentially rendering those cells insensitive to eATP. While eATP minimally affected calcium flux directly, it potentiated ionomycin-induced responses in young donors but not in aged counterparts. Conversely, $CD4^+$ T cells from aged donors exhibited heightened ionomycin responsiveness but reduced sensitivity to ATP. $CD4^+$ T cells from aged donors also showed minor amplification of mitochondrial potential after 24h activation with anti-CD3/CD28 in presence of eATP, with minimal effects on mitochondrial and cellular ROS. ATP-primed $CD4^+$ T cells from both, young and aged donors, displayed increased migration and significantly elevated granzyme B expression and secretion, along with enhanced production of IL-17A, IL-4, perforin, and granulysin.
Conclusion: These findings highlight age-related alterations in $CD4^+$ T cell responses to eATP, suggesting a potential role for purinergic signaling in immune aging. Elevated P2Y receptor expression in aged $CD4^+$ T cells may contribute to dysregulated immune metabolism and compromised functions.

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965 – WS58.5

Spatial and functional association of CD69 leukocyte activation receptor with Peroxiredoxin-1

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The early leukocyte marker CD69 is an immunoregulatory molecule that controls T cell activation, retention and differentiation. Recently, we have identified that the binding of oxLDL to CD69 induces the expression of PD-1 through the activation of NFAT. To decipher the molecular mechanisms of CD69-driven T cell regulation, we have carried out a "peroxidase-catalyzed proximity labeling" strategy. We have generated a fusion protein of CD69 with the ascorbate peroxidase APEX2 and stably over-expressed this construct in a CD69 knock-out (KO) T cell line of Jurkat previously generated by CRISPR-Cas9 technology. Among a number of CD69-spatially neighboring proteins, there was an unexpected enrichment in peroxiredoxins (PRDXs) such as PRDX2, PRDX4 and especially PRDX1. PRDXs are antioxidant enzymes that prevent cell damage caused by reactive oxygen species (ROS). In addition, PRDX1 acts as a molecular chaperone under oxidative stress conditions, enhances natural killer cell cytotoxicity, suppresses oncogenic proteins and it is a key regulator of lipid peroxidation. The association of CD69 with PRDX1 was validated by a pull-down assay in activated naïve CD4+ T lymphocytes obtained from wild-type mice compared to CD69-deficient mice. We have also evaluated the lipid peroxidation content in Jurkat and primary T lymphocytes by flow cytometry. Activation with anti-CD3/CD28 enhanced lipid peroxidation specifically in CD69+ T cells, since both KO Jurkat and mouse CD4+ T cells CD69-deficient showed less lipid peroxidation. Moreover, Jurkat overexpressing CD69 have increased lipid peroxidation that activated wild-type cells. These results may indicate a novel role for CD69 controlling the regulation of lipid peroxidation through its association with PRDX1, thereby modulating the activation and cellular fate.

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876 – WS58.6

A novel interaction between E-cadherin and PD-1 leads to inhibition of T cell function

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Purpose: E-cadherin is a member of the cadherin superfamily widely known to participate in cell-cell adhesion processes. Interestingly, studies have demonstrated its ability to modulate functional responses of NK cells and T cells by interacting with the inhibitory receptor KLRG1. Like KLRG1, PD-1 is also an inhibitory receptor expressed on T cells that regulates T cell activation and promotes immune tolerance. Since E-cadherin is extensively expressed on the surface of epithelial cells, it is important to investigate its interaction with PD-1 and its role in peripheral tolerance.
Methods: The N-terminal ectodomains of E-cadherin and PD-1 were expressed and purified followed by interaction analysis using Surface Plasmon Resonance. Fluorophore-conjugated E-cadherin tetramers were used to check for interaction with PD-1 expressed on T cells via flow cytometry. The effect of E-cadherin on T cell activation and differentiation was investigated to understand the functional role of the interaction. The available structures of PD-1 and E-cadherin were analyzed followed by molecular docking and mutagenesis studies to map their binding interface.
Results: Biophysical studies with either mouse or human E-cadherin and PD-1 showed a concentration-dependent increase in response indicating an interaction between the two proteins. Moreover, E-cadherin was able to bind to PD-1-expressing T cells independently of KLRG1 expression and proportionate to the level of PD-1 expression, confirming the interaction between E-cadherin and PD-1 on T cells. The engagement of PD-1 by E-cadherin inhibited T cell activation, proliferation, and cytokine production, demonstrating the inhibitory effect of this novel interaction. The effect of E-cadherin mutants on T cells showed that the canonical homodimeric interface of E-cadherin participates in its interaction with PD-1.
Conclusion: E-cadherin and PD-1 can interact through their N-terminal ectodomains resulting in T cell inhibition. Given the expression of E-cadherin in the peripheral tissue, it is important to investigate the biological implication of the novel PD-1: E-cadherin interaction in maintaining peripheral tolerance. Also, the role of this interaction in immune-evasion strategies by tumor cells needs to be studied in-depth which may lead to the identification of novel therapeutic targets.

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WS59 – T CELLS IN INFLAMMATORY AND INFECTIOUS DISEASES

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1134 – WS59.1

Blood Brain Barrier transmigration triggers inflammasome activation in Th lymphocytes during neuroinflammation

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Rationale: Multiple Sclerosis (MS) is a chronic autoimmune disease of the central nervous system (CNS), characterized by infiltration of immune cells into the brain. The disease is initiated when self-reactive T cells are activated in the periphery, after which they travel to the CNS through the blood brain barrier (BBB). Once inside, an inflammatory cascade is triggered, causing damage to neuronal tissue, which results in neurological disabilities. Up to now, the role of the inflammasome herein was mostly studied in innate immune cells. However, recently it was shown that inflammasome activation in adaptive immune cells plays a role in other autoimmune diseases. Here, we aim to identify whether inflammasome activation in T helper cells is increased in MS patients, and whether it contributes to disease pathogenesis and progression.
Methods/Results: RNA analysis of ex vivo samples of MS patients and healthy controls reveals that inflammasome related markers are upregulated in CD4 T cells of MS patients. To visualize inflammasome activation in vivo, we used a preclinical mouse model, experimental autoimmune encephalomyelitis (EAE), in ASC-reporter mice. Herein, we identified an increase in inflammasome activation in the CD4 T cell subsets Th17, Th17.1 and Tregs, over time **exclusively** in the CNS. Lightsheet microscopy of whole brains of healthy and EAE induced ASC-reporter mice show a clear increase in immune cells containing inflammasome activation during disease. In NLRP3 knock out mice, EAE induction resulted in significantly lower disease scores compared to WT mice. Importantly, this coincided with a decreased CNS infiltration of Th1 and Th17 subsets. Finally, using human in vitro BBB migration assays, we found that only Th cells show increased inflammasome activation after BBB transmigration. Also, inflammasome activation in Th cells increased their migration rate, linking the inflammasome to the propensity of Th cells to migrate across the BBB.
Conclusion: These results indicate that inflammasome activation in Th cells is crucially involved in autoimmune neuroinflammation. Importantly, it appears to be linked to BBB transmigration, which is an initiating player in EAE and MS. Therefore, inhibition of inflammasome activation specifically in Th cells could be a possible therapeutic modality to treat MS patients.

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1627 – WS59.2

CD29hi CD99hi T cells are upregulated in primary Sjögren's syndrome

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Purpose: Primary Sjögren's syndrome (pSS) is an autoimmune disease that is triggered by environmental factors in genetically susceptible individuals, resulting in a dysregulated immune response that leads to chronic inflammation and autoimmunity. This study aimed to explore the roles of CD29 and CD99 in human T lymphocytes and to clarify their significance in pSS.
Methods: Single-cell RNA-Seq data were employed to examine the expression of CD29 and CD99 in peripheral blood mononuclear cells. Flow cytometry was used to detect the expression of CD29 and CD99 in peripheral blood T lymphocytes of 24 pSS patients and 20 age- and gender-matched healthy controls. Based on RNA-Seq predictions, the expression of cytotoxic and activation-related molecules was analysed in both the CD29hi CD99hi and CD29int CD99int subsets. T lymphocytes were stimulated with anti-CD29 and anti-CD99 antibodies to elucidate the role of CD29 and CD99. CD29 and CD99 related cell subsets of pSS patients were detected, and their clinical effectiveness in diagnosing pSS was determined through Receiver Operating Characteristic (ROC) curves.
Results: Single-cell RNA-Seq data revealed a robust positive correlation between CD29 and CD99 in both CD4+T and CD8+T cells. Flow cytometry validated their co-expression, classifying T cells into CD29int CD99int and CD29hi CD99hi subsets. CD29hi CD99hi T cells demonstrated high expression of cytotoxic molecules and exhibited activation markers, with elevated levels of Ki-67, PD-1 and CD25. Stimulation with anti-CD99 and anti-CD29 antibodies increased granzyme B levels in CD29hi CD99hi CD8+T cells. CD29hi CD99hi CD8+T cells were significantly increased in pSS, indicating their potential for pSS diagnosis, as demonstrated by high AUC values in ROC analysis.
Conclusion: CD29 and CD99 showed significant co-expression in peripheral T cells, while CD29hi CD99hi T cells displayed characteristics of cytotoxic T lymphocytes and activated T cells. The changes in CD29hi CD99hi T cell subsets among pSS patients contribute to the early diagnosis of pSS.

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2230 – WS59.3

SARS-CoV-2-specific antibody and T-cell responses after COVID-19 BA.1 bivalent boosting

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SARS-CoV-2 variants of concern (VOCs) are a major issue for ongoing protection following COVID-19 vaccination and infection. In a longitudinal observational study of healthcare workers, we previously showed that T-cell responses to vaccination are well-maintained over time, and against VOCs, while antibody responses wane rapidly and are lost to emerging VOCs.
Here, we assessed the impact of the bivalent BA.1 COVID-19 vaccine (fourth dose=V4 received in autumn 2022) on T-cell and antibody responses using a multi-centre cohort, Protective Immunity from T-cells to COVID-19 in Healthcare workers (PITCH). This study uniquely compares responses in people who've received a bivalent vaccine dose with those having a parallel vaccination history, but did not receive the bivalent vaccine.
Peripheral blood mononuclear cells, plasma, serum, and nasal mucosal strips were collected at pre-V4 (V3+6 and 12 months) and post-V4 timepoints (V4+1 and 6 months). Individuals with no V4 were included at V3+18 months, equivalent to the V4+6-month timepoint. T-cell IFN-γ responses (ELISpot), circulating and nasal binding antibodies (MSD), and neutralizing antibodies to SARS-CoV-2 and relevant VOCs were measured.
Circulating IgG to spike and nucleocapsid were boosted by natural infection at pre-V4 timepoints (2022 UK summer/autumn). IgG levels were further increased by the bivalent booster, rapidly waning by V4+6 months. T-cell responses were well-maintained over time, with a slight boosting effect in spike and M+NP responses at V4+6 months, when circulating IgG and T-cell responses were similar in V4-boosted and non-V4-boosted individuals.
Neutralizing antibodies were boosted by V4, and a transient broadening of neutralizing capacity to VOCs BA.1, BA.2, XBB1.5 and BA.2.86 was noted one-month post-bivalent booster. Responses rapidly waned at V4+6 months, remaining slightly higher than the non-V4-boosted (V3+18) group.
Nasal IgG and IgA antibodies are being measured to give valuable insights into the boosting/broadening of mucosal antibody upon bivalent vaccination.
Our data demonstrate a transient broadening of neutralizing antibody responses due to the bivalent booster vaccine which might increase protection from new circulating VOCs during a narrow timeframe. We also highlight that the dynamics of T-cell and antibody responses to SARS-CoV-2 are now driven by a combination of natural infection events and vaccination.

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1516 – WS59.4

SIMON says: Using machine learning to understand correlates of protection for SARS-CoV-2 vaccines in non-human primate challenge studies.

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An automated machine learning platform, SIMON, can identify algorithms that model pathology and upper and lower respiratory tract viral control outcomes post-SARS-CoV-2 challenge. We have applied this platform to analyse the immune responses of ninety rhesus and cynomolgus macaques from various SARS-CoV-2 vaccine and re-challenge studies, to identify immune predictors of viral and pathological protection.
The immune predictors of protection identified include Spike binding and neutralising antibody, in support of the use of ELISA and MNA assays in human SARS-CoV-2 vaccine immunogenicity trials. The SIMON analysis has also extracted novel candidate CoPs in the adaptive cellular compartment including peripheral T cell subset frequencies. The frequency of the $CD14^{hi}CD16^{hi}$ monocyte population in the periphery, proposed to be associated with severe human COVID19 cases, has been demonstrated via SIMON to negatively impact pathology and viral load outcomes when present at high frequencies in the weeks following vaccination or primary challenge in NHPs.
SIMON has also been applied to identify early immune signatures that predict the emergence of high or low Spike binding titres. The $CD14^{hi}CD16^{hi}$ monocyte signature, identified to predict pathology, also predicted low Spike IgG titres. This points to restriction of the GC reaction when this monocyte population is in abundance, as has been reported in the context of co-infections. High baseline humoral immunity to human coronaviruses (HCoVs) was also identified as a predictor of low Spike IgG titres, providing an additional example of original antigenic sin. A systems immunology approach and the application of SIMON were vital to uncovering these findings, as the frequency of this monocyte population in the blood and the incidence of HCoV humoral immunity in macaques are both relatively low.
We have also applied SIMON to identify novel relationships between functional antibody, antibody isotype and antibody glycosylation in macaques, and the role of these compartments in providing clinical protection and mediating viral control upon challenge.
These findings encompass the longstanding value of a robust animal model in immunology and vaccinology research. The combination of this rich NHP dataset and the computational aptitude of SIMON has provided strong evidence for SARS-CoV-2 CoPs.

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2038 – WS59.5

Local skin enrichment of chemical-specific T cells as strategy for causative allergen identification

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Tattoos may initiate allergic reactions. Metals, e.g. nickel, contained in inks have been suspected as causative allergens to activate T cells responsible for clinical symptoms. Since a positive nickel patch test result does not necessarily indicate nickel as causative allergen, we here analyzed local skin T cell receptor (TCR) repertoires to confirm or exclude nickel as culprit in eczematous allergic skin reactions to tattoo.
Ni^{2+}-specific T cells detected by CD154 (CD40L)-based activation-induced marker (AIM) T cell assay using peripheral blood mononuclear cells and 200 µM $NiSO_4$ were sorted. Sequenced TCRs of activated T cells were quantified and compared to sequenced TCRs of matching blood randomly sorted T cells and healthy and inflamed skin biopsies of 3 patients with chronic tattoo allergic reactions and 1 nickel patch-tested patient.
We detected increased frequencies of Ni^{2+}-specific CD154+CD4+ T cells in positive nickel patch tested patient (1.3% allergic vs. 0.1% non-allergic) and tracked their TCRs in the skin repertoire. In the skin from the positive nickel patch test reaction, 28% and 45% and of Ni2+-specific TCRα and -β chains >2-fold enriched than in blood, respectively. Skin biopsies from allergic tattoo reactions lacked Ni2+-specific TCR enrichment, thus excluding Ni2+ as causative allergen. TCRs of unrelated antigen, e.g. tetanus toxoid, also lacked enrichment. In line with previous results, we observed an increased occurrence of the TRAV9-2 segment and histidine-containing complementary determining regions (CDR) 3 in both CD4+ memory T cell and inflamed skin TCR repertoires of the nickel allergic patient.
The local enrichment of Ni^{2+}-specific T cells in a small biopsy can identify or exclude nickel as culprit allergen *in situ* complementing patch test. This strategy combining AIM T cell assay and TCR sequencing can quantify chemical-specific T cell frequencies in blood and skin. Our approach may be transferred to other chemical sensitizers.

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1902 – WS59.6

Single-cell sequencing reveals disrupted skin tissue resident memory T cell dynamics in allogeneic hematopoietic stem cell transfer and acute graft-versus-host disease

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Skin tissue-resident memory T cells (TRM) are important regulators of tissue homeostasis, but also implicated in inflammatory processes, including graft-versus-host disease (GVHD) that can occur after hematopoietic stem cell transplantation (HSCT). To date, there is lack of adequate human models to study tissue-instructed T cell reprogramming and TRM involvement in tissue-specific disease mechanisms. To investigate the role of TRM in GVHD pathology, we study patients undergoing allogeneic HSCT and with acute (a)GVHD. While most host immune cells are eliminated before HSCT by myeloablative conditioning, host skin TRM can survive this treatment and coexist in the skin next to transplanted donor T cells. In these patients with skin T cell chimerism, cells of host and donor can be distinguished by genetic differences.
We hypothesize that comparison of pre-existing host and developing donor TRM after transplantation under steady state und inflammation will unravel new pathways involved in regulating tissue residency and pathogenic signaling. Furthermore, we believe that interactions with structural skin cells and other immune cells are crucial in this regulation.
Here, we collected longitudinal blood and skin samples of HSCT patients and untreated aGVHD patients. We performed single-cell RNA/TCR sequencing to define host/donor genotype by SNP-based demultiplexing, investigate transcriptional changes in immune and non-immune cells, predict cell-cell interactions in the skin and assess dynamics of T cell clonality.
Among skin T cells, we found few donor cells in the skin by day 14, whereas they dominated during aGVHD. Shared donor T cell clones in skin and blood on day 100 displayed a TRM gene expression profile enriched in skin. However, specific subsets of the TRM pool found in healthy skin at steady state were declining after transplantation and in GVHD. This disrupted TRM homeostasis in aGVHD was accompanied by profound transcriptional changes in non-hematopoietic skin cells, including decreased expression of genes regulating extracellular matrix organization and upregulation of chemotaxis and antigen presentation pathways.
Conclusively, our study suggests a selective loss of certain TRM subsets after transplantation and has the potential to uncover new principles of tissue-instructed regulation of TRM development, maintenance, and pathogenicity under homeostatic conditions and in cutaneous inflammation.

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WS60 – MACROPHANGES IN INFECTIOUS AND AUTOIMMUNE DISEASES

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1818 – WS60.1

Loss of synovial tissue macrophage homeostasis precedes rheumatoid arthritis clinical onset

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Purpose: In this study we performed an in-depth investigation into the myeloid cellular landscape in the synovium of Rheumatoid Arthritis (RA) patients, 'individuals-at-risk' of RA (IAR) and healthy controls to examine the largely unexplored nature and contribution of synovial-tissue macrophage subsets in the pathogenesis of RA.
Methods: Single-cell synovial-tissue suspensions from RA (n=41), IAR (n=5) patients and healthy controls (n=11) were obtained through arthroscopy. Synovial tissue macrophage subsets were examined by advanced multiparameter flow cytometric analysis, single-cell and bulk RNA-sequencing, metabolic and functional assays.
Results: Flow-cytometric analysis demonstrated for the first time, the presence of a CD40-expressing CD206+CD163+ macrophage population dominating the inflamed RA synovium, associated with disease-activity and treatment response. In depth RNAseq and metabolic analysis demonstrated that this macrophage population is transcriptionally distinct, displaying unique inflammatory, and tissue-resident gene signatures, has a stable bioenergetic profile, and regulates stromal cell responses. Single cell transcriptomic profiling of synovial tissue cells from RA patients and healthy individuals was also performed to give a unique myeloid atlas from health to disease. scRNAseq profiling of 67908 RA and healthy synovial-tissue cells identified nine transcriptionally distinct macrophage clusters, further classified into four subpopulations; TREM2high, TREM2low, FOLR2high, IL-1Bhigh. Two clusters: IL-1B+CCL20+ and SPP1+MT2A+ were identified as pro-inflammatory macrophage populations. Interestingly both these clusters are enriched in RA compared to healthy synovial tissue, display heightened CD40 gene expression, are capable of shaping stromal cell responses, and importantly are enriched pre-disease onset in IAR. Functionally, RA synovial tissue myeloid cells are potent producers of pro-inflammatory mediators (reversed by CD40-signalling inhibition), significantly correlate with disease activity and treatment response and are capable of inducing an invasive phenotype in healthy synovial-fibroblasts. Crucially inflammatory myeloid signatures present in active RA synovium identified in phenotypic and single cell transcriptomic analysis, are an early phenomenon, occurring prior to clinical manifestation of disease in individuals 'at-risk' of RA (positive for ACPA) while inflammatory macrophage subsets identified in scRNAseq are also enriched early in disease.
Conclusion: Combined, these findings identify the presence of early pathogenic myeloid signatures that shapes the RA joint microenvironment and represents a unique opportunity for early diagnosis and therapeutic intervention.

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441 – WS60.2

Long-lasting protection against listeriosis and peritonitis by trained immunity

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Objective: We demonstrated that trained immunity, the memory-like property of the innate immune system, protects from a large panel of infections. Moreover, protection from listeriosis was maintained 9 weeks after the induction of training. Our objective was to determine whether trained immunity confers significantly longer protection from bacterial infections.
Methods: Mice were trained by intraperitoneal injection of β-glucan and challenged up to 7 months later, a considerable period in terms of mouse lifespan, with *Escherichia coli* intraperitoneally or *Listeria monocytogenes* intravenously. Peritoneal fluid, blood, bone marrow and spleen were collected to quantify bone marrow long-term and short-term hematopoietic stem cells (HSCs), multipotent progenitors (MPPs), leukocyte sub-populations and bacteria, to measure the antimicrobial activity of leukocytes, and to perform RNA seq on neutrophils.
Results: The induction of trained immunity induced an accumulation in the peritoneal cavity of small peritoneal macrophages that persisted 3 months, and a less pronounced recruitment of PMNs. Trained immunity increased bone marrow long-term-HSCs and myeloid-biased progenitor MPP3 as well as blood monocytes and neutrophils. Cell numbers returned to control levels after 7 months. Blood collected 3 months after training controlled better the growth of *Listeria monocytogenes* and produced more pro-inflammatory cytokines, including IIL-1β, IFNγ and TNF ($P<0.05$-0.01), in response to lipopolysaccharide than control blood. Peritoneal cells collected 7 months after training produced more IL-6 in response to lipopolysaccharide, CpG oligonucleotide and heat-killed bacteria than control cells ($P<0.01$). The transcriptome of bone marrow neutrophils trained for 6 months was enriched in gene pathways associated with wound healing and homeostatic processes. In the model of *Escherichia coli* peritonitis, mice trained 7 months prior to infection showed reduced bacteremia ($P=0.04$) and increased survival ($P=0.056$). In the model of systemic listeriosis, mice trained 3 and 7 months before infection showed decreased bacteremia ($P<0.05$) and increased survival (3 and 7 months: $P=0.002$ and $P=0.08$).
Conclusions:. Training has long-term effects on the number and reactivity of innate immune cells and provides long-lasting protection against lethal peritonitis and listeriosis. The underlying mechanisms of long-term imprinting are currently being investigated.

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2187 – WS60.3

Rheumatoid arthritis and psoriatic arthritis circulatory monocytes display metabolic changes, with dysfunctional circadian rhythm and mitochondrial dynamics

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Purpose: Rheumatoid Arthritis (RA) and Psoriatic Arthritis (PsA) are distinct in clinical presentation and molecular profile. As crucial innate effector cells, we investigate the role of circulating RA and PsA monocytes in metabolic reprogramming, immune activation, circadian rhythm and mitochondrial dynamics.
Methods: PBMCs and CD14 monocytes were isolated from RA, PsA and healthy controls (HC). Frequency and immune/metabolism markers (PDL-1, HIF1a, pS6, pAKT, Glut1, BTLA) on monocyte subsets were assessed by flow cytometry. Metabolic analysis was performed on basal and LPS stimulated monocytes by RT-PCR and Seahorse-XFe-technology. In parallel, genes involved in circadian rhythm (CLOCK, BMAL1, PER1, PER2, NFIL3) and mitochondrial fission and fusion (DRP1, MFN1) were assessed by RT-PCR.
Results: Baseline ECAR following LPS stimulation was induced, with minimal effect on OCR, resulting in a significant shift of RA and PsA monocytes towards glycolysis ($p< 0.05$). Differential expression of PDL-1, pS6 and HIF1α were observed between RA and PsA monocytes, with higher expression in PSA than RA in classical monocytes. LPS induced metabolic genes, with fumarase induction significant in PSA ($p< 0.05$) and HIF1α more significant in RA ($p< 0.001$). RA monocytes also demonstrated increased endogenous NDufB5 expression ($p< 0.05$). Supporting mitochondrial dysfunction in RA, endogenous MFN2 was significantly increased in RA ($p< 0.05$), with induction of mitochondrial fission regulator DRP1 upon LPS stimulation. Endogenous circadian rhythm genes CLOCK, BMAL1 and PER2 were increased in RA (ns) and PSA ($p< 0.05$) over HC, with LPS-stimulation reducing expression to that of HC. Finally, downstream effector NFIL3 was induced with LPS in RA monocytes ($p< 0.05$), with no effect observed for PsA.
Conclusion: RA and PsA CD14+ monocytes display a shift towards a glycolytic phenotype. Differential metabolic marker expression is observed between monocyte subsets. Altered regulation of genes involved in circadian rhythm suggest differences in the cellular clock, with endogenous levels increased in RA and PsA. This was paralleled by altered mitochondrial dynamics, with significant induction of mitochondrial fission regulator DRP1 in RA monocytes. Taken together, this data supports differential metabolic dysregulation of RA and PsA monocytes, effects that may involve changes in the cellular clock, inducing monocyte pathogenic mechanisms.

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742 – WS60.4

Macrophage reprogramming in chronic organ disease – a lesson from the liver

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Purpose: Kupffer cells are a specialized population of tissue-resident macrophages with high phagocytic activity, acting as sentinels in the hepatic sinusoids to detect and remove bacteria, dead or dying cells, and aged platelets from the circulation. Furthermore, they are involved in antigen presentation and immune tolerance to protect from immunity against food-, gut- or commensal bacteria-derived antigens. On liver injury, Kupffer cell numbers decrease and get replaced by monocyte-derived macrophages, however the long-term consequences of these changes remain largely obscure. Therefore, we aimed to phenotypically and functionally characterize changes of the liver myeloid compartment in response to acute and chronic liver injury.
Methods: We leveraged intravital immunostaining and multi-color spectral laser scanning microscopy to study cell-cell interactions and macrophage scavenging function in mouse models of acute and chronic liver damage. Multiplex spectral flow cytometry was used for in-depth immunophenotyping of the myeloid and lymphoid compartment. T cell activity was assessed by assessment of proliferation, cell phenotyping and measuring antigen specific cytotoxic T cell responses.
Results: Intravital microscopy revealed stark alterations in liver macrophage cellular crosstalk with neutrophils and CD4+ T cells as well as impaired macrophage scavenging function. We could link these alterations with pronounced changes in macrophage phenotype showing reduced expression of scavenging markers Tim4, CD68, CRIg, CD163, CLEC2 and CD206. Functional and activation profiling of the T cell response showed a significant increase in T cell immunity following acute liver damage. Surprisingly, in chronic liver injury, we found a clear suppression of both CD4 and CD8 T cell activity, which was only partly dependent on antigen presentation during liver injury. Mice with chronic liver injury were more susceptible to sepsis and showed higher mortality following bacterial challenge, underpinning the biological and clinical relevance of our findings.
Conclusion: Acute and chronic liver injury induce distinct alterations in macrophage spatiotemporal cell-cell interactions. Furthermore, in response to injury, liver macrophages lose their scavenging function, and this translates into increased susceptibility to infection. Changes in macrophage function were also associated with altered T cell immunity, which highlights the importance of liver macrophages as central hubs of the hepatic immune response.

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2204 – WS60.5

Functional heterogeneity of tissue resident macrophages of distinct origin during polymicrobial sepsis

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Life-threatening sepsis is characterized by a dysregulated systemic immune response and multi-organ failure upon infection. Mechanisms regulating the immune system's competence to fight the systemic infection without harming the host are still poorly understood. Especially the role of tissue-resident macrophages (TRM) as key players in the defense against invading pathogens and source of immune modulatory cytokines need further investigation. In recent years it has been shown that TRM are either of embryonic or adult hematopoietic stem cell (HSC)-derived origin. However, the functional impact of ontogeny of TRM in polymicrobial sepsis is largely unknown.
Conditional depletion and repopulation studies using Clodronate-Liposomes reveal that – in contrast to embryo-derived TRM - HSC-derived macrophages are a heterogenous population with varying expression of immune modulating receptors like Tim-4 and VSIG4, and a distinct ability to clear bacteria via phagocytosis. The exchange of embryo-derived TRM by adult HSC-derived macrophages is associated with higher mortality in the peritoneal contamination and infection (PCI) sepsis model demonstrating a protective role of embryo-derived TRM.
These findings imply that the ontogeny of tissue-resident macrophages determines their function and that homeostasis of macrophages of embryonic origin is key for shaping the immune response and survival of sepsis.

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1501 – WS60.6

Immunological tools for the prediction of poor outcome in Oligoarthritis

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Purpose: Oligoarthritis is the most common form of Juvenile Idiopathic Arthritis. The disease course and outcome within two years from diagnosis is vary variable, with most patients exhibiting an oligoarticular course, more benign and likely to achieve remission, and a significant proportion (30-40%) developing a polyarticular course (pcJIA), which is more severe and requires more aggressive treatment. New biomarkers are demanded for early discrimination of patients at risk of developing pcJIA. Cells causing inflammation and tissue-destructive effects in the joints of Oligoarthritis patients release extracellular vesicles (EV) both in plasma (PL) and synovial fluid (SF). A better characterization of inflammatory cells combined with that of EV content at disease onset may be valuable for the identification of early biomarkers predictive of pcJIA.
Methods: We utilized a strategy that combines classical approaches for the study of inflammatory cells in liquid biopsies and system biology–driven omic methods (miRNomic/proteomic) for the analysis of EV released by these cells. 97 treatment-naïve Oligoarthritis patients were enrolled in the study at onset and followed up for 24 months after diagnosis. EV-miRNA (EV-miR) and EV-protein (EV-Prot) expression profiling was carried out in PL and SF collected at disease onset. PL from 25 age-matched healthy children was used as control. Monocytes/Macrophages and T cell subsets were phenotypically characterized in peripheral blood (PB) samples from 26 patients and compared between those undergoing different clinical course.
Results: Omic approaches identified a signature of 7 EV-miR and 112 proteins expressed both at a systemic and local level able to discriminate new-onset patients from controls with a high potential diagnostic value. Supervised machine learning method and WGCNA analysis demonstrated the power of EV-miR 29a,-miR 223 and of 16 protein clusters in the stratification of patients undergoing different disease course. Cytoflurimetric analysis showed different proportions of activated CD4/CD8, effector memory CD8, T regulatory cells and M1/M2 monocyte/macrophages expressing the inflammatory hypoxic receptor TREM1 between the outcome groups, both in SF and PB samples
Conclusion: Our data provide new immunological indicators of pcJIA in new-onset Oligoarthritis patients that include, EV-miRs and EV-Prots and specific cell subsets.

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WS61 – CANCER IMMUNE REGULATION AND EVASION

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1334 – WS61.1

Exploring the impact of CD300e in colorectal cancer: insights from obesity-related murine models

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The etiology of colorectal cancer (CRC) is multifaceted and intricately linked with lifestyle factors, particularly diet and obesity. High-fat diet (HFD)-induced obesity entails a persistent low-grade inflammation and dyslipidemia, fostering insulin resistance, steatosis, and lipotoxicity, thereby activating proliferation pathways in colonic epithelial cells. Recent research underscores a significant correlation between CD300e activation and impaired macrophages' antigen presentation. Interestingly, the greater part of tumor-associated macrophages in CRC patients display CD300e^{high}/MHC-II^{low} expression pattern, suggesting that CD300e may act as an immune checkpoint. Though categorized as an orphan receptor, sphingolipids emerge as potential ligands for both mouse and human CD300e, prompting the investigation of how CD300e affects CRC in the presence of obesity-associated metabolic dysregulation. Thus, we employed a murine model of obesity-related CRC in CD300e knock-out (KO) mice. Unexpectedly, HFD-fed mice exhibited no significant genotype-based disparities, in contrast to the standard diet (STD)-fed control groups, where KO mice displayed a remarkable reduction in tumor burden and an enrichment in IFN-γ-producing CD8$^+$ T cells in local lymph nodes, indicative of a boosted anti-tumor response compared to wild-type (WT) controls. Additionally, systemic immune profiling revealed a higher neutrophils-to-lymphocytes ratio in WT mice, a recognized adverse prognostic marker in CRC patients. Given the constrained size of tumors, immune cell infiltration was assessed in a subcutaneous model of CRC, confirming an enhanced anti-tumor immune response in KO mice characterized by pronounced MHC-II expression on macrophage surfaces, coupled with elevated IFN-γ-producing CD4$^+$ and CD8$^+$ T lymphocytes infiltration.
The absence of discernible variation in a HFD setting finds corroboration in an ongoing study highlighting a protective effect of CD300e in obesity, proposing that CD300e binding sphingolipids foster their scavenging, thus reducing the accumulation and the subsequent detrimental effects. Supporting this hypothesis, liver histology from HFD-fed KO mice revealed signs of lipotoxicity and pronounced immune cell infiltration, with a milder phenotype observed in WT mice.
In summary, these findings emphasize the pivotal role of CD300e in shaping a pro-tumor microenvironment, positioning it as a promising target for immunotherapy. Nonetheless, it is crucial to account for potential divergent effects of the receptor in the presence of comorbidities.

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395 – WS61.2

Type 2 innate lymphoid cells fuel breast cancer development by inhibiting anti-cancer immunity

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The tumor microenvironment (TME) is an important regulator of breast cancer (BC) development. It is composed of acellular and cellular factors, among others immune cells like type 2 innate lymphoid cells (ILC2s). It has been shown that ILC2s are enriched in the TME of BC patients compared to healthy individuals, and in the 4T1 BC mouse model, ILC2s inhibited the immune response against the tumor cells at both the primary and the metastatic tumor site. However, the exact molecular mechanisms of how ILC2s regulate BC immunity remain largely unknown.
To reveal the phenotype of ILC2s in BC, we used the polyoma middle T antigen transgenic mouse model, mimicking luminal BC development. Single-cell (sc)RNA sequencing and flow cytometry experiments showed that ILC2s (expressing *Gata3*, *Rora*, *Il2ra*, and *Il7ra*) accumulated in the mammary fat pad (MFP) during BC development. Furthermore, the MFP ILC2s phenotypically changed during tumor development by increasing PD-1, c-KIT, and Neuropilin-1, while downregulating KLRG1 and Sca-1 expression. Both, healthy MFP and tumor ILC2s, expressed interleukin-5 and -13 after PMA/Ionomycin restimulation. We detected the same ILC2 phenotype in the tumors of $BRCA1^{mut}p53^{-/-}$ mice, which mimic the basal BC subtype. Next, we tested if ILC2s directly regulate cancer cell growth *in vitro*. Co-culturing the BC cell line 'VO' with healthy MFP or tumor ILC2s for 48 hours increased the number of viable VO cells. To assess the functional relevance of ILC2s in BC *in vivo*, we co-injected ILC2s and VO cells orthotopically into FVB mice and measured the tumor growth. While 85% of the mice that were co-injected with VO cells and ILC2s developed a tumor, 50% of the VO-only recipients rejected the tumor cells. ILC2-co-injection induced an increased infiltration of $Gr\text{-}1^{+}CD11b^{+}$ neutrophils, which could potentially inhibit anti-cancer immunity.
In summary, we showed that ILC2s accumulate, phenotypically change, and promote an immune-inhibitory environment during BC development. Additionally, ILC2s can directly promote BC cell growth *in vitro*. Further experiments will provide knowledge about ILC2s as an important player in the BC TME and identify novel molecular mechanisms that can be used to develop new therapeutic strategies to treat BC patients.

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564 – WS61.3

IL4I1 expression in macrophages leads to their pro-tumor profile and T-cell exhaustion in the course of tumor progression.

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Purpose: Deciphering how IL4I1 promotes pro-tumor properties of melanoma-associated macrophages.
Methods: Spectral flow-cytometry, imaging, co-culture, *in vivo* neutralization
Results:
1. Tumor-associated macrophages (Mφ) overexpress IL4I1 during melanoma progression
We established the pro-tumoral role of the phenylalanine oxydase IL-4 induced gene 1 (IL4I1) in mice transgenic for the human RET oncogen developing a spontaneous metastatic melanoma. Here we show an upregulation of IL4I1 expression in a fraction of Mφ in the course of RET melanoma progression.
2. Mechanism of IL4I1 induction in melanoma
To decipher the mechanism involved in IL4I1 expression in the tumor microenvironment, Mφ were cultured in presence of soluble factors derived from RET primary tumor. Among those, only recombinant TNF-α strongly induced IL4I1 in Mφ *in vitro*. Furthermore, TNF-α neutralization *in vivo* decreased IL4I1 expression by melanoma-associated Mφ in RET mice. Taken together, our data revealed a role of TNF-α in driving IL4I1 expression.
3. Mφ-specific deletion of IL4I1 delays melanoma growth
Next, we set-up conditional mice in which IL4I1 is specifically inactivated in Mφ. After transplantation with B16 melanoma cells, IL4I1$^{M\varphi KO}$ mice exhibited a reduced tumor growth compared to littermates. The incidence of both primary melanoma and cutaneous metastasis was also significantly delayed in RET-IL4I1$^{M\varphi KO}$ mice compared to RET control mice, highlighting the pro-tumoral properties of IL4I1^{+} Mφ.
4. IL4I1 deficiency favors anti-tumor properties of tumor-infiltrating T cells and reprograms Mφ toward an anti-tumoral phenotype.
The delay of tumor development in RET-IL4I1$^{M\varphi KO}$ mice is associated with reduced expression of exhaustion markers by tumor-infiltrating CD8^{+} T cells. Moreover, the genetic inactivation of *il4i1* in Mφ strongly decreased pro-tumoral markers expressed by Mφ themselves, but also permits to these Mφ to acquire anti-tumoral properties.
Conclusion: Our data reveal the key role of IL4I1^{+} tumor-associated Mφ in tumor aggressiveness through altered anti-tumoral functions of Mφ and CD8^{+} T cells. As IL4I1 is expressed by Mφ in most of human solid tumors, this study paves the way for targeting specific Mφ subsets to improve anti-cancer immunotherapies.

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2044 – WS61.4
TNFR2 expression by Tregs is critical for suppressing tumor immunity by regulating inflammatory cytokines

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Foxp3+ regulatory T cells (Tregs) are immunosuppressive cells that inhibit tumor immunity. Their mechanism of action is complex and only partially understood. Here, we show that expression of the tumor necrosis factor (TNF) receptor type 2 (TNFR2) by Tregs is essential for limiting anti-tumor immunity. Genetic and inducible ablation of TNFR2 in Tregs leads to a reduction in tumor growth within a few days, which is associated with a rapid and by-stander effect on tumor-infiltrating T cells and myeloid cells. This anti-tumor effect has been confirmed in different mouse models. Mechanistically, Tregs and conventional T cells produce more TNF and IFNg, while monocytes, macrophages and neutrophils are more activated and produce increased levels of iNOS. Experiments using effector or blocking reagents show a direct anti-tumor effect of TNF and iNOS, which was confirmed by scRNAseq analyses. In conclusion, our work surprisingly shows that one of the main mechanisms of suppression of anti-tumor immunity mediated by Tregs depends on their expression of TNFR2. Tregs expressing TNFR2 limit tumor rejection by inhibiting TNF and iNOS production.

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1344 – WS61.5

A Second Tumor Escape System mediated by HHLA2: Biological and Therapeutic Implications on effector cells.

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Purpose: HHLA2, a newly discovered B7 family's member has emerged as a promising target in cancer immunotherapy. Its upregulation in many cancers, and notably in PD-L1 negative tumoral cells suggests a potential role in tumour evasion through its inhibitory receptor KIR3DL3. But KIR3DL3 is tough to highlight at mRNA and protein levels. HHLA2 also binds CD28H activating receptor on NK and T cells, although CD28H and KIR3DL3 have non-identical fixation sites on HHLA2. We investigated a new HHLA2 potential tumour immune escape mechanism based on the downmodulation of CD28H activating receptor.
Methods: We developed cell lines (K562 and HEK-T cells) expressing either the full length HHLA2 or HHLA2-IgV1's portion. Both cell lines expressing HHLA2 were able to bind CD28H- and KIR3DL3-recombinant molecules, while the one expressing HHLA2-V1 only binds CD28H-recombinant protein but not KIR3DL3.
Results: We found that HHLA2 expression results in a rapid loss of membrane CD28H on NK and T cells further followed by its loss intracellularly in both cell types. This was shown to be dependant of the HHLA2-CD28H contact and to correlate with the amount of HHLA2 expressed by the cell lines. However, cells expressing only the HHLA2-V1 portion have no impact on CD28H expression even if they were able to bind CD28H. Monensin highlighted the involvement of specific protein transport for CD28H loss in T cell and to a lesser extend in NK cells, while Brefeldin A and Bafilomycin A1 are ineffective for both. Other inhibitors are currently tested to decipher the mechanism underlying CD28H loss. We also recently confirmed these observations with cells naturally expressing HHLA2, i.e. ccRCC cell lines injected subcutaneously into immunodeficient mice, since they only express HHLA2 *in vivo*. A-498 freshly excised tumours showed mRNA expression of HHLA2 and extracted cells bound the recombinant proteins CD28H-Fc and KIR3DL3-Fc. Importantly, co-culture of this cells with T and NK cells also led to downmodulation of CD28H.
Conclusion: Based on these results, we hypothesise that overexpression of HHLA2 by tumour cells would result in the downmodulation of CD28H. We expect our findings to lead to treatment strategies that would preserve the HHLA2-CD28H activating interaction.

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1626 – WS61.6

Investigating the Immunomodulatory Role of Epithelial YAP/TAZ in Gastric Metaplasia

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Purpose: Gastric cancer is one of the leading causes of cancer-related deaths and its prevalence has been associated with infection by *Helicobacter pylori*. *H. pylori* persistence leads to chronic inflammation which can lead to the development of preneoplastic conditions such as gastric metaplasia. This results in the elimination of parietal cells and the appearance of spasmolytic polypeptide/trefoil factor 2-expressing metaplasia (SPEM) cells at the base of the gastric glands which have been linked to tumour development. Initiation and progression of SPEM is immune-driven, with dependence on the activation of key players of type 2 inflammation such as innate lymphoid cells 2 (ILC2) and M2 macrophages. However, processes driving epithelial cell fate and immune status during regeneration of the gastric epithelium remain unclear.
Methods: The transcriptional effectors yes-associated protein 1 (YAP) and transcription coactivator with PDZ-binding motif (TAZ) of the Hippo pathway were conditionally deleted in the gastric epithelium using YAPfl/fl;TAZfl/fl;PGC-CreERT mice (YAP/TAZ KO). YAP/TAZ KO and littermate controls were subjected to high-dose tamoxifen (HDT) treatment to induce tissue damage as well as YAP/TAZ deletion. At various time-points following treatment, stomachs were collected and processed for histological analyses, cytokine multiplex, and immune profiling of the lamina propria using spectral flow cytometry.
Results: We show that epithelial YAP/TAZ play an essential role in controlling cell fate during gastric tissue regeneration in this chemical injury model. Loss of YAP/TAZ in the stomach epithelium resulted in persistence of gastric metaplasia upon tissue injury as well as chronic immune cell infiltration. Immunophenotyping experiments showed that unlike SPEM initiation and progression, tissue regeneration was independent of type 2 immunity. Instead, loss of YAP/TAZ resulted in a tissue destructive response associated with chronic recruitment of macrophages and B cells.
Conclusion:
These results suggest that epithelial YAP/TAZ may control cell fate decision as well as inflammation, highlighting the importance of immune-epithelial cell interactions in tissue regeneration and neoplasia development.

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WS62 – T CELL REGULATION AND FUNCTION III

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664 – WS62.1

Roles and functions of the Foxk1 transcription factor to control T cell responses

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T lymphocytes play a key role in the immune response and their functions are intimately linked to metabolic programs. During immune responses, T cells undergo a metabolic reprogramming characterized by increased aerobic glycolysis, nutrient uptake and macromolecules synthesis. These processes are crucial for cell growth, differentiation, and acquisition of effector functions. In this regard, the AKT-mTOR signalling pathway has been shown to be an essential hub for regulating T cell metabolism and, consequently cell fate.
Using a quantitative phosphoproteomic approach, we have identified a new transcription factor called Foxk1 as being highly phosphorylated in T cells upon T Cell Receptor (TCR) engagement. Our results also indicate that Foxk1 phosphorylation and nuclear translocation is dependent of the AKT-mTOR kinase activities. Using T-cell specific Foxk1 deficient mice ($Foxk1^{-/-}$), we demonstrated that Foxk1 is required for full T cell activation. Foxk1-deficient T cells exhibited reduced proliferation and cytokine secretion following TCR stimulation. Furthermore, T cells from $Foxk1^{-/-}$ mice were less prone to acquire an effector like phenotype than wild-type cells when challenged in vivo.
Conversely, Foxk1 overexpression in T cells enhanced their effector functions in a TCR-dependent manner. In CD8+ T cells, this effect also results into enhanced cancer cell killing capacity *in vitro*, and improved tumor rejection *in vivo*.
Global proteomic analysis of TCR-stimulated CD4+ and CD8+ T cells from Foxk1 deficient mice revealed defective expression of critical proteins involved in metabolism, and in particular of effectors and enzymes of the glycolysis pathway. Accordingly, Foxk1 deficient T cells had reduced maximal mitochondrial respiratory capacity compared to wild-type and exhibited an impaired ability to enhance aerobic glycolysis upon TCR stimulation or glucose supplementation.
Altogether, these results indicated that Foxk1 is a major regulator of T cell metabolism and thus, of T cell effector functions. Its molecular targeting can be envisioned as a part of T-cell reprogramming to fight cancer or reduce auto-immune reactions.

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604 – WS62.2

Characterization of DNA Methylation dynamics during human thymic T lymphocyte development

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The importance of DNA methylation (DNA-meth) for the regulation of the stability and plasticity of mature T cell subsets has been established in recent studies, such as the DNA-meth-regulated activity of the FOXP3-TSDR for the Treg phenotype or the progressive loss of DNA-meth in heterochromatic parts of the genome during differentiation and proliferation of peripheral CD4+ T cells. However, an in-depth understanding of the DNA-meth mediated control of T cell development in the thymus is so far lacking.
To address this lack of knowledge, we sorted 8 defined pediatric human thymic T cell progenitor subsets using a 19-color flow-cytometry panel and generated genome-wide DNA-meth profiles. Analysis of these datasets revealed that major DNA-meth remodeling occurs at two distinct developmental steps: from progenitors (DN1 and DN2) to the rearrangement populations (DN3, ISP4, early DP and late DP) and subsequently to mature T cells (SP4 or SP8). In addition, individual progenitor and mature T cell populations display distinct, cell-type specific DNA-meth signatures, indicating that DNA-meth mediated gene expression control is a driver of the ongoing developmental processes. In contrast, the DNA-meth landscape among rearrangement populations does not change significantly. To our surprise, the DNA-meth landscape in the heterochromatin remained stable throughout thymic differentiation of T cells, in contrast to what has been observed in mature peripheral T cells. Finally, we used our identified DNA-meth profiles of ex vivo isolated human thymocytes to verify the success of human T cell development in a humanized mouse model. We could show that populations from both systems cluster according to their common cellular phenotype, confirming that, in this model, the transplanted human hematopoietic stem cells (HSCs) undergo appropriate T cell development in the murine thymus. Still, big differences on the mean DNA-meth level could be observed, indicating substantial DNA-meth alterations during HSC-derived thymic precursor development.
Taken together, our data revealed a dynamic involvement of DNA-meth mediated mechanisms to the appropriate regulation of the complex T cell developmental cascade. Further, the generated reference DNA methylomes of thymic T cell subsets allow the validation of in vivo and in vitro model systems for human T cell development.

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1680 – WS62.3

Not so TOP after all: cytokine 3'untranslated regions dictate responsiveness to mTOR-mediated translation in human T cells

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T cells are key contributors to clear our body from infected and malignant cells. When T cells respond to target cells, they undergo profound transcriptional and translational alterations. A key regulator of the protein turnover and degradation is the evolutionary highly conserved kinase mechanistic target of rapamycin (mTOR). mTOR signalling acts on T cell differentiation and homeostasis. It also supports T cell activation, and promotes the production of effector molecules, such as the key pro-inflammatory cytokines IFNγ, TNFα and IL-2. In this study, we uncovered a novel pathway that mTOR employs to regulate cytokine production in primary human T cells. Instead of the well-known 5' Untranslated region (5'UTR)-mediated mTOR regulation through TOP motifs, we found that mTOR inhibition reduces the expression of IFNγ, TNFα and IL-2 in a 3'UTR-dependent manner. Mutation studies revealed that, albeit the presence of non-classical TOP motifs in the cytokine 3'UTRs, mTOR-mediated regulation acts through AU rich elements (ARE). RNA binding protein (RBP) pulldown essays from activated T cells uncovered that LSM7 and DDX21 interact with the *IL2* 3'UTR in an ARE-dependent manner, and that this interaction is lost upon mTOR blockade with Torin. Concordantly, LSM7 or DDX21 deficiency in primary human T cells leads to amplified cytokine expression. Mechanistically, mTOR inhibition does not affect the stability of cytokine mRNA, but rather appears to induce alternative adenylation. In conclusion, we uncovered a novel mode of action of mTOR signalling modulating cytokine production through the 3'UTR.

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1872 – WS62.4

A truncated LAT isoform lacking the 5 distal tyrosines still allows the development of gamma delta T cells

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Purpose: Previous results generated by our group showed that mutation of a negatively charged segment of LAT (Linker for the Activation of T cells) induced an increase of proximal intracellular signals, but downstream signals such as Ca^{2+} influx or MAPK pathways were partially inhibited. We therefore decided to generate the corresponding knockin strain of mice (LAT-NIL), in order to analyze the impact of these mutations on thymic development and T-cell functions.
Methods: LAT-NIL knockin mice were generated by means of a CRISPR/Cas9 nuclease approach for direct mutagenesis of Lat exon 7 sequence using an ssODN template harboring the desired mutations. The phenotype of homozygous and heterozygous mutant mice was analyzed by flow cytometry, using wild-type mice as controls.
Results: Homozygous LAT-NIL mice showed an almost total block of thymic development at the DN3 stage. RT-PCR performed with RNA obtained from thymocytes of LAT-NIL mice showed that the cDNA is shorter than WT-cDNA, and sequencing of this cDNA showed that exons 6 and 7 were deleted during the RNA maturation. This generated a frameshift introducing a premature STOP codon, which generated a shorter form of LAT adaptor (145 residues, with the last 39 coming from a frameshift) lacking the essential 5 distal tyrosines. Although we have not been able to detect the presence of this truncated LAT protein in LAT-NIL thymocytes, the analysis of spleens and lymph nodes from LAT-NIL mice always had a small population of gamma-delta CD3+CD8+ T cells, although they were not able to proliferate or activate.
Conclusion: Overall, our data reveal that the LAT adaptor is a crucial element in the TCR and pre-TCR signaling cassettes, and that a truncated form of LAT is not able to promote the development of alpha-beta T cells, although it does allow the development of a population of gamma delta, CD8 positive T cells.

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1097 – WS62.5

ISG20L2 is a novel RNA-based regulator of T cell function

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Purpose: We aim to understand the functional role of ISG20L2, a 3' to 5' exoribonuclease, in T cell function.
Methods: Pull down assay with biotinylated miRNAs, recombinant protein expression, miRNA degradation assays, primary human and mouse T cell isolation and culture, Jurkat T cell KO clones and ISG20L2-deficient T cell mouse models generated by CRISPR-Cas9, messenger and small RNAseq, qPCR, flow cytometry, western blot and confocal microscopy
Results: ISG20L2 appears to play a crucial role in CD4 T cell function by the degradation of a specific repertoire of RNAs. ISG20L2 expression is modulated by TCR engagement and IFN I stimulation and its silencing leads to increased basal expression of CD69 and IL2 secretion. However, its absence impairs several key processes during immune synapsis, including CD25 upregulation, CD3 synaptic accumulation, and MTOC translocation towards the antigen-presenting cell during immune synapsis. Additionally, ISG20L2 regulates the expression of multiple immunoregulatory molecules such as AHR, NKG2D, CTLA-4, CD137, TIM-3, PD-L1, and PD-1. Knockout of ISG20L2 results in increased levels of these molecules in Jurkat T cells, suggesting its role in immune regulation. This effect is further supported by the upregulation of CD137 and PD-1 on the cell surface of activated human primary cells lacking ISG20L2.
The essential physiological function of ISG20L2 is highlighted by the fact that global knockout of ISG20L2 in mice is not viable. Initial analysis of a mouse model with conditional knockout of ISG20L2 in CD4-positive cells confirms its involvement in modulating the expression of CD3 and the repertoire of immunoregulatory molecules. This suggests that ISG20L2 is crucial for orchestrating immune responses and maintaining immune homeostasis.
Conclusion: The dysregulation observed in key molecules for T cell responses supports a role for ISG20L2 as a novel RNA-based regulator of T cell function.

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99 – WS62.6

S1P-signaling inhibition suppresses Th1-like Treg generation by rewiring mitochondrial metabolism

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Inflammatory environments are known to induce Treg plasticity, i.e., the generation of dysfunctional IFNγ+Tbet+ Th1-like Tregs. Th1-like Tregs show defective function and increased frequencies in autoimmune conditions including multiple sclerosis (MS). We previously identified the PI3K/Akt/FoxO pathway as a main regulator of Th1-like Treg plasticity, but the pathways restraining the generation of dysfunctional Th1-like Tregs are mostly unknown. Fingolimod is a functional agonist of sphingosine-1-phosphate (S1P) receptors, blocking lymphocyte egress from secondary lymph organs and used to treat MS. Data suggest that fingolimod affects immune cells beyond migratory properties, including increasing Treg proportion in treated MS patients. Preliminary data suggest that Th1-like Tregs upregulate several genes involved in S1P signaling. However, the effects of S1P signaling on Treg stability is unknown. Here we show that S1P signaling inhibition inhibits the generation of Th1-like Tregs and rescues their suppressive function. This is mediated by a decrease in mTOR and S6 phosphorylation and is reproduced by mTORC1 inhibition with rapamycin. Because mTOR plays an important role in immunometabolism, we investigated metabolic pathways in Tregs and Th1-like Tregs treated with fingolimod ex vivo and showed that Th1-like Tregs undergo mitochondrial uncoupling that is restored by S1P-signaling inhibition. The in vitro S1P-signaling inhibition results are validated in vivo, as Tregs from fingolimod-treated MS patients display decreased Th1-like Treg frequency, increased suppressive function, and restoration of mitochondrial metabolism.
These results highlight the involvement of mitochondrial metabolism in Treg plasticity and identify S1P-signaling inhibition as a target to suppress the generation of dysfunctional Th1-like Tregs.

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WS63 – GENETIC AND ENVIRONMENTAL TRIGGERS OF AUTOIMMUNITY

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490 – WS63.1

Unraveling the Enigmatic Role of atypical ANCAs: A Novel Pathophysiological Mechanism in Ulcerative Colitis - Targeting Neutrophil Extracellular Traps

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Background and Aims: Ulcerative colitis (UC) is an inflammatory bowel disease characterized by chronic colon inflammation. Neutrophils and neutrophil extracellular traps (NETs) have been linked to UC. Atypical Anti-Neutrophil Cytoplasmatic Antibodies (a-ANCAs) are biomarkers for UC diagnosis, but after decades their target antigens are unknown. This study aims to investigate if these antibodies target NETs and their potential role in UC pathophysiology.
Methods: Serum samples and biopsies from UC patients and healthy volunteers were collected. Neutrophils from healthy controls were induced to form Lytic (PMA) and non-lytic (LPS-treated platelets) NETs. Indirect immunofluorescence (IIF) was used to detect a-ANCAs. Macrophage clearance were measured by IIF and real time live cell imaging and measured cytokine expression via Real-Time Quantitative PCR. Immunohistochemistry was employed to visualize NETs in colon tissue.
Results: a-ANCAs exclusively bound to ethanol-fixed neutrophils, with DNAse or trypsin treatment reducing binding. Binding was DNA-dependent and localized within DNA-protein complexes. Enhanced binding to non-lytic NETs was observed. In inflamed colon tissue, a-ANCAs recognized NETs linked to crypt abscesses. This antibodies impeded NET clearance and triggered pro-inflammatory responses in macrophages. Patients with this antibodies experienced worse UC future outcomes as resistance to treatment, presence of extraintestinal manifestations, or post-surgery activity.
Conclusion: A novel perspective through the discovery of a distinct antibody, Anti-NETs antibodies (ANETAs), which interact with NETs, forming immune complexes that inhibit NETs clearance by macrophages and induce pro-inflammatory responses, potentially contributing to UC pathophysiology. This "Proinflammatory Loop induced by ANETAs" (PLANETAs) mechanism could have implications for UC disease monitoring and therapy development.

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514 – WS63.2

EBV infection accumulates T-bet+ B cells that migrate to the CNS and cause MS-like lymphocyte infiltration in brain tissue of humanized mice

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Epstein-Barr virus (EBV) is a common human γ-herpesvirus, which persistently infects more than 95% of the adult human population. There is strong epidemiological evidence that links EBV infection and its altered immune control to the autoimmune disease multiple sclerosis (MS). However, the mechanism by which EBV alters the immune system to increase MS risk remains largely unknown. Our aim is to study primary EBV infection in an *in-vivo* model to understand how alterations in the immune system during infection and migration of infected B cells might constitute a prerequisite for the development of MS. To that end we use HLA-DRB1*1501(HLA-DR15) and HLA-A*0201(HLA-A2) knock-in immunodeficient mice, carrying the main genetic risk factor of MS, HLA-DR15. Upon reconstitution with human immune system components from HLA-DR15 positive $CD34^+$ hematopoietic progenitor cells we can mimic symptomatic primary EBV infection in these animals.
Primary EBV infection leads to a significant increase of T-bet^+ $CXCR3^+$ atypical B cells (ABCs). This increase of ABC during infection positively corelates with viral burden in the animals. Furthermore, measurement of viral particles and *in-vitro* bioluminescence imaging revealed migration of EBV infected cells to the central nervous system (CNS) in highly infected animals. This migration of EBV infected cells is accompanied by the infiltration of ABCs and activated lymphocytes into the CNS. Single cell RNA sequencing indicated that a large population of the ABCs is a clonally expanded reservoir for EBV and shows upregulation of chemotaxis genes such as CCL3. Transfer of T-bet+ EBV transformed lymphoblastoid cell line into non-reconstituted mice confirmed that those cells can migrate into the CNS independently of other lymphocytes and are *ex-vivo* able to attract activated T cell.
These findings suggest that EBV infection drives the generation of a clonally expanded $CXCR3^+$ T-bet+ B cell population which can migrate into the CNS and might attract activated T cells. In certain predisposed individuals this could lead to the infiltration and local stimulation of cross-reactive and MS autoantigen specific T cells that potentially triggers MS pathology.

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1559 – WS63.3

Human genetic and environmental factors affect type I interferon protein production in a pathway specific manner.

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Human antiviral immunity is highly variable with significant consequences for disease susceptibility; yet the factors that contribute to such variation remain poorly understood. Here, we used samples and data from 1000 donors of the Milieu Interieur cohort to investigate factors associated with variation in the antiviral type-I interferon (IFN-I) response. We used ultrasensitive digital ELISAs (Simoa) to quantify IFNa and IFNb protein secretion in response to stimulation with agonists that activate antiviral TLRs including TLR3/MDA5 (polyIC), TLR7 (Gardiquimod), TLR7/8 (R848), TLR9 (ODN), as well as live influenza A virus (IAV) and Sendai virus. Using linear modelling controlling for age, we found that females produced more IFN-I in response to TLR7/8, IAV and Sendai virus, while males exhibited a stronger TLR3/IFIH1 response. We did not observe a sex difference in the TLR9 response. In response to all stimuli, we found that younger donors produced more IFNa and IFNb in response to stimulation compared with older donors. By testing 136 demographic variables we found strong environmental associations for the IAV IFN-I response including vaccine history, influenza antibody titre and serostatus. We also identified associations for smoking specifically with TLR7, but not TLR8, and between BMI, abdominal circumference and weight and the TLR3/IFIH1 IFNb response. To test for potential genetic associations we performed genome wide association studies of IFN-I responses, including age, sex, pDC and CD14+ monocyte counts as well as genetic PCs as covariates. We found that genetic variation in *IKZF1* is significantly associated with TLR7, TLR8 and TLR9 responses. The variants we identified in IKZF1 are associated with decreased IFN-I responses and are response quantitative trait loci for IKZF1 expression. Many of these variants have previously been associated with increased risk of autoimmune disease including systemic lupus erythematosus. We also found variants in *IRF7* that are associated with the response to TLR3/IFIH1, and in *SIGLEC14* and *LILRB2* that are associated with the TLR9 IFNa response. Ongoing work is focused on analysing sex hormone and sex chromosome effects, to understand what are the drivers of sex differences in IFN-I responses, and on performing fine mapping to identify causal variants in our analysis.

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572 – WS63.4

The T cell response against the EBV is shaped by HLA risk alleles in multiple sclerosis

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Purpose: Epstein-Barr virus (EBV) infection and the presence of HLA risk alleles are the major environmental and genetic risk factors for the development of multiple sclerosis (MS), respectively. We hypothesize that MS-related HLA (super)types present different EBV epitopes on EBV-infected B cells, resulting in an altered anti-EBV response by T cells. In this study, we aimed to link these environmental and genetic risk factors in MS by performing in depth characterization $CD8^+$ T cells in the context of risk and protective HLA alleles in MS.
Methods: For the characterization of EBV-specific $CD8^+$ T cells, 55 natalizumab-treated MS patients and 54 healthy controls were screened for presence of disease protective HLA-A*02 and risk HLA-B*07 allele using flow cytometry. MHC class I-coupled EBV and CMV peptides (n=10) were tetramerized with fluorochrome labeled streptavidins for separate detection of virus-specific T cells. The tetramers were included within an elaborate 38-color spectral flow cytometry panel including lymphocyte lineage markers, chemokine receptors, co-inhibitory and co-stimulatory receptors and brain-homing associated markers.
Results: HLA-A*02^+-restricted $CD8^+$ T cells were directed towards a wide variety of latent and lytic EBV peptides, while EBV-specific $CD8^+$ T cells restricted to HLA-B*07 were only found for the EBNA3A peptide. Using unsupervised clustering, EBV-specific $CD8^+$ T cells showed differential marker expression between HLA-A*02 and HLA-B*07 restricted cells. Interestingly, co-expression of co-inhibitory receptors TIGIT, CD160, KLRG1, PD1 and 2B4, was most pronounced on HLA-B*07-restricted EBV-specific $CD8^+$ T cells in MS patients, pointing towards a more exhausted phenotype. Additionally, brain homing and tissue residency-associated markers were found on these cells. CD20 was increased on EBV-specific T cells compared to other effector memory $CD8^+$ T cells and CCR5, similarly to the co-inhibitory receptors, was elevated especially on HLA-B*07-restricted EBV-specific $CD8^+$ T cells in MS patients.
Conclusion: Together, we show a link between HLA genetic risk and the T-cell response against EBV in MS. Our findings reveal a distinct phenotype of EBV-specific $CD8^+$ T cells, which is influenced by disease-related HLA alleles.

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1362 – WS63.5

Integration of in silico analysis and in vitro functional analysis reveals Tissue Factor Pathway Inhibitor 2 (TFPI2) as a master transcriptional regulator and potential target to impede infliximab resistance in adult ulcerative colitis patients.

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Purpose: Infliximab (IFX), an anti-TNF-α targeting agent, is used as the standard biological therapy for moderate-severe cases of ulcerative colitis (UC). However, approximately 40% of patients do not respond to IFX. While transcriptional alterations have been widely associated with non-response to IFX, the precise factors that regulate this remain poorly understood. The aim of this study was to perform a robust *in silico* analysis to identify master transcriptional regulators (MTRs) which regulate gene expression changes in IFX non-responsive UC patients, followed by their investigation in an *in vitro* model to establish their functional role in UC pathogenesis.
Methods: Differentially expressed genes (DEGs) identified from four independent public datasets were applied to the GeneXplain platform to identify transcription factors (TFs) enriched for the DEGs. This was followed by an upstream analysis to identify MTRs which regulate expression of these genes through the TFs. Finally, in a novel IBD-like *in vitro* system comprising of normal rectal epithelial cells treated with TNF-α, the role of these MTRs in driving an UC-associated phenotype was explored.
Results: Over 200 TFs were identified in both upregulated and downregulated genes in IFX non-responders across all data sets. RELA, NANOG, and FOSJUN were the top 3 TFs enriched for upregulated genes in three out of the four datasets. Using the TF data, the upstream analysis identified CXCL8, SELE, PTGS2, FCGR3, and TFPI2 as the top five MTRs. Furthermore, of the five MTRs, only TFPI2 was significantly upregulated in our IBD-like *in vitro* model. siRNA-mediated knockdown of TFPI2 in this model resulted in a significant decrease in genes including *TNFAIP6, TNFAIP3, FCGR3B, BIRC3* and *CXCL5*, all of which were predicted *in silico* to be upregulated by TFPI2. Intriguingly, downregulation of TFPI2 resulted in significant amelioration of pro-inflammatory cytokines including IL-1β, IL-10, IL-6 and IL-8.
Conclusion: This study for the first time identifies key MTRs, such as TFPI2, which act as master regulators of genes whose upregulation drives poor response to IFX. Therefore, these results indicate these MTRs, particularly TFPI2, as potential therapeutic targets whose perturbation in UC patients would likely result in improved response to IFX.

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1495 – WS63.6

Maternal autoimmunity drives autoreactivity and potential neuropsychiatric sequelae in offspring independently of genetic factors

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Systemic lupus erythematosus (SLE) is a chronic autoimmune disease, targeting multiple organs, including the kidneys, skin, and lungs. However, up to 75% of patients experience neuropsychiatric manifestations ranging from headache to cognitive impairment and psychosis. This is proposed to be caused by cross-reactive autoantibodies targeting the cells of the CNS.
Particularly children of autoimmune mothers are at high risk of developing autism and learning disorders, since maternal transfer of autoantibodies, combined with genetic predisposition, can negatively impact the development and health of the offspring. Here, we used embryo transfers to examine the maternofoetal transfer of autoantibodies and immune activation in offspring, independently of genetic factors.
Healthy C57BL/6J embryos were implanted in either 564Igi females, a murine strain presenting with SLE-like disease due to an autoreactive B-cell receptor knock-in, or to healthy C57BL/6J females as controls.
Maternal transfer and endogenous production of both total and autoreactive antibodies were measured using solid-phase assays. Immune cell populations were analysed by flow cytometry, and the cortical microglia morphology along with astrocytic GFAP expression was visualized using immunohistochemistry and confocal microscopy and subsequently assessed via an image analysis pipeline.
Increased maternally transferred and endogenously produced (auto)antibody levels were detected in offspring born to autoimmune mothers, along with increased germinal center B cell populations in lymph nodes and spleen. However, no significant change in microglia morphology was observed between embryo transfer offspring, mothers, or controls.
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WS64 – REGULATORY T CELLS: DIFFERENTATION AND REGULATION

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115 – WS64.1

Selective ablation of thymic and peripheral Foxp3+ regulatory T cell development

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Foxp3$^+$ regulatory T (Treg) cells of thymic (tTreg) and peripheral (pTreg) developmental origin are thought to synergistically act to ensure immune homeostasis, with self-reactive tTreg cells primarily constraining autoimmune responses. Here we exploited a Foxp3-dependent reporter with thymus-specific GFP/Cre activity to selectively ablate either tTreg (ΔtTreg) or pTreg (ΔpTreg) cell development, while sparing the respective sister populations. We found that, in contrast to the tTreg cell behavior in ΔpTreg mice, pTreg cells acquired a highly activated suppressor phenotype and replenished the Treg cell pool of ΔtTreg mice on a non-autoimmune C57BL/6 background. Despite the absence of tTreg cells, pTreg cells prevented early mortality and fatal autoimmunity commonly observed in Foxp3-deficient models of complete Treg cell deficiency, and largely maintained immune tolerance even as the ΔtTreg mice aged. However, only two generations of backcrossing to the autoimmune-prone non-obese diabetic (NOD) background were sufficient to cause severe disease lethality associated with different, partially overlapping patterns of organ-specific autoimmunity. This included a particularly severe form of autoimmune diabetes characterized by an early onset and abrogation of the sex bias usually observed in the NOD mouse model of human type 1 diabetes. Genetic association studies further allowed us to define a small set of autoimmune risk loci sufficient to promote β cell autoimmunity, including genes known to impinge on Treg cell biology. Overall, these studies show an unexpectedly high functional adaptability of pTreg cells, emphasizing their important role as mediators of bystander effects to ensure self-tolerance.

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161 – WS64.2

The activation and functional states of TIGIT+CD226+ Regulatory T cells (Tregs) and their role in autoimmunity

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Foxp3+ regulatory T cells (Tregs) are crucial in maintaining immune tolerance and preventing autoimmunity, with TIGIT an important co-inhibitory receptor in Treg suppressive function. Sharing the same ligand (CD155/PVR), the effect of co-stimulatory CD226 expression on Tregs is less well defined. Furthermore, variable expression of TIGIT and CD226 on CD4+ T cells and Tregs have been linked to worsened disease activity in various inflammatory conditions, suggesting altered expression patterns impact regulatory maintenance.
Here we investigated the co-expression of TIGIT and CD226 on Tregs and effector T cells in autoimmunity, through spectral flow cytometry analysis of peripheral blood (PB), and synovial fluid (SF) of inflamed joints, from individuals with the childhood-onset condition Juvenile Idiopathic Arthritis (JIA). Synovial Tregs had increased co-expression compared to PB in JIA (mean %TIGIT+CD226+ of Foxp3+, SF 60.2% vs PB 19.4%, $p<0.0001$). Furthermore, a double positive Treg subset was detected in PB of individuals with clinically active JIA but was near absent in children who were clinically inactive. This suggests co-expression of these co-receptors could represent altered Treg response to certain inflammatory stimuli. Indeed, TIGIT+CD226+ Tregs from both SF and PB displayed a memory phenotype (>90% CD45RA-) with a heightened activation state, through increased expression of CD71, 4-1BB, PD-1 and CD39.
To further delineate the properties of TIGIT+CD226+ Tregs, we stimulated primary CD4+ T cells from healthy individuals *in vitro* with anti-CD3/CD28 beads or artificial antigen presenting cells and assessed Foxp3+ Treg phenotype. The percentage of TIGIT+CD226+ Tregs increased over time (mean 16.1% to 27.4% from day 1-4) with high expression of TNF superfamily receptors/ligands GITR, TNFR2, OX40, CD40L and 4-1BB found predominantly on CD226+ Tregs. Whilst TIGIT expression appeared key in maintaining CD25 and CD39 expression on CD226+ Tregs, TIGIT+CD226+ Tregs had reduced Foxp3 and Helios median fluorescence intensity (MFI) compared to TIGIT+CD226-, suggesting altered transcription factor-dependent profile.
We therefore propose TIGIT+CD226+ co-expression represents a highly activated Treg state in response to continuous inflammatory stimuli, such as in the inflamed joint. However, this state could represent altered functional stability of Tregs and could present as a possible target in monitoring or restoring tolerance in autoimmunity.

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575 – WS64.3

A binary Cre transgenic approach to study the differentiation and function of tissue-Treg cells

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Foxp3-expressing regulatory T cells (Treg) are critical for immune homeostasis by restraining excessive inflammation. Treg cells located in non-lymphoid tissues (tissue-Treg cells) possess not only immune regulatory but also tissue repair and regeneration functions, such as supporting insulin sensitivity in the adipose tissue and the regeneration of injured muscle or brain. To perform these functions, tissue-Treg cells need to undergo a tissue adaptation process and are characterized, e.g., by the expression of the interleukin 33 (IL-33) receptor (ST2) and killer cell lectin-like receptor subfamily G1 (KLRG1). We have recently characterized the differentiation process of tissue-Treg adaptation as a two-stage process starting in the secondary lymphoid organs and compared human and mouse tissue-Treg cell differentiation (Delacher et al. Immunity 2020; Delacher et al. Immunity 2021). Still, the final steps of tissue adaptation within tissues as well as pathways required for the maintenance of tissue-Treg cells remain largely unknown. Even though single-promoter-driven Cre systems provide a powerful tool to study the role of individual genes involved in differentiation and function, they are often not exclusive for cell types, lineage subtypes or differentiation stages. Therefore, current Cre drivers are not able to distinguish between lymphoid-Treg and tissue-localized Treg cells. To overcome this limitation, we developed a binary transgenic intein-mediated Split-Cre system using complementation-competent C-terminal-Cre and N-terminal-Cre domains, whose expression is driven by two separate promoters: Foxp3-N-Cre and Klrg1-C-Cre. This approach results in the reconstitution of a functional Cre recombinase specifically in tissue-Treg cells as demonstrated by reporter expression and specific gene deletion of floxed targeted genes. Overall, we developed a novel intersectional genetic method that allows specific targeting of tissue-Treg cells, which will help to gain a deeper understanding of Treg cell tissue adaptation and their function.

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1127 – WS64.4

CREB regulates Foxp3+ST-2+ Tregs with enhanced IL-10 production

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Aim: Regulatory T-cells (T_{regs}) are characterized by the expression of Foxp3, a master regulator involved in the development and function of T_{regs}. Foxp3 expression is dependent on activity of the Treg specific demethylated site (TSDR), which contains a CREB binding site. We aimed to find out how Foxp3 specific CREB deletion affects T_{reg} expression and function.
Materials and methods: T_{regs} from *Foxp3^{cre}CREB$^{fl/fl}$* mice and wildtype mice were analyzed by flow cytometry. Cytokine analysis was performed by flow cytometry, ELISA and RT-qPCR. Gene expression analysis was performed using Affymetrix HTA2 assays, ATAC-sequencing, and Methylation-assays. For functional relevance, a CD4 T cell mediated transfer colitis was performed.
Results and Discussion: *Foxp3^{cre}CREB$^{fl/fl}$* mice showed increased frequencies of T_{regs} (CD25+/Foxp3+) in thymus, spleen and peripheral lymph nodes and in non-lymphoid organs including lung and colon, but decreased Foxp3 expression at the single cell level. Despite decreased Foxp3 expression, enhanced expression of the IL-33 receptor (ST-2), IL-10, IL-13, and CREM was observed. CREB deficient T_{regs} were highly suppressive *in vitro* and prevented disease activity in a CD4 T cell mediated transfer colitis in an IL-10 dependent way. At steady state conditions, increased ST-2 expression was found in T_{regs} of spleen, thymus, mesenteric lymph node, lung and colon of *Foxp3^{cre}CREB$^{fl/fl}$ROSA^{RFP}* mice compared to WT mice. *In vitro* expansion of T_{regs} revealed that *Foxp3^{cre}CREB$^{fl/fl}$ROSA^{RFP}* T_{regs} have higher expansion capacity compared to wild type T_{regs}, but with lower proliferation. In non-expanded cells no differences were found between the WT and *Foxp3^{cre}CREB$^{fl/fl}$ROSA^{RFP}* T_{regs} with regard to cell proliferation and apoptosis. Mechanistically, in co-operation with CREM, CREB expression in T_{regs} alters chromatin accessibility to the ST-2 region and thereby influences T cell specific immune responses mediated by IL-10.

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16 – WS64.5

Tachykinin receptor 1 antagonism promotes the Foxp3+ regulatory CD4 T cells and controls gut inflammation and autoimmunity

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Purpose: Neuroimmune communication at the gut-brain axis regulates gut inflammation and autoimmunity. Tachykinin receptors (TACRs) are known to express on neuronal and non-neuronal cells, and their expression is altered during inflammation. How TACRs signaling changes the phenotype and function of CD4 T cells and controls gut inflammation is not clearly understood.
Methods. Acute colitis was induced in naïve C57BL/6 mice by giving dextran sodium sulfate (DSS; 2% w/v) in drinking water. TACR expression on various tissues and immune cells was analyzed using quantitative RT-PCR, immunofluorescence staining, and multicolor spectral flow cytometry.
Results. Colonic biopsy samples of inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS) patients showed increased TACR1 and TACR2 expression compared to control individuals. In DSS-induced colitis, treatment with TACR1 antagonist (CP96345) reduced the gut inflammation and colitis. TACR2 and TACR3 antagonists (Osanetant) did not affect the colitis. Mice with DSS-induced gut inflammation showed significantly increased TACR1 expression in the colon and secondary lymphoid tissues compared to control mice. Multicolor spectral flow cytometry analysis showed that antagonizing the TACRs significantly reduced the frequency of effector CD4 T cells ($CD4^+CD44^+ROR\gamma t^+$) and promoted regulatory CD4 T cells ($CD4^+Foxp3^+CD73^+CD62L^+LAP1^+$) in the Peyer's patch compared to control mice. Further, CD4 T cells expressed TACR1, and antagonizing TACR1 during *in vitro* differentiation promoted the $Foxp3^+$ Treg differentiation. Multicolor spectral flow cytometry and tSNE analysis showed that antagonizing TACR1 gives a unique cluster of suppressive $Foxp3^+$Tregs. Adoptive transfer of TACR1 antagonist-treated Tregs showed significantly more stable and suppressed effector CD4 T cell-induced experimental colitis in immunodeficient mice.
Conclusion. The present work showed that tachykinin antagonism promotes the differentiation of regulatory CD4 T cells. We provided a new mechanism of TACRs-based strategy to control gut inflammation and treat gut-associated inflammatory diseases in clinics.

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512 – WS64.6

Ontogeny and functions of peripheral regulatory CD4 T cells

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Regulatory CD4 T (Treg) cells are key players in the prevention of autoimmune diseases. Treg cells include T Lymphocytes that are naturally produced in the thymus, called thymic Treg (tTreg) cells, and cells that have differentiated from naive CD4 T (CD4 T_N) cells in secondary lymphoid organs (SLOs) called peripheral Treg (pTreg) cells. Although they share a similar phenotype (characterized in particular by the expression of the transcription factor Foxp3), they are only partially redundant in inhibiting immune responses. Our aim is to gain a better understanding of the biology of pTreg cell subsets to obtain clues for manipulating their generation or their function in pathological contexts.
To explore pTreg cell generation, *in vitro* Treg cell polarisation assay were performed and the heterogeneity of the Treg cells obtained was assessed by scRNAseq. T-cell specific deficient mice were generated and characterized.
Our results demonstrate that pTreg cell generation is controlled by both the CNS1 enhancer in the Foxp3 gene and by 2 members of the Foxo transcription factor family. While in the absence of either of these elements, CD4 T_N cells are still able to differentiate into pTreg cells, their combined lack abolishes this ability suggesting that some Treg cells arise through Smad dependent mechanisms and others from Foxo dependent signaling. This heterogeneity has been confirmed in a scRNAseq experiment performed on Treg cells induced *in vitro* from WT, $CNS1^{KO}$ and $Foxo1/3^{KO}$ CD4 T_N cells. These results led us to generate $CNS1^{WT}$ and $CNS1^{KO}$ mice lacking Foxo1, Foxo3 or both. While deficiencies in Foxo1 or Foxo3 alone is not deleterious to the mice, the deletion of both leads to the development of a multi-organ autoimmune disease that is further exacerbated by an additional CNS1 deficiency. Consistently, Treg cell numbers in these various deficient mice are gradually reduced with deletions of Foxo1, Foxo3 and CNS1. Taken together, these results tend to demonstrate the existence of a heterogeneity, or even a dichotomy, of Treg cells induced from CD4 T_N cells.
This study reveals the complementary roles of the Foxo transcription factor and of the regulatory element CNS1 in pTreg cell ontogeny and heterogeneity.

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WS65 – ADOPTIVE T CELL THERAPY

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2063 – WS65.1

Advancing CD8+ Treg cell therapy to the clinic for the treatment of kidney transplanted patients

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Purpose: CD8+ Treg cell therapy is effective in preclinical models but has never been evaluated in a clinical trial. We designed a phase 1/2a clinical trial of cell therapy using GMP manufactured autologous polyclonal CD8+ Tregs to treat kidney transplanted patients from living donors.
Methods: CD8+ cells were isolated from peripheral blood by Clinimacs system, then CD8+CD4-CD45RClow/-CD56- cells were sorted by MACSQuant Tyto cell sorter. Cells were stimulated weekly with anti-CD3 and CD28 mAbs and cultured for 3 weeks in X vivo 15 supplemented with human serum, rapamycin, IL-2 and IL-15. Cytotoxicity was assessed by Annexin V/DAPI staining and suppressive ability was assessed in vitro and in vivo in NSG mice irradiated at 1.5 Gy and co-injected with human PBMCs.
Results: First, we set up a method for the isolation of CD8+Tregs from peripheral blood of healthy individuals using positive magnetic selection and flow cytometry in a safe closed system. We determined optimal clinical-grade culture conditions to preserve high proliferation rate, baseline phenotypic profile, and suppressive function in vitro and in vivo in a model of acute GVHD in NSG mice. We verified that they were resistant to classic maintenance immunosuppressive drugs, persistent and efficient in vivo but not cytotoxic. The method was validated on cells from patients with renal insufficiency, then transferred in the GMP facility with 3 validation runs demonstrating the phenotypic and functional stability of CD8+ Treg, meeting quality controls and release criteria.
The clinical study is a first-in-human, one-arm, open-label, prospective trial in patients with end-stage chronic renal failure requiring primary kidney transplantation, with 3 escalating doses of CD8+ Tregs administered the day before the transplantation without induction treatment and associated with classic maintenance immunosuppression. Patients are monitored for long-term safety, immunosuppression burden and occurrences of infections. Transcriptomic and proteomic analyses on blood, biopsy, and urine samples will inform on persistence and migration of Tregs to the graft. Recruitment will start mid-2024.
Conclusion: We designed a clinically compatible manufacturing process of CD8+Tregs and the first-in-human clinical trial evaluating the safety and hints of efficacy of CD8+Treg cell therapy in kidney transplant patients.

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1190 – WS65.2

Use of nonengineered multiantigen specific Tissue-resident memory T cells for adoptive cellular therapy in gastrointestinal patients with liver metastases

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Purpose: One of the major challenges for adoptive cell transfer (ACT) is the ability of T cells to infiltrate the tumor and to be able to persist for a long term. Tissue resident memory (T_{RM}) T cells are endowed with increased functionality and cytotoxicity compared to non-T_{RM} cells. Due to their efficacity and long-term persistence, the use of T_{RM} cells in ACT could be a useful approach.
Methods: Our team developed a reproducible, well-validated protocol for *in vitro* generation of multiantigen specific T_{RM} cells from patients' peripheral blood mononuclear cells (PBMCs). Monocyte-derived dendritic cells were loaded with CD4 and CD8 peptide mix and co-cultured with autologous PBMCs in the presence of T_{RM} cells polarizing cytokine cocktail. This **a**ntigen **s**pecific **T_{RM}** cells (AST_{RM}) generation protocol was tested on a cohort of patients presenting gastrointestinal cancers (cholangiocarcinoma, hepatocellular carcinoma, metastatic colorectal cancer, and stomach cancer).
Results: For the 15 tested patients, a CD4 and CD8 T_{RM} phenotype was observed ($CD69^+CD103^+$) and ($CD69^+CD103^-$). These two T_{RM} subsets highly expressed tissue homing biomarkers ($CXCR6^+CD101^+CD49a^+$). All generated T_{RM} cells highly expressed activation biomarkers and highly expressed PD1 which is a hallmark of T_{RM} cells and not associated with T cell exhaustion. However, no expression of LAG-3 nor TIGIT was observed. Seven of the tested patients (45%) presented TERT specific CD4 T_{RM} cells. NY-ESO1 and TERT specific CD8 T_{RM} cells were also observed. Remarkably, these *in vitro* generated T_{RM} cells were highly functional compared to antigen specific non T_{RM} cells. Over 50% of these generated CD4 and CD8 T_{RM} cells were polyfunctional as they co-produced IFNg, TNFa and IL-2. Interestingly, these T_{RM} cells were also highly cytotoxic as they were able to enact cancer cell lines eradication, mirrored by high expression of Annexin/7AAD.
Conclusion: On the light of these results, our team will be soon conducting a phase Ib proof-of-concept study "MERIT trial" that will allow the investigation for the first time of a novel therapeutic strategy using nonengineered multiantigen-specific T_{RM} cells combined to anti-PD-1 to treat patients with liver metastases.

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294 – WS65.3

Chasing Antigen-Specific T Cells with High Avidity (CATCH)

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Cellular immunity to a specific antigen can be detected and characterized by the analysis of the quantity or functions of specific T cells either isolated *ex vivo* from blood or tissues or from *in vitro* systems. T cells express a highly variable set of specific immune receptors (T cell receptors, TCR) to recognize various peptides mostly in the context of HLA molecules. The strength and duration of the interaction of TCR with peptide-HLA complexes determine initial developmental steps of T cells, but are also decisively pivotal for the activation, following differentiation steps and the maintenance of functionally competent T cells that react efficiently in secondary challenges. For immediate productive immune reactions immune receptors with high affinity for their targets are indispensable, in particular for applications in malignant diseases. We here introduce the **CATCH** assay, the cytometric assessment of the CD3/TCR complex downregulation, as a novel technology to easily characterize functional avidities of T cell receptors directly in a precise and fast manner (Loyal et al., Science, 2021). By combining flow cytometric activation induced marker (AIM) assays with the analysis of CD3 downregulation we demonstrate efficient analysis and isolation of high avidity pathogen and tumor specific T cells within 4 hours covering a broad antigen concentration range. The method can be directly combined with functional analytics, but also be implemented for TCR engineering in human and mice. **CATCH** enables the high throughput *ex vivo* characterization of an individual's or a cohorts' T cell population quality. Integration of the **CATCH** assay into clinical studies and immune checkpoint inhibitor therapies harbors substantial potential for improving diagnosis and treatment success rates.

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437 – WS65.4

Optimized platform for the production of personalized T cells with transgenic TCRs for a chronic lymphocytic leukemia therapy development

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The adoptive transfer of autologous T cells genetically modified to express tumor-specific T cell receptors (TCRs) is a highly personalized therapy in development, facing significant challenges in the time required from identification, design, and manufacturing to patient administration. To address this, we propose to establish optimized methods for neoantigen-specific TCR identification and subsequent induction as transgenic TCRs in T cells. This will facilitate the development of a personalized advanced therapy focused on chronic lymphocytic leukemia (CLL), which is the most common leukemia in adults and has a high incidence in elderly individuals.
The project encompasses three main areas: (i) identification of candidate TCRs; (ii) lentiviral vector production and purification for efficient TCR delivery; and (iii) transgenic TCR transduction and validation. In the first part of the project, we have optimized processes for neoantigen affinity prediction for the HLA molecules and identification of neoantigen-reactive T cells.
Tumoral mutations in CLL patients were identified to determine neoantigens and bioinformatic predictions for both class I and class II HLA molecules were performed using NetMHCpan-4.1 and NetMHCIIpan-4.0, respectively. Additionally, for HLA-I peptides, we applied an immunogenicity prediction using the PRIME2.0 bioinformatic tool. Combining these two algorithms, between 2-5 peptides per patient (per HLA class) were chosen.
Candidate peptides were used to stimulate T cells. A 14-day activation protocol was established using T cells from healthy donors, with efforts to shorten it to 7-10 days and validated using T cells from patients. Direct peptide stimulation of peripheral blood mononuclear cells was compared with stimulation using monocyte-derived dendritic cells. CD154 and CD137 markers were chosen for assessing specific CD4+ and CD8+ T cell activation, respectively. Our results demonstrate successful optimization for identifying neoantigens from CLL tumors and selecting neoantigen-specific T cells.
Ultimately, this study seeks to develop a robust platform for personalized CLL therapy, extendable to other oncological diseases. The findings are expected to contribute to the advancement of cancer immunotherapy and personalized medicine.

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390 – WS65.5

A folate receptor alpha specific, CD28/CD40-based chimeric costimulatory antigen receptor (CoStAR™), enhances anti-tumour activity of T cells and tumour infiltrating lymphocytes.

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Tumour infiltrating lymphocyte (TIL) therapy for treatment of refractory metastatic melanoma, has shown clinical efficacy in a number of clinical trials, and has recently received FDA approval. However, extending the clinical benefit to patients with other cancers has presented a challenge. Insufficient costimulation in the tumour microenvironment can lead to T cell anergy and exhaustion, leading to loss of anti-tumour activity. Here, we describe the construction and functional testing of a chimeric costimulatory antigen receptor (CoStAR) which enhances TCR signals in T cell and TILs upon antigen engagement. CoStAR comprises of a tumour associated antigen specific single chain antibody fragment (scFv) fused to the signalling domains of CD28 and CD40. Transfer of a FRα specific CoStAR to T cells augments T cell activity in a TCR-mediated signal 1 dependent manner. CoStAR also enhanced proliferation, even in the absence of exogenous IL-2. CoStAR activity was dependent on the engagement of surface anchored FRα and neither blocked nor activated by the soluble form, which can be found at high levels in patient serum. Peptide titration assays demonstrated that CoStAR mediated costimulation enhanced overall responses to peptide, but did not lower the activation threshold, a phenomenon shared with physiological costimulation. Using an *in vivo* tumour xenograft model, CoStAR enhanced control of tumour growth, and improved host survival. TIL from multiple cancer indications could be efficiently and reproducibly engineered to express CoStAR, resulting in augmented activity in response to target antigen expressing cell lines and autologous tumour digest. CoStAR represents a novel approach to enhancing TIL activity with synthetic costimulation, in turn increasing anti-tumour activity.

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1954 – WS65.6

Adoptive PD1+ tumor-infiltrating lymphocyte therapy for metastatic TNBC: production results of the safety run-in phase of the TILS001 clinical trial

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Purpose: Compared to other breast cancer types, metastatic triple-negative breast cancer (mTNBC) carries a poor prognosis with rapid progression and lower survival rates. The TILS001 clinical trial (NCT05451784) evaluates the safety, tolerability, and effectiveness of infused PD1+ T-cells (NUMARZU-001). TILS001 is a single-arm, multicenter phase I/II study evaluating PD1+ TILs infusion as a treatment for advanced or mTNBC. The characterization of NUMARZU-001 in the first 3 infused patients is outlined below.
Methods: Three sequential phases before TILs infusion are done. Initially, a molecular prescreening phase is done to evaluate PD1 mRNA expression. Following this, in the prescreening phase, a preexpansion from a lesion is carrying out. Finally, after achieving full expansion of PD1+ TILs, patients undergo a non-myeloablative lymphodepleting chemotherapy regimen, followed by TILs infusion and subcutaneus IL-2 treatment.
Results: At data cut-off (September 2023), molecular prescreening was conducted on 51 pts, with 18 undergoing prescreening, and 3 received treatment. The median age was 49 years. One patient had received 4 prior treatment lines, while the other two had received 2 lines. Preexapnsion and full expansion were done in a centralized way without incidences. CD4+ T cells expansion and enrichment of Central Memory populations were observed.
Conclusion: Overall, our results show that clinical-grade production of TILs for advanced or mTNBC patients are feasible, and that the obtained products meet the current quality standards of the field. NUMARZU-001 has shown a manageable safety profile in the safety run-in phase. Recruitment is ongoing to increase number of treated patients and evaluate its efficacy further.

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WS66 – CANCER IMMUNE EVASION AND RESISTANCE

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247 – WS66.1

Identification of self-reactive T follicular helper cells in ovarian cancer patients

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T follicular helper (Tfh) cells support the process of antibody affinity maturation within germinal centers (GCs), thereby facilitating the selection of protective high-affinity antibody variants. Recent studies demonstrate the presence of B cells and GC in various tumor types correlates strongly with better prognosis and response to immune checkpoint blockade (ICB), yet less is known about Tfh cells and their specificity in this context. Potential sites for Tfh-mediated tumor-specific affinity maturation include GCs within tumor-draining lymph nodes (tdLNs) and GC-like tertiary lymphoid structures (TLS) formed at the tumor site. Recently, our laboratory has identified and characterized MMP14-specific antibody-forming cells in ovarian cancer patients, which arise from T cell-driven antibody affinity maturation processes. These findings prompted us to investigate whether autoreactive T cells within the tumor microenvironment (TME) or tdLN could contribute to B cell selection. To address this, we developed a method to isolate and TCR-sequence self-reactive T cells *ex vivo* from ovarian cancer patients, using MMP14 as a model tumor-associated self-antigen. By performing single-cell genomics on matched tumor and tdLN samples, we can elucidate the nature and origin of the identified MMP14-specific T cells based on their TCR sequences. We found shared and unique cell population between the tdLNs and the TME. Most strikingly, CXCL13-expressing Tfh cells are found solely in the TME of ovarian cancer patients but not in the tdLN.
The identification of autoreactive Tfh cells in our study holds potential for understanding the adaptive immune response in ovarian cancer and the breakdown of tolerance within the TME. Furthermore, these findings may pave the way for novel therapeutic approaches for ovarian cancer patients by enhancing interactions between self-reactive T helper cells and B cells.

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2128 – WS66.2

Probing tumour immune evasion via local immune profiling with fine needle aspirates in hepatocellular carcinoma (HCC)

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Purpose: Harnessing tissue-resident immunity for tumour clearance requires approaches that rescue local effector CD8+ T cells from intrinsic dysfunction, while overcoming extrinsic suppression by regulatory immune counterparts within the tumour niche. Thus, sampling immune cells sequestered in tumours is critical to the discovery of targetable regulatory pathways and subsets. In HCC, diagnostic biopsies often contain a mixture of immune cells from HCC and surrounding liver, and their invasive nature has restricted their application. We hypothesized that fine needle aspiration (FNA) of liver tumours would offer a minimally invasive method to monitor local tissue-compartmentalised immune responses to dissect primary and secondary immunotherapy resistance mechanisms.
Methods: Patients with advanced HCC were consented to provide matched blood, FNA and biopsy. Peripheral blood mononuclear cells (PBMC) from blood (n=25) and tumour infiltrating leukocytes (TIL) from FNA (n= 25) and biopsy (n=15) were isolated for *ex-vivo* spectral multi-parameter flow cytometric characterisation of effector and regulatory immune populations.
Results: FNA reproducibly yielded a broad range of viable immune subsets local to the tumour microenvironment (TME). Crucially, FNA were able to extract tissue-resident $CD8^+$ T cells ($T_{RM,}$ $CD69^+CD103^+CD8$) that could not be sampled in blood. The majority of tumour $CD8^+T_{RM}$ sampled before immunotherapy expressed immune checkpoints including PD-1, Tim-3 and 2B4, accounting for significantly higher expression of these therapeutic targets on the global $CD8^+$ T cells extracted from FNA or biopsies than from blood. Within the myeloid compartment, dendritic cells were reduced whereas granulocytic / polymorphonuclear myeloid derived suppressor cells (g-MDSC/PMN-MDSC) were significantly expanded in tumour FNA compared to blood, allowing characterisation of their dominant homing and immunoregulatory mechanisms to define novel therapeutic targets.
Conclusion: We show that minimally invasive FNAs are capable of comprehensively sampling the distinct immune landscape of HCC compartmentalised at the site of disease, including the expression of key immunotherapeutic targets (T-cell checkpoints and g-MDSC). The resulting data have allowed characterisation of immunotherapeutic targets for future personalised refinement of targeted immunotherapy to overcome tumour immune escape.

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112 – WS66.3

CX3CR1 loss by CD8 tumor infiltrating lymphocytes mediates immune evasion in colorectal cancer

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Purpose: This study aimed to investigate the regulation of CX3CR1 expression on CD8 T cells within the tumor microenvironment (TME) of colorectal cancer (mCRC) liver metastases and its implications for antitumor immunity. Furthermore, immune evasion mechanisms relying on the CX3CL1/CX3CR1 axis were investigated.
Methods: TGF-β-dependent pathways regulating CX3CR1 expression on CD8 T cells were explored in vitro and assessed by Flow Cytometry, RT-qPCR, and Chromatin Immunoprecipitation (ChIP). Peripheral blood mononuclear cells (PBMC) and tumor infiltrating lymphocytes (TILs) from mCRC patients were collected and their TCR diversity was analysed, as well as protein and transcriptomic CX3CR1 and miR-27a-5p expression. A Jurkat T cell-NALM6 in vitro immunological synapse model was developed to analyse mechanisms involving the CX3CL1/CX3CR1 axis in the regulation of T cell activation. **Results:** TGF-β was found to decrease CX3CR1 expression in CD8 T cells in a miR-27a-5p dependant manner independently of SMAD2/3. Our findings, based on mCRC patient-derived samples, underscored a significant variation in CX3CR1 expression between peripheral blood CD8 T cells and their associated TILs from CRC liver metastases. Moreover, cancer patients displayed heightened CX3CR1 expression in their antigen specific peripheral CD8 T cells. Transcriptomic analyses revealed a robust correlation between CX3CR1 expression and cytotoxic effector molecules such as perforin and granzyme. Furthermore, CX3CR1-CX3CL1 binding was found to increase TCR-induced signalling and to enhance effector functions of T cells.
Conclusion: Our results suggest the improvement of T cell activation following CX3CR1 engagement with its ligand CX3CL1. Thus, the loss of CX3CR1 within the TME, driven by TGF-β signalling in a miR-27a-5p dependant manner, should be considered as an immune evasion mechanism in cancer. Understanding the role of CX3CR1 in the modulation of the immunological synapse and its implications for antitumor immunity might lead to the development of new immunotherapeutic strategies.

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775 – WS66.4

IFN-γ predisposes acute myeloid leukemia to therapy resistance

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Acute myeloid leukemia (AML) patients frequently relapse following frontline therapies such as high-intensity chemotherapy and hypomethylating agents. Currently, the mechanisms underlying this therapy resistance remain elusive. We recently discovered that AML blasts present MHC peptides that are recognized by T cells at diagnosis, leading to their activation and subsequent secretion of cytokines. While such a response might help eradicate leukemia, we hypothesized that pro-inflammatory cytokines secreted by immune cells might also contribute to therapy resistance. Therefore, we performed large-scale transcriptomic analyses comparing blasts obtained at diagnosis from patients who either responded or did not respond to conventional cytarabine + anthracycline therapy. Our analyses revealed an upregulation of IFN-γ signaling signatures in patients resistant to therapy. Similar signatures were observed in patients resistant to the hypomethylating agent 5-azacytidine. Consequently, patients expressing high IFN-γ signaling scores at diagnosis had lower survival rates. Additionally, treating multiple AML cell lines with IFN-γ in vitro significantly decreased the cytotoxic activity of high doses of chemotherapeutic agents; treated cells exhibited faster proliferation rates following chemotherapy exposure than untreated cells, suggesting that IFN-γ signaling may accelerate relapse in patients. In conclusion, our findings suggest that inhibiting IFN-γ signaling might help overcome therapy resistance in AML.

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1704 – WS66.5

Lack of TREM2 during anti-PD1 therapy reprograms intestinal macrophages and microbiota to enhance tumor rejection

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Immune checkpoint therapy (ICT) is successfully used to activate anti-tumor T cell responses in the treatment of several types of cancer. However, while many patients respond durably to ICT, a significant number remain unresponsive, prompting the investigation of complementary therapeutic avenues to improve ICT. Reprogramming tumor-associated macrophages (TAMs) by either blocking or deleting the macrophage receptor TREM2 attenuates tumor growth and promotes ICT. Another strategy to improve checkpoint therapy relies on the intestinal microbiota. Here, we found that the synergistic effect of TREM2 deficiency with anti–PD1 is dependent on the microbiota. Anti-PD1 combined with TREM2 deficiency induces proinflammatory programs in intestinal macrophages and a concomitant expansion of *Ruminococcus gnavus* (*R. gnavus*) in the gut microbiota. Gavage of wild-type mice with *R. gnavus* recapitulated enhancement of anti-PD1-mediated tumor elimination occurring in the absence of TREM2. The intestinal proinflammatory environment coincided with expansion, increased circulation, and migration of TNF-producing CD4+ T cells to the tumor bed. Thus, TREM2 remotely controls anti-PD1 checkpoint blockade through modulation of the intestinal immune environment and microbiota; *R. gnavus* is a potential probiotic agent for overcoming resistance to anti-PD1.

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1034 – WS66.6

Liver metastases of colorectal carcinoma contain different subsets of tissue resident memory CD8 lymphocytes correlated with distinct risk of relapse following surgery and mediate immune checkpoint responses

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Purpose: Tissue resident memory (T_{RM}) T cells have emerged as key players in cancer immunosurveillance, and their presence has been linked to a favorable clinical outcome in solid cancer patients. Liver metastases exhibit a highly immunosuppressive tumor microenvironment, however the role and clinical impact of T_{RM} cells infiltration in colorectal cancer remain elusive.
Methods: An exhaustive profiling using multiparametric flow cytometry was conducted on tumor infiltrating lymphocytes isolated from 27 patients' colorectal cancer liver metastases (CRC-LM) and compared to 16 peripheral blood samples of CRC-LM patients. Cytokine production was also evaluated in *in vitro* activated T_{RM} and non-T_{RM} cells. Prognostic value of T_{RM} cells was also assessed in a well-defined cohort of CRC-LM.
Results: Here we identified two subsets of T_{RM} cells expressing CD103 and/or CD69 showing significantly higher expression of tissue residency and activation biomarkers. $CD103^+CD69^+$ T_{RM} cells subset showed almost exclusive expression of tumor reactivity biomarkers PD-1 and CD39. Supporting this observation, $CD103^+CD69^+$ T_{RM} cells showed a more oligoclonal TCR repertoire. Both T_{RM} subsets presented higher cytotoxic and functional capacity compared to non-T_{RM} cells. Our study showed that only the presence of $CD103^+CD69^+$ T_{RM} cells was associated with longer recurrence free survival of colorectal cancer patients with liver metastases. Moreover, treatment with anti-PD-1 and anti-CTLA-4 therapy in CRC murine models resulted in expansion of this intratumoral T_{RM} population.
Conclusion: Taken together, our work demonstrates the existence of a phenotypic heterogeneity of T_{RM} cells in colorectal cancer liver metastases. In this study, we identified a population of $CD103^+CD69^+$ T_{RM} cells exhibiting the characteristics of tumor reactivity and correlated with better patients' prognosis, with potential implication in optimal therapeutic strategies determination.

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WS67 – GLYCOSYLATION

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354 – WS67.1

Investigating the effects of targeting stromal cell sialylation on the tumour immune microenvironment in colorectal cancer

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Purpose: The tumour microenvironment (TME) of colorectal cancer (CRC) contains tumour cells, immune cells and stromal cells. The TME of CRC subtype CMS4 has been associated with poor prognosis, immunosuppression and more recently, sialylation. Sialylation is a post-translational process in which sialic acids (SA's) are added to cell surface glycoproteins forming sialoglycans. Sialoglyans bind immune cell inhibitory receptors called Siglecs, downregulating anti-tumour immunity. In our research, we are investigating the role of sialylation in stromal cell-mediated immunosuppression in CMS4 CRC.
Methods: We performed stromal-immune coculture assays in primary human ex vivo experiments including cancer and normal-associated fibroblasts (CAFs and NAFs) from CRC patients and NK cells. Cocultures included sialyltransferases inhibitor (SI) and sialidase (Sia) targeting of stromal cell sialylation. NK cell Siglec expression and NK cytotoxicity of HCT116 cancer cells were assessed via flow cytometry. We then conducted an in vivo study of stromal-rich CRC including SI and Sia targeting of stromal cells. Tumours, spleens and lymph nodes were analysed via flow cytometry including immune cell panels for T cells, NK cells, macrophages and dendritic cells. Analysis included Siglec expression and activational/functional marker expression in stromal-rich and SA-depleted stromal groups.
Results: Human ex vivo stromal-NK cocultures showed an induction of Siglec 9 on NK cells in the presence of CAFs, which is significantly reduced with targeting of SA on stromal cells. SA removal significantly increased NKG2D activation on NK cells. In vivo, growth of SA-targeted tumours was significantly lower than stromal-rich group. Flow cytometry analysis showed significantly higher numbers of CD49b+ NK cells and CD11b+ macrophages in SA-targeted groups. NK cells had significantly higher levels of granzyme B in tumours with stromal cell sialylation targeting. These effects were seen in the tumour but also systemically in the secondary lymphoid organs.
Conclusion: Together this data shows in multiple models of CRC that stromal cells can modulate the activity of immune cells including NK cells trough the Siglec/Sialic acid axis. Targeting stromal cell sialylation increased numbers and activation of NK cells and macrophages in vivo. Targeting stromal cell sialylation may be the key to reversing immunosuppression in stromal-rich CRC.
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2040 – WS67.2

N-glycosylation shapes the thymic differentiation of gd T-cells

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Purpose: γδ T-cells differentiate in the thymus and play key roles in tissue homeostasis and in immune responses. Many of these properties are imprinted during their thymic maturation, and thus dissecting the underlying molecular mechanisms is essential to optimize the performance of γδ T-cells in immunotherapies. In this regard, glycosylation is a post-translational modification involved in multiple aspects of the immune system. However, its impact on γδ T-cell biology remains poorly defined, therefore we investigated the role of *N*-glycosylation in the development of γδ T-cells.
Methods: We used qRT-PCR and a lectin-based approach to investigate the regulation of *N*-glycosylation by γδ thymocytes, employing thymic organ cultures to address the role of metabolism in its modulation. Lastly, we used glycoengineered mouse models, lacking the early glycan-branching enzymes Gnt-I and Gnt-II in lymphocytes, to study the impact of this pathway on the differentiation of γδ T-cells along with *in vitro* TCR stimulation of immature γδ thymocytes to dissect its role in TCR activation levels.
Results: We show that γδ thymocytes committing to IFN-γ production (γδIFN) downregulate the *N*-glycosylation program, whereas IL-17-producing (γδ17) cells exhibit higher expression levels of critical enzymes of this pathway. As result, embryonic γδIFN thymocytes display a greater content in mannose *N*-glycans compared to their γδ17 cell counterparts. Importantly, we show that aerobic glycolysis blockade alters the *N*-glycome of developing γδ T-cells. Lastly, in glycoengineered mouse models, we observed a substantial impairment in γδ17 cell differentiation in the embryonic and adult thymus associated with defects in the regulation of their lineage transcriptional program. This was accompanied by an increase in the minimal threshold of TCR activation upon *N*-glycosylation inhibition in immature γδ thymocytes.
Conclusion: Globally, our results suggest that *N*-glycosylation program is upregulated in thymic γδ17 cells, being modulated by their metabolic profile and impacting their differentiation through the regulation of the γδ17 transcriptional program and the threshold levels required for TCR activation. In summary, our results shed new light on the biological role of *N*-glycosylation in thymic γδ T-cell development, which may condition their functional performance in immune responses.

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169 – WS67.3

Siglec-1/CD169+ macrophages orchestrate immune reaction and matrix deposition in pancreatic cancer

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Purpose: Pancreatic cancer (PDAC) is set to be the 2nd deadliest cancer by 2030. PDAC is both a stromal and immune disease. With 80% of the tumor mass being stromal and most of the immune cells in the tumor microenvironment (TME) being macrophages. Several reports have revealed the importance of macrophage reprogramming in pancreatic cancer. Therefore, understanding the communication leading to pro- or and tumoral macrophages may be the key to unleash the potential of immunotherapy in PDAC.
Methods: Here we performed studies with primary cell lines issued from LSL-Kras$^{G12D/+}$; p16$^{fl/fl}$;Ptf1a-Cre; (KIC mice), stromal cells from LSL-Kras$^{G12D/+}$;Ptf1a-Cre (KC mice). The cells were orthotopically injected in CD169$^{DTR/DTR}$ recipients. We also performed single cell RNAseq analysis data on human PDAC single cell data set comprising of more than 70 patients. Mice were injected with diphtheria toxin in order to specifically deplete CD169 expressing cells. The pancreatic tumour growth and the changes in the local immune cells were analyzed by flow cytometry, immunohistochemistry and immunofluorescence.
Results: We found that CD169^{+} macrophages were highly abundant in pancreatic cancer in Humans and mice. We show here that stromal cells could polarize CD11b^{+}F4/80^{+} macrophages toward an immunosuppressive phenotype with expression of CD169. CD169^{+} macrophages could produce large amounts of stromal TGFBI that directly stimulated CXCL12 production by stromal cells, which attracted CD8^{+} T cells. Inhibition of CD169 by a DTR approach in an orthotopic pancreatic cancer model decreased matrix deposition and CD8^{+} T-cell infiltration within the tumor microenvironment.
Conclusion: These data suggest that CD169^{+} macrophages play a key role in sustaining the stromal reaction in pancreatic cancer and that specific targeting of this component might be beneficial to patients as an neoadjuvant therapy.

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904 – WS67.4

Glycosylation of γδT cells defines its activity and function associated with autoimmunity

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Purpose: *N*-glycosylation is a post-translational modification where glycans are added to proteins of essentially all cells. Evidence has shown the prominent role of glycans in T-cell biology. We demonstrated that human and murine thymocytes display different glycan structures that define developmental stages. Moreover, mannosylated *N*-glycans in thymocytes lead to defects in T-cell development, promoting the γδ-lineage, leading to increased susceptibility to inflammation and infection (Vicente M. et al., CellMolImmunol 2023). In addition, at the periphery, we observed that IL-17-producing γδT-cells recognize specific glycan structures associated with Systemic Lupus Erythematosus (SLE) disease (Alves I. et al., ScienceTransMed 2023). However, how altered glycans in γδT-cells could influence their response remains unknown. In this work, we explored the role of altered *N*-glycans expression on γδT-cell activity and function and its impacts on autoimmunity.
Methods: We started to analyze public RNA-seq data from healthy individuals and to characterize the glycosylation profile of γδT-cells from the peripheral blood of autoimmune patients. Moreover, to study the biological impact of altered glycans in γδT-cells´ function, we performed *in vitro* culture assays taking advantage of glycoengineered conditional KO mice developed in the group, in which selected glycogenes are deleted in lymphocytes. Finally, to disclose the impact of glycans on autoimmunity, we induced specific autoimmune diseases in the glycoengineered mice models.
Results: The public RNA-seq data from healthy individuals has revealed that peripheral γδT-cells exhibit a high dependency on the expression of glycogenes, namely those involved in the early *N*-glycan branching. Furthermore, the γδT-cells' glycosylation profile from the peripheral blood of autoimmune patients (including SLE) revealed a remarkable alteration in the *N*-glycosylation pathway. Moreover, *in vitro* culture assays with abnormal glycosylated γδT-cells revealed an association between the sequential *N*-glycosylation pathway and differential activation phenotypes of γδT-cells. Finally, using different glycoengineered mice, we demonstrated that truncation of complex *N*-glycan structures on γδT-cells defines its activity and cytokine production, contributing to an autoimmune-like disease in mice models.
Conclusion: In conclusion, we demonstrated the importance of glycans in regulating T-cell response and the impact on autoimmunity.

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2069 – WS67.5

The interaction between TLR4 and sialic acid governs monocyte phenotype variations

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Purpose: Osteoarthritis (OA) is a chronic, low-grade inflammatory joint disease characterized by pain, swelling, and reduced mobility. It affects up to 10% of the population, making it the leading cause of disability today. Despite its widespread impact, treatment options remain elusive due to the intricate and multifaceted nature of the disease. In OA, degenerated cartilage exhibits aberrant glycosylation, which can activate monocytes and T-cells, yet the mechanisms are unclear. We hypothesized that the monosaccharide Sialic acid (Sia) positioned at the outermost end of glycans on both cells and tissue proteins, could be involved in the disease pathogenesis, as it dictates immune regulation, including cellular communication, migration, and recruitment.
Methods: Using fluorescence-coupled metabolic labelling, we assessed Sia biosynthesis by flow cytometry in chondrocytes (Tc28a2) and monocytes (U-937) after different stimulations (LPS, IL1B/TNFa or TNFa alone). Subsequently, we assessed the removal of Sia on both human primary monocytes and porcine cartilage explants after treatment of the enzyme neuraminidase (Neu). The cellular effects of removing Sia on monocytes were compared to those induced by LPS and M-CSF in the presence or absence of the TLR4 inhibitor TAK-242. We evaluated the expression of Sialic acid immunoglobulin-like lectin (siglecs) 5, 9, and 15 by flow cytometry, ELISA, and confocal microscopy. For tissues, cartilage degeneration was assessed by measuring release of glycosaminoglycans using the DMMB assay.
Results: Metabolic labelling revealed that, in contrast to monocytes, chondrocytes increased their Sia biosynthesis only after IL1B/TNFa stimulation, not by LPS or TNFa alone. Removal of Sia by Neu generated comparable monocyte populations to LPS stimulation, characterized by a higher number of $CD16^{low}$ Siglec 15^{+} monocytes. Conversely, M-CSF-stimulation exhibited a greater abundance of $CD16^{high}$ Siglec 15^{high} monocytes. Blocking TLR4 resulted in Neu- and LPS-treated cells adopting a similar phenotype to that induced by M-CSF. DMMB results revealed a concentration-dependent release of glycosaminoglycans in explants treated with Neu.
Conclusion: Our study unveiled cell-specific molecular pathways linked to sialylation. Furthermore, a close association between Siglec 15 and TLR4 was observed. Neuraminidase demonstrated a concentration-dependent ability to degrade cartilage extracellular matrix, indicating its potential pathogenic mechanism in OA.

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914 – WS67.6

T cell glycosylation as a key player during colitis-associated carcinogenesis

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Purpose: Inflammatory Bowel Disease (IBD)-associated carcinogenesis (CAC) is a major concern in the clinical management of patients with chronic IBD. Inflammation is a driving factor in IBD and cancer, but the mechanism underlying cancer progression remains poorly understood. Dysregulation of mucosal glycosylation has been described as a key regulatory mechanism associated with colon inflammation and colorectal cancer (CRC) development. Our group showed that colonic IBD $CD4^+$T cells glycosylation are deficient in β1,6-*N*-acetylglucosamine branched *N*-glycans, observed by a downregulation in *MGAT5* expression, associated with disease severity. On the other hand, aberrant expression of *MGAT5* in CRC was associated with immune escape.
Whether colitis and cancer differential glycosignatures constitute a new mechanism converting T cells from hyperactivated to an immunosuppressive phenotype in CAC, remains unknown.
Methods: We performed *in-silico* analysis of a publicly available human and mice dataset. A well-characterized cohort of 64 CAC patients at different stages of carcinogenesis (colitis, dysplasia and cancer) was assessed to evaluate colon immune cells' *N*-glycosylation profile. *In-vivo* studies (using AOM/DSS to induce CAC) were conducted in glycoengineered mice. The glycoprofile and immune profile of colon T cells was analyzed.
Results: *In-silico* analysis showed that the glycome profile switches in CAC towards more complex forms of *N*-glycans, emphasizing its importance in the CAC pathogenicity. Additionally, our cross-sectional study showed a distinct glycoprofile in the stroma, characterized by an increase of complex *N*-glycans in carcinoma compared to active colitis. Interestingly, higher complex *N*-glycans expression on the immune compartment of colitis samples was able to predict the progression in the carcinogenic cascade, with 87.9% specificity and 64.3% sensitivity.
Moreover, when glycosylation was remodeled, we observed a tumor development decrease in the CAC mouse model. Indeed, glycoengineered mice showed an increase in T cells infiltrate and pro-inflammatory cytokines. Interestingly, these colonic T cells on wild-type mice presented a switch on *N*-glycans composition along disease course.
Conclusion: We show for the first time that colonic T cells display a distinct glycosignature along the CAC cascade, impacting tumor development. This work reveals that complex *N*-glycans can be used as a biomarker to improve the clinical management of IBD patients.

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WS68 – TRANSLATIONAL CANCER IMMUNOLOGY

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2147 – WS68.1

Lymphatic-derived oxysterols promote immunity and response to immunotherapy in melanoma

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In melanoma, lymphangiogenesis correlates with metastasis and poor prognosis and promotes immunosuppression. However, it also potentiates immunotherapy by supporting trafficking of immune cells. Lymphatic endothelial cells (LECs) are highly plastic cells that shape their phenotype according to distinct microenvironments, which likely define their functional properties, such as immunomodulation. Here, we show in a lymphangiogenic murine melanoma model that LECs upregulate the enzyme Ch25h, which catalyzes the formation of 25-hydroxycholesterol (25-HC) from cholesterol and plays important roles in regulating lipid metabolism, gene expression, and immune activation. LECs represent the main source of extracellular 25-HC in tumors, leading to the inhibition of PPAR-γ in intra-tumoral macrophages and monocytes. This prevents their immunosuppressive phenotype and instead promotes their conversion into proinflammatory myeloid cells that support effector T cell functions. We identify here in mechanistic detail a novel LEC function that supports anti-tumor immunity, which can be therapeutically exploited in combination with immunotherapy.

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2170 – WS68.2

Profiling CRC liver metastasis immune microenvironment at steady state and after pre-surgical therapy

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Purpose: Colorectal cancer (CRC) is the third most common cancer with more than 50% of patients developing metastases to the liver (CRC-LM), poorly responding to current therapies and leading to high risk of recurrence. This project undertakes a deep profiling of the immune landscape of CRC-LM from previously untreated or therapy treated patients, addressing the steady state immune contexture and the impact of pre-surgical therapy on its composition.
Material and methods: To dissect the CRC-LM microenvironment upon different therapeutic regimens, high dimensional 26-29 color flow cytometry panels were deployed to assess distinct immune cell subsets from tumor lesions, peritumoral tissue, distant normal liver and paired autologous whole blood obtained from patients.
Results: We showed a significant enrichment of intratumor $CD163^+$ $CD206^+$ HLA-$DR^{Low/Dim}$ M2-like cells, tissue resident $CD103^+CD69^+CD39^+CD8^+$ T cells co-expressing activation/exhaustion markers and activated $CD4^+$ Treg cells, encompassing both conventional and unconventional T cells.
Pre-surgical therapy and particularly the angiogenesis targeting regimens reshape both the T and the myeloid intratumor compartments, resulting in: i) the expansion of non-tissue resident and less exhausted LAG-$3^{high}CD39^{low}$ $CD8^+$ T cells at the expenses of large tissue resident exhausted LAG-$3^{neg}CD39^{high}$ populations; ii) the relief of local immunosuppression by reducing putative pro-tumor M2-like macrophages and Treg frequencies.
Conclusions: Our results define the immune landscape of CRC-LM and suggest pre-surgical therapy reshapes the immune composition, enforcing an immune-inflammatory response that may lead to reduced immunoregulation and increased anti-cancer T cell stimulation. Flow cytometry data are being integrated with genomics, bulk RNA-seq, spatially resolved multiplexing tissue analysis and correlated with clinical data. Finally, we are coupling at single cell level the mRNAseq, the surface phenotype and the clonal evolution of the main CD8 T cell subsets present at the steady state and reshaped by pre-surgical therapy.

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Complement activation sustains tumor progression and invasiveness in human and mouse colorectal cancer - CRC

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The complement system emerges as a major player of tumor-promoting inflammation. Here, we investigated its contribution to colorectal cancer (CRC) development, where its role is still debated. Indeed, it has been reported that complement activation may exert pro or anti-tumoral function by regulating tumor cell functions or the tumor microenvironment (TME) through several mechanisms.
We investigated the role and mechanisms of complement in CRC by exploiting transplantable primary and metastatic MC38 CRC models in mice deficient of complement components. *C3*$^{-/-}$, *MBL1/2*$^{-/-}$ and *C4*$^{-/-}$ mice showed reduced susceptibility to primary tumor, as well as to CRC liver metastasis development, while C1q and factor B deficiency had no impact, indicating the importance of C3 and lectin pathway activation.
We investigated C3-downstream events and observed that the deficiency of complement 5a receptor 1 (C5aR1) in MC38 cancer cells reduced tumor growth *in vivo*, while its deficiency in mice did not, suggesting its relevance in tumor cell function but not in the TME in this context. In line with these results, *in vitro* experiments showed that MC38 cells express C5aR1 and respond to C5a in invasion assays.
Then, we explored the relevance of these results in human CRC. We observed a negative correlation between the expression of C5aR1, but not C3aR nor C5aR2, and survival of CRC patients from the human TCGA dataset. By analyzing C5aR1 expression on a cohort of CRC patient-derived tissues, we found that C5aR1 was mainly expressed by tumor cells and its expression was associated with lymph node metastasis, stage II to stage III transition and poor clinical outcome. In agreement with these results, C5aR1 expression by tumor cells and its association with tumor progression and poor prognosis have been also reported in lung, gastric and breast cancer.
Overall, these results confirm that the role and target of complement in CRC depends on the pathogenesis of the tumor, and highlight that complement and lectin pathway activation are components of CRC progression and metastatic process, suggesting C5AR1 as a potential therapeutic target in this disease.

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Investigating the immune profile of early stage HER2+ breast cancer patients receiving chemotherapy and HER2-targeted therapies.

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Purpose: Trastuzumab (T) was the first mAb therapy approved for the treatment (tx) of HER2+ breast cancer (BC). The HER2+ BC subtype makes up 15-20% of all BCs. T directly inhibits HER2 signalling activity and engages innate immune cells like natural killer (NK) cells through Fc receptor (FcR)-mediated antibody-dependent cell-mediated cytotoxicity (ADCC). Lapatinib (L) is a small molecule tyrosine kinase inhibitor (TKI) targeting HER2 and EGFR. T and L have been combined with chemotherapy in the clinic based on pre-clinical data reporting enhanced HER2 inhibition and augmented ADCC response. This is an overview of our comprehensive immune-based translational investigation of blood and tissue samples from the Irish Phase II ICORG/CTRIAL-IE 10-05 HER2+ BC neo-adjuvant (Neo-Adj) clinical trial.
Methods: ICORG/CTRIAL-IE 10-05 (NCT01485926) (n=88) compared T, L and TL in combination with chemotherapy (docetaxel/carboplatin). Serum, plasma, peripheral blood mononuclear cells (PBMCs) and formalin fixed paraffin embedded (FFPE) tumour biopsies were collected from patients. *Ex vivo* T-ADCC function of PBMCs was determined in co-culture assays (Guava flow cytometer). The phenotype of circulating PBMCs was determined (CytoFLEX platform). FcRs were genotyped (Agena MassArray technology). Serum tumour auto-antibodies (AAb) were detected (HD-NAPPA platform). Plasma chemokine profiles were assessed (Multiplex ELISA). RNA-Seq NK cell gene expression profiles were generated (CIBERSORT). Tumour infiltrating lymphocyte (TIL) data and clinico-pathological data were available. Data were assessed pre- *vs.* post-Neo-Adj tx, and by pathological complete response (pCR).
Results: Tumour NK gene cell signatures and peripheral immune cells (T cell/NK cell/monocyte, B cells) displayed significant alterations post-Neo-Adj therapy, primarily confined to the No pCR cohort. FcR genotype status did not associate with pCR. Neo-Adj tx attenuated *ex vivo* PBMC T-ADCC. Lower pre- and post-tx levels of the chemokine CCL17 were associated with the pCR cohort. AAbs targeting p53 were detected in patients with p53 tumour mutations. AAbs associated exclusively with pCR, partial response and non-responders were detected. No pCR patients with pembrolizumab-sensitive PBMCs emerged as a potential biomarker.
Conclusions: Translational studies from the ICORG/CTRIAL-IE 10-05 trial have identified distinct immune profiles associated with pCR, as well as potential biomarkers of response to HER2-targeted therapy/chemotherapy for exploration in larger datasets.

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1219 – WS68.5

Flow cytometric analysis of TILs from different layers of human Glioblastoma samples revealed an enrichment of T cells expressing PD-1 and tissue-resident markers in the intermediate and marginal layers

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Purpose: Glioblastoma is the most common malignant brain tumor in adult population, for which immunotherapy show reduced efficacy. Current knowledge on immunotherapy failure is limited and detailed information about immune infiltrates in glioblastoma are urgently needed to personalized therapeutic strategy.
Methods: We enrolled 33 glioblastoma patients collecting peripheral blood (PB), total tumor resection or tumor samples from the central necrotic area, the intermediate and the marginal tissue through 5-aminolevulinic-acid (5-ALA) assisted surgery. T cells obtained from different samples were evaluated phenotypically, for immune-checkpoints and tissue-residency markers (Trm) expression, and functionally by assessing their cytokine production profile (TNF-α, IFN-γ, GMC-SF, IL-17, IL-10). Patients overall survival (OS) were retrospectively assessed and correlated with biological data.
Results: Flow-cytometric analysis showed a significantly higher frequency of T lymphocytes expressing PD-1, CD69 and CD103 in glioblastoma. In particular, we observed a preferential enrichment of CD8 expressing PD-1 and Trm markers (CD69 and CD103) in intermediate and marginal tumor areas, in which glioblastoma cells showed an active 5-ALA metabolism. T cells functionality was assessed by cytokine production, which results higher in glioblastoma compared to PB samples. In particular, the glioblastoma PD-1 positive T cells population maintained a good effector potential after restimulation ex-vivo. Interestingly, the higher frequency of IFN-γ-producing T cells was observed in the intermediate tumor layer. Notably, in the 5-ALA cohort a strong direct correlation between CD103 and OS in the necrotic layer, tipically not enriched in Trm effector cells, was observed. This result suggest an important role of Trm cells in tumor core, which however is apparently hardly infiltrated.
Conclusion: In this study we observed an enrichment in CD8+ Trm like T cells expressing PD-1, which retain a good effector potential, in the intermediate and marginal tumor areas compared to the necrotic core. Obtained results highlight the relevance of a comprehensive analysis of glioblastoma immune infiltrate to better understand the reasons for reduced efficacy of immunotherapy and to identify new biomarkers, prognostic factors and immunotherapy targets.

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440 – WS68.6

Understanding the mechanism of Cancer-associated fibroblast subpopulation reprograming-mediated immunomodulation in Colorectal cancer.

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Background: Consensus Molecular Subtype 4 (CMS4) of Colorectal cancer (CRC) has the worst patient outcome and significant therapy resistance in all of CRC. CMS4-CRC is characterised by an inflamed immunosuppressive tumour environment (iTME) and enriched with stromal cells. Cancer-associated fibroblasts (CAFs) which are the most abundant cells of the stromal cells in CRC are a heterogeneous population of cells, and their specific spatial-temporal functions are not well-defined. Two of the main CAF subgroups which are identified as myoCAFs and iCAFs are characterised by TNFα and TGFβ signatures, respectively. Understanding the mechanisms of CAF remodelling the iTME is crucial for the development of new therapies to target specific CAFs. We hypothesise that TNF-conditioned tumour secretome (TNF-TCS) and TGF-conditioned tumour secretome (TGF-TCS) model CAFs enhance their immunomodulation properties differentially and influence the NK cell functions in the CRC TME.
Methods: Using TCS and TNFTCS conditioning normal fibroblasts (NAFs), the RNA level screening was assessed by RNA sequencing. Using co-culture assays, we assessed the impact of the secretome from TNF-TCS and TGF-TCS conditioned MSCs on CMS4 CRC cell lines and primary NK cell activation and cytotoxicity of CRC cell lines by flow cytometry.
Results: We observed that both of TGF receptor and TNF receptor are highly expressed on CAFs. The expression of the chemokines is highly upregulated in TNF-TCS-conditioned NAFs. Following conditioning with TNF-TCS conditioned MSCs secretome, NK cell expression of the activation markers was significantly downregulated. The data from the cytotoxicity assay suggested that TNF-TCS conditioned MSC secretome impairs the NK function. TNF-TCS-conditioned MSC secretome also upregulated immune checkpoint markers, PD-L1 and PDL2, on both CMS4 tumour cells, suggesting two-way crosstalk that enhances immunosuppression in the CRC TME.
Conclusion: Subgroups of CAFs show the different functions and the potential for reprogramming in the iTME modification. TNF-TCS increase chemoattractant factors in NAFs. TNF-TCS-conditioned MSC secretome suppresses NK function and increases the immune checkpoint ligand expression on tumour cells. TGF-TCS may be involved in another mechanism to modify CAF immunomodulation function.
Using approaches to target CAF immunosuppressive signalling, we will define the role of targeting CAFs in developing novel immunotherapies.

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WS69 – CANCER IMMUNOTHERAPIES

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970 – WS69.1

Development of a soluble form of TIGIT with immune modulatory property

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Purpose: Tumor cells often express different ligands that interact with their corresponding receptors to inhibit T and NK cell function. Nectin-4, which is negligibly expressed in healthy adults is overexpressed in tumor cells in variety of malignant cancers and is widely considered as a cancer-specific biomarker. Nectin-4 preferentially interacts with an inhibitory receptor TIGIT that is highly expressed by both tumor-infiltrating T and NK cells. Nectin-4 is the only ligand that is not shared by both TIGIT and the activating receptor DNAM1 (also known as CD226), thus making it an important therapeutic target. Deciphering the molecular, structural, and functional basis of this interaction is, therefore, necessary to understand the tumor-immune cells crosstalk and design novel immunotherapeutic strategies.
Method: The ectodomain of TIGIT and nectin-4 were expressed and purified, followed by their interaction studies using surface plasmon resonance (SPR)-based biosensor. Structure-guided mutagenesis studies were done to map the nectin-4 binding interface on TIGIT. Based on the structural data, a novel TIGIT mutant exhibiting increased affinity toward nectin-4 was engineered.
Results: Our studies indicate that the membrane distal IgV domain of TIGIT and nectin-4 are sufficient to mediate the interaction between them and suggest a weak interaction as demonstrated by SPR-based analyses. Few residues of TIGIT that might play an important role in nectin-4 recognition were selected from the modeled complexes and mutated for mapping the binding interface. SPR-based studies reveal a decrease in binding response for all the TIGIT mutants in comparison to the wild type. Based on the biophysical data and structural analysis of the modeled complex, a novel TIGIT mutant has been engineered that shows higher affinity towards nectin-4 compared to wild-type TIGIT.
Conclusion: Targeting TIGIT:nectin-4 pathway is of prime importance for immunotherapy studies to design optimized therapeutics to treat cancer. Deciphering the molecular and structural basis of the TIGIT: nectin-4 interaction provided deeper insights into the structure-guided engineering of soluble TIGIT that preferentially interacts with nectin-4 expressed on cancer cells. The efficacy of the engineered high-affinity TIGIT in inducing NK and T cell cytotoxicity will be tested in cancer cell lines.

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327 – WS69.2

Targeting SUMOylation pathway triggers IFN-I-dependent activation of Natural Killer cells against Acute Myeloid Leukemias

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Natural Killer (NK) cells play a pivotal role in cancer immune surveillance. Patients with Acute Myeloid Leukemia (AML) presents a defective phenotype and function of NK cells facilitating AML immune escape. Enhancing NK cells activity in AML patients, either by activating endogenous NK cells or by adoptive transfer of ex vivo activated NKs, shows promising potentials for AML treatment. Several studies reported that SUMOylation, a protein posttranslational modification regulating the function and fate of thousands of proteins, plays critical roles in the control of anti-cancer immune responses. TAK-981, a first-in-class inhibitor of SUMOylation undergoing phase I/II clinical trials for cancer, is indeed emerging as an immunomodulatory drug.
In this study we demonstrate that targeting the SUMO pathway with TAK-981 activates primary NK cells from healthy donors and AML patients, increasing their cytotoxicity against AML cells and their ability to produce cytokines (IFN-γ, TNF-α, FasL). In addition, we show that TAK-981 increases *in vivo* the anti-leukemic activity of ex-vivo expanded human cord blood NK cells injected in AML mice models. At the molecular level, TAK-981 first induces *IFNB1* gene in NK cells, leading to the secretion of type I Interferon (IFN-I), which binds to the Interferon receptor IFNAR. This induces Interferon-Stimulated Genes (ISG) and activates NK cells *in vitro* and *in vivo*. Furthermore, inhibition of SUMOylation by TAK-981 stimulates IFN-I secretion by monocytes, contributing to NK cell activation in a paracrine manner.
Altogether, our results suggest that targeting SUMOylation with TAK-981 represents a promising strategy to reactivate AML patients' NK cells and enhance the efficiency of NK cells-based therapies NK for AML patients.

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254 – WS69.3

Using nanoparticles as artificial antigen presenting cells to activate human CD4 T cells for immunotherapy

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Introduction: CD4 T cell-based adoptive cell transfer (ACT) therapies have clinical success across multiple cancer types, including metastatic melanoma and epithelial cancer. One of the challenges of CD4 T cell-based ACT therapy is finding methods to expand tumor antigen-specific T cells. Therefore, engineering artificial platforms for antigen-specific T cell expansion is critical since it provides greater control over studying T cell biology and has significant translational relevance. We developed a nanoscale platform that expands murine and human antigen-specific T cells *in vitro*. This platform allows us to study the effector and helper function of CD4 T cells through precise tuning of material parameters, including shape, size, and ligand patterning.
Methods: Our lab utilizes artificial antigen-presenting presenting cells (aAPCs) – iron dextran particles conjugated with MHC class II and the co-stimulatory molecule to activate T cells. This aAPC platform provides T cells with two critical signals for T cell activation, which permits the activation of cognate antigen-specific CD4 T cells *ex vivo*. We isolate human CD4 T cells from PBMCs of healthy donors. We then stimulate CD4 T cells with HLA II aAPCs in media with inflammatory cytokine and assess the specificity, effector function, and memory profiles through tetramer staining, cytokine release assays, and peptide-pulsed lymphoblastoid cell line killing assays.
Results: Co-culturing Class II aAPCs and human CD4 T cells significantly increases the frequency of cognate antigen-specific CD4 T cells. Specifically, we successfully expanded tetanus toxoid (p30)-specific and herpes simplex virus (HSV)-specific HLA-DP4-positive CD4 T cells from healthy human PBMCs with aAPC. By the end of the 21-day expansion period, the antigen-specific cells showed substantial proliferation, escalating from under 1% to approximately 10% for HSV and exceeding 30% for p30. The resulting antigen-specific CD4 T cells display high levels of effector cytokine production, including TNF-a, IFN-g, IL-2, and granzyme B. Furthermore, these CD4 T cells demonstrated antigen-specific cytotoxic capabilities and are predominantly effector memory T cells.
Conclusion: These data show that aAPC platform can expand antigen-specific CD4 T cells from a rare precursor population. Therefore, this technology is beneficial in expanding rare neoantigen-specific cells for ACT for CD4 T cell-enriched cancers.

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731 – WS69.4

Potent CD8+ T cell effector functions induced by NaCl in cancer immunotherapy

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CD8$^+$ T cells play a central role in anti-tumor immunity but inevitably become dysfunctional or "exhausted" in the tumor microenvironment. Ionic metabolism is emerging as a novel regulator of CD8$^+$ T cells in anti-tumor immunity. Here we show that sodium chloride (NaCl) counteracts T cell dysfunction to promote cancer regression. NaCl supplementation during CD8$^+$ T cell culture induced potent effector differentiation, IFN-γ production and cytotoxicity while maintaining gene networks responsible for stem-like plasticity. Accordingly, adoptive transfer of tumor-specific T cells resulted in enhanced persistence and superior anti-tumor immunity in humanized models. In mice, high salt diet (HSD) administration reduced growth of experimental tumors in a CD8$^+$ T cell-dependent manner, by inhibiting terminal differentiation, and by enhancing the effector potency of CD8$^+$ T cells. Mechanistically, NaCl enhanced glutamine consumption that was critical for cellular reprogramming. In humans, CD8$^+$ T cells undergoing antigen recognition in tumors and predicting favorable response to checkpoint blockade immunotherapy resembled those induced by NaCl. Collectively, we identified NaCl metabolism as a major regulator of CD8$^+$ T cell effector function, with potential translational use in cancer immunotherapy.

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1967 – WS69.5

A novel combined treatment approach for pancreatic ductal adenocarcinoma enhances immune response and tumour reduction

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Purpose: Pancreatic ductal adenocarcinoma (PDAC) is aggressive and resistant to standard treatments. In our study, we aimed to enhance immune cell infiltration into tumors by combining therapies. This involved targeting the activation of the Hippo pathway, specifically YAP-1 and FOSL-1 genes, using lipoplexes carrying siRNA, alongside with conventional chemotherapy and epigenetic inhibitors. PDAC progression is influenced by immune cell invasion and genetic alterations. Our results show a significant increase in immune cell infiltration compared to the control group, where immune cells remained outside the tumor. This indicates that our strategy promotes immune cell penetration into the tumour microenvironment, enhancing the anti-tumoral response and offering hope for improved treatment outcomes.
Methods: We prepared lipoplexes carrying four siRNAs targeting YAP-1 and FOSL-1 genes using cationic liposomes made of DODAB and MO. This was combined with chemotherapy using Entinostat and low doses of Gemcitabine. We meticulously tested this treatment on human PDAC and mouse organoids and xenograft mouse models. Cell viability in organoids was assessed using immunofluorescence assays, while xenograft mice received lipoplexes targeting YAP-1/FOSL-1 followed by chemotherapy, with daily tumour growth monitoring. Subsequent histological analysis, RT-PCR, and Western blotting were performed on tumour and organoid samples to examine stromal changes, immune cell infiltration and confirmation of gene/protein silencing.
Results: Pre-treatment using liposomes aimed at YAP-1 and FOSL-1 led to a notable decrease in tumor size and collagen levels in the tumor's tissue, mainly due to changes in cancer-related fibroblasts (CAFs). Further treatment with Entinostat and Gemcitabine resulted in a significant reduction in tumor volume compared to using each therapy alone. These results were strongly supported by experiments conducted on models of PDAC, including xenografts and organoids. Additionally, histology and immunofluorescence tests indicated a rise in immune cells infiltrating the tumors. Our treatment notably increased the presence of immune cells within tumours, facilitating the success of subsequent immunotherapies.
Conclusion: Our study demonstrates the effectiveness of combining siRNA treatment, chemotherapy, and epigenetic inhibitors to enhance immune cell infiltration in PDAC tumours. This approach significantly reduces tumour size and collagen levels, indicating the potential for improved treatment outcomes through enhanced immune response and targeted therapy.

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2103 – WS69.6

CD69-deficiency confers susceptibility to anti-PD1-induced myocarditis

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The advancement of immunotherapies has changed cancer treatment. Immune checkpoint inhibitors (ICIs) are monoclonal antibodies targeting PD-1 and PD-L1. ICIs stimulate the immune system, by inhibiting regulatory T cells, to attack cancer cells that evade the immune response. However, the mechanism of action of ICIs could lead to undesirable events (irAEs). During immunotherapy, autoimmune T cell are activated. Therefore, many systemic autoimmune diseases may arise, including myocarditis, which is the rarest, yet most fatal, with a mortality rate of 60%. There is a lack of animal models to study ICI-myocarditis, and specific biomarkers to predict this disease. Our group identified mmu-miR-721 as a biomarker for acute myocarditis in mouse and humans. The main objectives are to analyse and validate the *cd69*$^{-/-}$ mouse model for the study of the pathophysiology of ICI-myocarditis in tumor-bearing mice under immunotherapy, and to validate mmu-miR-721 as an early biomarker of this condition.
cd69$^{-/-}$ mice were used since our group has described how these animals develop exacerbated Th17 responses and consequently severe myocarditis. We analyzed the subsequent immune response in peripheral blood, lymph nodes and heart by flow cytometry, as well as the cardiac dysfunction by echocardiography.
We proposed an animal model of susceptibility to the development of myocarditis induced by effective antitumoral treatment with anti-PD1. ICI treatment in these animals induces cardiac inflammation followed by cardiac dysfunction, compared to WT animals that do not develop any heart disease after anti-PD1 treatment. Immune infiltrate is detected in the heart and mediastinal lymph nodes, with high Th17 responses. We demonstrate that mmu-miR-721 biomarker levels are significantly elevated in plasma 10 days after initiating anti-PD1 therapy in *cd69*$^{-/-}$ mice, along with classical biomarkers of cardiac damage, such as troponin.
Anti-PD1 treatment in *cd69*$^{-/-}$ mice generates cardiac inflammation due to a specific immune response towards the heart. Moreover, biomarker mmu-miR-721 levels are significantly elevated at day 10 of the treatment in plasma of *cd69*$^{-/-}$ mice. This finding has clinical relevance since the mmu-miR-721 could be the first specific and early biomarker of ICI-myocarditis susceptibility and development.

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WS70 – IMMUNITY IN RESPIRATORY INFECTIONS

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924 – WS70.1

Developing bispecific antibody for sustained suppression of Influenza virus

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Targeting multiple viral proteins is pivotal for sustained suppression of highly mutable viruses. In recent years, broadly neutralizing antibodies that target the influenza virus hemagglutinin and neuraminidase glycoproteins have been developed, and antibody monotherapy has been tested in preclinical and clinical studies to treat or prevent influenza virus infection. However, the impact of dual neutralization of the hemagglutinin and neuraminidase on the course of infection, as well as its therapeutic potential, has not been thoroughly tested. For this purpose, we generated a bispecific antibody that neutralizes both the hemagglutinin and the neuraminidase of influenza viruses. We demonstrated that this bispecific antibody has a dual antiviral activity as it blocks infection and prevents the release of progeny viruses from the infected cells. We show that dual neutralization of the hemagglutinin and the neuraminidase by a bispecific antibody is advantageous over monoclonal antibody combination as it results in an improved neutralization capacity and augmented the antibody effector functions. Notably, the bispecific antibody showed enhanced antiviral activity in influenza virus-infected mice, reduced mice mortality and limited the virus mutation profile upon antibody administration. Thus, dual neutralization of the hemagglutinin and neuraminidase could be effective in controlling influenza virus infection.

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1393 – WS70.2

Myeloid-derived OLAH (oleoyl-ACP hydrolase) and its main catalytic products act as early biomarkers of life-threatening illness from diverse viral respiratory infections

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Purpose: Acute respiratory infections are characterized by varied disease severities and corresponding morbidity rates, and early identification of patients at risk for severe disease is integral to improving patient outcomes. We therefore set out to identify biomarkers of severe respiratory disease by studying cohorts with patients experiencing life-threatening respiratory symptoms due to acute viral infection.
Methods: Bulk and single-cell RNAseq and lipidomics were used to compare patients with life-threatening illness due to acute respiratory viral infections (including avian and seasonal influenza, SARS-CoV-2, and RSV) to healthy individuals or patients with less severe disease.
Results: Our anlayses identified *OLAH* (oleoyl-ACP hydrolase), a typically rarely expressed enzyme involved in fatty acid biosynthesis, as a biomarker of life-threatening disease across multiple viral infections. Among adults hospitalized with A(H7N9) influenza, those who succumbed to disease exhibited early elevations of peripheral *OLAH* transcription that persisted throughout the course of hospitalization. Likewise, single-cell analyses showed significant increases in *OLAH* among adults hospitalized with seasonal influenza compared to healthy subjects, particularly in peripheral monocytes and macrophages. *OLAH* was also identified as a biomarker for life-threatening disease outside of influenza infection; for instance, among children hospitalized with SARS-CoV-2, *OLAH* was the single-most differentially expressed gene increased in patients with life-threatening respiratory dysfunction compared to those with no-to-minimal respiratory dysfunction. Furthermore, lipidomics analysis of adults infected with SARS-CoV-2 showed significant upregulation of the main catalytic products of OLAH among hospitalized patients. Single-cell analyses of samples obtained from the lower respiratory tracts of children with acute lung failure due to seasonal influenza and/or RSV infection similarly showed upregulation of *OLAH* in monocytes, macrophages, and neutrophils, potentially implicating this peripheral biomarker in mechanisms underlying disease at the site of infection. Analyses of samples obtained from human challenge models of multiple respiratory infections consistently demonstrated that *OLAH* was not elevated during mild infections. Network analysis of the underlying datasets pointed to OLAH as a potential mediator of inflammation among specific myeloid subsets that traverse the respiratory tract and the periphery.
Conclusion: Upregulation of *OLAH* transcription during viral respiratory infection correlates with myeloid-mediated inflammation and provides an early biomarker of life-threatening illness.

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1130 – WS70.3

Leptin is expressed in lung granulomas of Mycobacterium tuberculosis-infected DBA/2 mice and impairs anti-mycobacterial capacity of immune cells

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Purpose: Tuberculosis (TB) is one of the deadliest infectious diseases worldwide. In 2021, about 10.6 million people fell ill with TB and *M. tuberculosis* (MTB) infection caused 1.58 million deaths. Host-direct therapies are thought as novel strategies to defeat TB, including the multi-drug resistant TB. We investigated whether leptin, an adipocyte-derived hormone, playing a major role in the regulation of feeding and energy expenditure and linking nutritional status with neuroendocrine and immune functions, may be a host target for novel TB therapies. In humans, low levels of circulating leptin, likely associated with a decreased body fat, have been associated with pulmonary TB and leptin-deficient C5BL/6 mice are more susceptible than wild-type mice to MTB infection. However, a moderate caloric restriction reduces systemic levels of leptin and promotes an immunometabolic reprogramming leading to protection against pulmonary MTB infection in DBA/2 mice, a strain highly susceptible to MTB infection.
Methods: DBA/2 mice were infected *in vivo* with MTB, severity of pathology was studied evaluating bacterial load by CFU assay and lung pathology by histology. Leptin was analyzed in serum and in granuloma lesions. Spleen cells infected *in vitro* with MTB or recovered from MTB-infected mice were cultured in the presence or absence of leptin to measure the anti-mycobacterial capacity of immune cells. The effects of leptin on autophagy was analyzed by western blot and on the metabolic profile by Seahorse analysis.
Results: We found that leptin was expressed in granulomatous lesions of the lungs and spleens of MTB-infected DBA/2 mice, and its expression correlated with disease severity. Leptin was mainly found in lung area rich in foam cells, a distinct sign of TB progression and a secure niche for bacterium survival. Mechanistically, leptin reduced autophagy, increased cell survival and promoted foam cell formation, all mechanisms limiting macrophage ability to kill the pathogen. In addition, leptin reduced glycolysis and tended to promote mitochondrial respiration in spleen cells of BCG-infected mice cultured with Lipopolysaccharide, a potent innate immune stimulus.
Conclusion: Leptin, an adipocyte-derived hormone, is expressed in lung granulomas and could promote MTB infection by impairing anti-MTB capacity of immune cells.

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1391 – WS70.4

High-parameter cytometry by time of flight (CyTOF) and unbiased bioinformatical analysis to identify biomarkers of Post-Covid Syndrome ME/CFS and of response to immunoadsorption therapy

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Coronavirus disease 2019 (COVID-19) infections can lead to symptoms of chronic fatigue and exertion intolerance, persisting for months and even years post-infection. This is known as Post-Covid Syndrome (PCS) or Long-Covid. Today, about every 10th person develops PCS after acute SARS-CoV2 infection and no prescribable therapies exist. The clinical appearance is very heterogeneous, pointing towards different PCS etiologies. A subset of PCS patients develops myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) a severe postinfectious sequela of various infections. Clinical studies provided evidence for a role of autoantibodies in ME/CFS. Recent reports connect severe COVID-19 and PCS with an aberrant immune response. Some patients show dysregulations in the B cell compartment and formation of autoantibodies and therefore might benefit from immunoadsorption (IA) therapy, clearing immunoglobulins from the circulation. Clinical studies in ME/CFS show that about two thirds of patients benefit from IA. However, detailed understanding why some patients benefit from immunoglobulin removal is lacking.
To reveal leukocyte alterations indicative of IA-response, cytometry by time of flight (CyTOF) was used to profile leukocytes in blood samples from PCS patients meeting ME/CFS criteria pre- and post-treatment and age- and sex-matched controls. PCS-ME/CFS patients undergoing IA divided into responders (increase of physical function assessed by SF-36) and non-responders. IA-responsive patients' SF-36 improved after 1 month of treatment. We observed signs of dysregulated B cell maturation and increased B cell activation in PCS-ME/CFS patients compared to controls. Importantly, we detected these alterations only in pre-treatment samples of IA-responsive patients potentially enabling patient stratification in future clinical trials. Furthermore, dysregulations in the B cell compartment normalized at the end of treatment, suggesting a causal link.
This study demonstrates B cell alterations as a potential biomarker for PCS-ME/CFS patients and autoantibody-targeting treatment. Currently, we perform single-cell transcriptome and mechanistic *in vitro* analyses to understand which factors might drive the dysregulation of the B cell compartment and its contribution to PCS development. However, the study also highlights the complexity of PCS and the need to identify biomarkers predicting therapy response.

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878 – WS70.5

Study of Bdellovobrio bacteriovorus as a live antibiotic against Mycobacterium tuberculosis.

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Purpose: Ineffectiveness of anti-tubercular drugs and growing frequency of MDR and XDR TB warrants the use of novel ways to treat *Mycobacterium tuberculosis* infection. *Bdellovibrio bacteriovorus*, a Gram-negative bacterium known for its unique role as an obligatory predator within the periplasmic space of other Gram-negative bacteria, could be looked upon as a viable adjuvant to antibiotics, particularly in combating multidrug resistant pathogens like: *Mycobacterium tuberculosis.*
Method: Culture and Differentiation of THP-1 cells was done using PMA. Maintenance and culturing of bacteria (*Mycobacterium tuberculosis* H37Ra and *Bdellovibrio bacteriovorus*) was done for infection experiments. Determination of multiplicity of infection (MOI) was done via Calcein AM staining using Flow Cytometry. Spot clearance assay on H37Ra lawn was done to check for the direct killing capacity of *Bdellovibrio bacteriovorus*. THP-1 cells were treated with *Bdellovibrio bacteriovorus* before and after H37Ra infection with appropriate controls. Mtb clearance capacity was checked by colony forming units/assay (CFU). Intracellular cytokine and surface marker was assessed for macrophage phenotype profiling using Flow Cytometry followed by transmission electron microscopy study for bacterial colocalisation in macrophages.
Results: Our study is the first to explore the predatory potential of Bdellovibrio bacteriovorus against Mycobacterium tuberculosis. Bdellovibrio pretreatment showed Mtb clearance capacity in both extracellular and intracellular settings. Pretreatment of macrophages with *Bdellovibrio bacteriovorus* before H37Ra infection was found to significantly enhance intracellular mycobacterial clearance than the post treated and control groups as depicted by CFU counts. Bdellovibrio treatment also showed significant shift from necrosis to apoptosis and pyroptosis, which might be contributing towards the observed reduction in Mtb CFU count. However, we could not demonstrate any appreciable changes mediated by Bdellovibrio on immune environment within the macrophages. Furthermore, we found that *Bdellovibrio bacteriovorus* and H37Ra colocalize within macrophages as observed through Transmission Electron Microscopy, indicating the potential contribution of direct predation of H37Ra bacilli by *Bdellovibrio bacteriovorus* to the substantial reduction in CFU illustrated by the CFU assay.
Conclusion: These promising preliminary findings suggest an interesting interplay between *Bdellovibrio bacteriovorus* and macrophages, which could be further explored as a potential therapeutic agent against *Mycobacterium tuberculosis* using appropriate animal models.

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281 – WS70.6

Tissue-specific immunophenotyping reveals divergent immune responses to Mycobacterium tuberculosis in lung parenchyma and vasculature of tuberculosis mice model

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Purpose: The role of resident lung immune cells against *Mycobacterium tuberculosis* (*Mtb*) still remains unclear. The aim of this study is to perform deep immunophenotyping in the lung parenchyma and vasculature and to investigate the differences in the response against *Mtb* depending on the organ and tissue of origin of the cells in a chronic TB mice model.
Methods: Female (n=13) and male (n=13) C57Bl/6 mice were infected by aerosolization (Inhalation Exposure System, Glas-Col) with *Mtb* H37Rv and sacrificed at 6 weeks post-infection. Mice were intravenously administered with anti-CD45 antibody prior to euthanasia to distinguish extravascular from intravascular fractions of the lung. Blood and lungs were extracted, and immune cells were obtained after erythrocyte lysis and Percoll density gradient centrifugation, respectively. Cells were stimulated overnight with PMA/Ionomycin, ESAT-6/CFP-10 (*Mtb*-specific), or PPD (mycobacteria-specific) and stained for surface and intracellular antigens with 33 antibodies related to local and central memory in T, B and NK cells. Samples were acquired using full spectrum Cytek Aurora 5-laser and analysed with FlowJo.
Results: We will focus for this abstract on the expression of inflammatory and memory markers on major cell subsets. For both specific stimuli, $CD4^+$ and $CD8^+$ T-cells expressed significantly higher levels of IL-17 and lower levels of IL-22 in blood than in the lung. Within the $CD4^+$ T-cells cells of the lung, there was a prevalence of pro-inflammatory cytokines IFN-γ and TNF, and a reduction of PD-1 in the vasculature region of the lung compared to its parenchymatic counterpart ($p<0.05$). Tissue-resident memory T-cells ($CD69^+CD101^+$ and $CD69^+CD103^+$[$p<0.05$], $KLRG1^-$[$p<0.01$]) were enriched in the parenchyma for both CD4 and CD8 lymphocytes. Additionally, we also observed that this tissue had lower levels of NK cells ($CD3^-NKp46^+$), with a predominantly immature phenotype ($CD27^+CD11b^{low}$;$p<0.05$), and a higher proportion of B cells with a regulatory phenotype ($CD3^-B220^+CD1d^+IL\text{-}10^+$;$p<0.01$), when compared to blood-originated immune cells.
Conclusions: Following *Mtb* infection, immune cells from lung parenchyma display specific tissue-residency and anti-inflammatory phenotypes in comparison to lung and peripheral vasculature, which express more canonical pro-inflammatory cytokines. The role of these and other immune populations will be further clarified by non-supervised multiparametric analyses.

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WS71 – VACCINES FOR VIRAL INFECTIONS

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2272 – WS71.1

Investigating long-term T cell memory responses to COVID-19 vaccines in people living with HIV

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South Africa has 7.5 million people with HIV (PWH), and an estimated 500 new infections every day; 67% of PWH are virally suppressed. Some vaccines elicit suboptimal responses in PWH. Ongoing systemic immunological dysfunction even in well-controlled HIV may influence COVID-19 vaccine responses. Few studies have characterised cellular immunity in PWH following COVID-19 vaccination.
The overall aim of the study is to monitor long-term immunological memory in PWH. This involves understanding the durability of T cell memory and identifying potential defects in cellular immunity in PWH.
Spike-specific T cell memory responses were examined longitudinally in PWH and compared to HIV-uninfected participants, in healthcare workers (n=90) from the Sisonke Ad26.COV2.S trial who received a homologous or heterologous (BNT162b2) vaccine boost. T cell responses were measured by flow cytometry.
The study participants were stratified into three groups based on their CD4:CD8 ratio, those within the normal range (≥ 0.9), and those exhibiting partial or poor immune reconstitution. Six months after boosting, spike-specific CD4 T cell frequencies decreased compared to baseline. However, there was a trend towards an increase in the proportion of CD8 T cell responders six months after booster vaccination, likely attributable to the vaccine response. Comparable T-cell profiles were observed in participants with both well and partially reconstituted CD4:CD8 T-cell ratios. However, participants with poor CD4:CD8 ratios had a lower proportion of CD4 and CD8 T-cell responders. Ongoing studies include the phenotyping of T cell memory subsets, T cell activation and exhaustion profiles as well as measuring durability and function (proliferative capacity, cytotoxic potential) of spike-specific T cells at 6 months versus 24 months post booster vaccination.
Participants living with HIV with well-reconstituted immune profiles exhibit robust T cell responses after booster vaccination, in the context of hybrid immunity. However, participants with poor immune reconstitution displayed deficiencies in both CD4 and CD8 T cell responses, both at baseline (reflecting the memory response post-primary vaccination) and post-booster vaccination. This study demonstrates the heterogeneity of SARS-CoV-2 T cell responses in people with HIV and highlights the importance of monitoring this population.
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298 – WS71.2

Role of antigen bio-distribution and persistence in early and long-term immune responses to the yellow fever vaccine in non-human primates

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Purpose: Despite recent advances in vaccine development, gaps remains to understand the mechanisms driving long-term memory response to vaccines. The purpose of the work is to characterize the role of vaccine antigen persistence in generating durable immunity. Using the live-attenuated yellow fever (YF)-17D vaccine, one of the most efficient and durable vaccine, we explored molecular and cellular interactions in tissues of immunized non-human primates.
Methods: Two groups of cynomolgus macaques were either vaccinated with a commercial dose of live-attenuated vaccine or with the same dose of a β-propiolactone-inactivated vaccine adjuvanted with alum. Animals also received a 5-Iodo-2'-deoxyuridine (IdU) treatment to follow the mobilization and persistence of immune cells following immunization. Longitudinal systemic immune responses were characterized by mass cytometry, as well as viral load by qPCR, and neutralizing antibodies. At necropsy, different types of tissues were collected at several timepoints to follow antigen persistence by immunohistofluorescence (IHF) and *in situ* hybridisation; and to explore tissue immune responses by imaging mass cytometry (IMC). Results were compared to data obtained from tissues of macaques infected wild type Asibi strain.
Results: Low and transient viremia is observed in vaccinated animals (reaching 10^3 to 10^4 copies/mL) at approximately day 3 pi. Longitudinal analysis of immune responses by mass cytometry shows a high mobilization of innate and adaptive immune cells at early time-points. Adaptive T and B cells show persistent activation in the long term (up to 12 month pi) as demonstrated by the persistence of IdU^+ cells. Neutralizing antibodies were shown to persist for the live-attenuated vaccine whereas they decreased significantly around 3 months pi for the inactivated vaccine. By comparing these strategies, we identified differences in regulatory T cells and three types of circulating follicular helper cells ($CD127^+$ $PD\text{-}1^-$, $CD127^{mid}$ $PD\text{-}1^-$ $CD25^+$, and $CD127^{lo}$ $PD\text{-}1^+$) that could affect antibody persistence. In the tissues, YF antigens were detected at early timepoints mainly in the liver, although at lower rates than the infected animals, and in the spleen.
Conclusion: Our data enhance the understanding of the immunological mechanisms of YF-17D vaccination. Together, they highlight the relevance of specific immunological mechanisms that occur after vaccination.

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1929 – WS71.3

Repeated SARS-CoV-2 mRNA vaccination results in decreased capacity of spike-specific serum antibodies to activate NK cells in older adults

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To ensure prolonged protection against severe disease outcomes in the face of continued circulation of SARS-CoV-2 variants, many public health authorities have recommended a repeated booster vaccination regimen, especially in older adults. Previous research has shown that repeated vaccination results in a continued increase in post-vaccination antibody binding concentrations and neutralization titers. However, it has also been reported that repeated mRNA vaccination results in class-switching to IgG4. Importantly, little is known about the effect of repeated vaccination on the quality of the antibody response beyond neutralization, more specifically, the capacity of the induced antibodies to mediate Fc-dependent effector functions.
For the current analysis, we have assessed the capacity of serum antibodies to mediate SARS-CoV-2 spike-specific antibody dependent phagocytosis (ADCP), complement deposition (ADCD), and NK cell activation (ADNKA) in older adults (n=38, 65-77 years of age) at one month after completing the primary mRNA vaccination series, and at one month following the first and third mRNA booster dose. In addition, we measured SARS-CoV-2 spike-specific IgG binding concentrations, IgG avidity, and subclasses IgG1, IgG2, IgG3 and IgG4.
Although we observed a continued increase in the absolute functionality of the antibodies induced by repeated vaccination, we found that the capacity of the antibodies to mediate ADNKA and ADCD decreased after the third booster dose when corrected for IgG concentration. Repeated mRNA vaccinations also increased IgG4 responses over time in older adults. For ADNKA, the relative decrease in functionality associated with a decreased IgG3/IgG4 ratio.
Together, these data provide novel insights into the development of antibody quality upon repeated vaccination within a relatively short time period in the older adult population. Since increasing evidence suggests that Fc-mediated effector functions contribute to protection, these insights are important for the design of the most optimal (mRNA) vaccination policies in the event of repeated booster schedules.

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204 – WS71.4

Mesoporous Silicon Microparticles (MSMPs) as a new adjuvant in a vaccine against the SARS-COV-2 virus

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Purpose: Our main purpose is to study the role of Mesoporous Silicon Microparticles (MSMPs) as a new adjuvant in a vaccine against SARS-CoV-2 based on the viral spike protein (S1). In this work we analyzed how MSMPs induce humoral and cellular response in wild type mice and protection against SARS-CoV-2 infection and COVID -19 dissease in K18-hACE2 transgenic mice. MSMPs immunoestimulatory effects were always compared with $Al(OH)_3$.
Methods: To achieve our objectives we immunized mice with MSMPs-S1 and studied humoral and cellular response, detecting IgG1, IgG2a and IgG by ELISA and IFNγ production by flow cytometry after stimulated splenocytes with Peptivator_S. Furthermore, we infected intranasally immunized K18-hACE2 mice with SARS-CoV-2 and studied viral replication in lungs and brain by qPCR. Disease development also was analyzed through clinical score, survival rates and weight variations during 15 days.
Results: Our findings indicate that MSMPs act as an adjuvant by enhancing humoral and cellular response, even better than $Al(OH)_3$ especially in the case of cellular response. In humoral response we detected higher levels IgG1, IgG2a and IgG antibodies than mice immunized with S1 and same leves of these antibodies as in mice immunized with $Al(OH)_3$-S1 except for IgG2a after boost. In this case we detected higher leves of IgG2a in the serum of mice immunized with MSMPs than in the serum of mice immunized with $Al(OH)_3$. In cellular response, mice immunized with MSMPs-S1 had higher level of IFNγ than mice immunized with $Al(OH)_3$-S1 and S1. After infection, we observed that mice immunized without adyuvants died within the first fives days. On the other hand, mice immunized with MSMPs-S1 or $Al(OH)_3$-S1 didn´t die, had less weight change and lower clinical score than those immunized with S1 protein. Finally, we analized the presence of SARS-CoV-2 in differents organs and detected higher viral load in non-immunized mice and mice immunized with S1 protein than in mice immunized with MSMPs-S1.
Conclusion: In conclusion our results demonstrate that MSMPs are a promising candidate as novel adyuvant in vaccine complex, enhancing both humoral and cellular response and providing protection against SARS-CoV-2 infection.

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230 – WS71.5

Vaccine-induced virus neutralizing antibody responses against seasonal Influenza virus H1N1 strains are not enhanced upon subsequent pandemic H1N1 infection.

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The first exposure to influenza is thought to shape the B cell antibody repertoire, leading to preferential enhancement of these initial responses upon subsequent exposures to viral variants. Here, we investigated whether this principle holds when there is a large genetic and antigenic difference between primary and secondary influenza virus antigens. Because humans have usually experienced a complex influenza virus exposure history, this was investigated in influenza-naive cynomolgus macaques. Two groups of six macaques were immunized four times with influenza virus-like particles (VLP) displaying either one (monovalent) or five (pentavalent) different hemagglutinin (HA) antigens derived from seasonal H1N1 (H1N1) strains. Four weeks after the final immunization, animals were challenged with pandemic H1N1 (H1N1pdm09). While immunization resulted in high virus neutralizing responses against all VLP-based vaccine strains, there was no cross neutralization against H1N1pdm09, and all animals became infected. No reduction in virus load in nose or throat in the monovalent or pentavalent vaccine group was detected. After infection, strong virus neutralizing responses were induced against H1N1pdm09. However, there was no increase in virus neutralizing titers against four of the five H1N1 vaccine strains and only a modest increase was seen against the influenza A/Texas/36/91 vaccine strain. After H1N1pdm09 infection, both the monovalent and pentavalent vaccine groups showed higher virus neutralizing titers against two H1N1 strains of antigenic intermediate distance between the H1N1 vaccine strains and H1N1pdm09 than the naive control group. Furthermore, both vaccine groups had higher HA-stem antibodies early after infection. In conclusion, immunization with VLPs displaying HA from antigenically distinct H1N1 variants resulted in increased breadth of the immune response upon subsequent H1N1pdm09 challenge, which however was limited to antigenic intermediate variants.

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1410 – WS71.6

Adaptable live-attenuated SARS-CoV-2 OTS vaccines show preclinical safety and broad protection

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Purpose: There is still a need for adaptable and highly efficacious vaccines that would protect against the wide spectrum of SARS-CoV-2 variants of concern (VOCs), as well as can be administered easily to provide both systemic and local mucosal immunity.
Methods: We developed live-attenuated vaccines (LAV) based on recoding the SARS-CoV-2 genome to enrich for "one-to-stop" (OTS) codons. Further, in order to increase the immunogenicity, we introduced additional mutations in the non-structural-protein-1 (nsp1), and the deleted the open reading frames (ORF) 6, 7ab and 8. Finally, by deleting the spike polybasic cleavage site (PCS), we aimed to reduce LAV replication in the lung and prevent vaccine shedding. The attenuation of the vaccine candidates as well as the protection they elicited were demonstrated in cell lines, nasal- and bronchial epithelial cells, in vivo K18-hACE2 mice and Syrian hamster models. Here we present the protection provided by the OTS-modified LAV in the lethal K18-hACE2 transgenic mice model.
Results: The adjustable OTS-modified LAV protected the K18-hACE2 mice efficiently against wild-type (WT) SARS-CoV-2 and other VOCs upon intranasal administration. Furthermore, LAV immunization resulted in faster virus clearance after SARS-CoV-2 challenge compared to mRNA vaccines, a block of transmission of WT SARS-CoV-2, and significantly reduced transmission of currently circulating variants.
Conclusion: To conclude, OTS-modified SARS-CoV-2 LAVs represent a new generation of live vaccines that are intranasal administered at the natural site of infection, provide efficient and innovative infection control, and conceptionally are readily applicable to many other emerging viruses.

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WS72 – INNATE IMMUNITY IN CANCER II

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1735 – WS72.1

Dissecting molecular drivers of NK cell dysfunction to enhance anti-tumor activity

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Purpose: Natural Killer (NK) cells are a critical arm of the innate immune response, serving as a powerful weapon against viral infections and cancer. However, approximately 13±6% of NK cells do not express classical inhibitory receptors during their education and are rendered naturally 'anergic,' exhibiting reduced effector functions. The molecular cascades regulating NK cell anergy remain mainly unknown. Furthermore, unlike T-cells, in which the dysfunctional immune cell state in tumors, known as 'exhaustion,' has been extensively studied, this state is not well characterized in NK cells. It is also unclear whether NK cell 'exhaustion' is completely distinct from 'anergy.' Our study aimed to unravel the molecular mechanisms and etiology that drive NK cell dysfunction, providing insights for manipulating these cell populations to enhance anti-tumor responses.
Methods involved RNA sequencing and protein-level analysis. This was followed by the utilization of 3D culture systems, in vivo pancreatic cancer models, and lipid-based nanoparticles (LNPs) to reprogram 'anergic' and 'exhausted' NK cells in their respective microenvironments. This was achieved by gene silencing the intrinsic regulators identified to govern this dysfunction, demonstrating the potential for reprogramming NK cells.
Results: Here, we profile the dysfunctional phenotypes and identify the transcription factor, Egr2, and diacylglycerol kinase, DGKα, as key intrinsic regulators of NK cell dysfunction. Egr2 silencing restored anergic NK cell cytotoxicity through the EGR2-DGKα-MAPK-pERK axis, potentially modulating SHP-1 activity and calcium levels. Utilizing a nanoparticle-based method, we reprogram dysfunctional NK cells in their native milieu, boosting their anti-tumor activity. Our data imply commonalities between the dysfunctional states 'anergy' and 'exhaustion.'
Conclusion: Targeting the transcription factor Egr2 represents a promising approach to rewiring the overall functional circuitry of NK cells, potentially overcoming dysfunction-associated transcriptional imprints, and augmenting the efficacy of immunotherapies. This research is pivotal in elucidating and restoring the anti-tumor activity of dysfunctional NK cells, offering crucial insights into their intricate biology within solid tumors and unlocking the full potential of NK-based therapies. Regulation of NK cell dysfunction at the molecular and transcriptional levels potentiates the development of next-generation immunotherapies for chronic infections and cancer.

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259 – WS72.2

Anti-CD20 mediated apoptosis in B-cell lymphoma impairs "don't-eat-me" CD47 signaling and boosts Fc receptor-mediated phagocytosis

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The IgG1 anti-CD20 monoclonal antibody rituximab (RTX) is a well-established treatment for B-cell non-Hodgkin lymphomas (NHLs). However, not all B-cell NHLs respond to RTX treatment. Thus, there is an urgent need for new strategies to enhance RTX-mediated anti-tumor immune responses.
The efficacy of RTX depends crucially on the phagocytosis of antibody-opsonized tumor cells by monocytes/macrophages. The phagocytic process is regulated by the "don't-eat-me" CD47 molecule, which dampens the phagocytic activity. In this study, we demonstrate that by impairing CD47 signaling, we can significantly enhance RTX-induced phagocytosis of human $CD20^+$ B-cell lymphoma cells by human monocytes.
Our results reveal that an IgG2 isotype of RTX (RTX-IgG2) in combination with RTX-IgG1 or RTX-IgG3, can significantly induce enhanced phagocytosis of $CD20^+$ B-cell lymphoma cells in comparison with single use of RTX-IgG1 or RTX-IgG3. This effect was associated with CD20-dependent apoptosis by RTX-IgG2. Likewise, the apoptosis inducer staurosporine (STR) could enhance phagocytosis of lymphoma cells when combined with RTX-IgG1 or RTX-IgG3. Notably, we observed that apoptosis mediated by RTX-IgG2, as well as by STR, downregulated CD47 expression on the lymphoma cells. Indeed, microscopic analysis revealed that the expression of CD47 was decreased and scattered in patches on the cell membrane of RTX-IgG2 or STR-treated lymphoma cells. In contrast, RTX-IgG1 and RTX-IgG3 did not induce apoptosis and alteration of CD47 expression on the lymphoma cells. Aligned with this finding, we show that CD47-blocking antibodies, either with a functional or silenced Fc domain, on lymphoma B-cells enhance the phagocytic activity induced by RTX-IgG1 or RTX-IgG3 in monocytes.
Based on this principle, we further demonstrate that RTX-IgG2 can enhance the efficacy of other tumor-targeting antibodies, such as IgG1 anti-PD-L1, in stimulating phagocytosis of $CD20^+$ B-cell lymphoma cells.
In summary, our study demonstrates that RTX-IgG2 enhances Fc receptor-mediated phagocytosis of $CD20^+$ B-cell lymphoma cells through CD20-dependent apoptosis and downregulation of CD47. This finding emphasizes the potential of RTX-IgG2 as a valuable agonist in B-cell NHL treatment. Combining RTX-IgG2 with RTX-IgG1, or potentially any other tumor targeting antibody, offers an exclusive target-specific approach to boost the efficacy of $CD20^+$ B-cell lymphoma therapies.

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75 – WS72.3

Lipid-Associated Macrophages Are Induced by Cancer-Associated Fibroblasts and Mediate Immune Suppression in Breast Cancer

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Purpose: Tumor-associated macrophages (TAM) play a detrimental role in triple-negative breast cancer (TNBC). In-depth analysis of TAM characteristics and interactions with stromal cells, such as cancer-associated fibroblast (CAF), could provide important biological and therapeutic insights.
Methods and Results: Here we identify at the single-cell level a monocyte-derived STAB1+TREM2high lipid-associated macrophage (LAM) subpopulation with immune suppressive capacities that is expanded in patients resistant to immune checkpoint blockade (ICB). Genetic depletion of this LAM subset in mice suppressed TNBC tumor growth. Flow cytometry and bulk RNA sequencing data demonstrated that coculture with TNBC-derived CAFs led to reprogramming of blood monocytes towards immune suppressive STAB1+TREM2high LAMs, which inhibit T-cell activation and proliferation. Cell-to-cell interaction modeling and assays in vitro demonstrated the role of the inflammatory CXCL12-CXCR4 axis in CAF-myeloid cell cross-talk and recruitment of monocytes in tumor sites.
Conclusion: Altogether, these data suggest an inflammation model whereby monocytes recruited to the tumor via the CAF-driven CXCL12-CXCR4 axis acquire protumorigenic LAM capacities to support an immunosuppressive microenvironment.

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107 – WS72.4

Wiring the transcription factor network to prime natural killer anti-tumor immunity in triple-negative breast cancer

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Background: Natural Killer (NK) are characterized by high transcriptional plasticity which is tightly connected with their effector functions. We and others demonstrated that some epigenetic drugs, such as BET inhibitors, drive an efficient immune response by modulating NK transcriptional programs and enhancing their overall anti-tumor response. This is particularly relevant in Triple-Negative Breast Cancer (TNBC) in which we demonstrated that NKs are the principal immune components that predict a divergent patient response to neoadjuvant chemotherapy. Thus, the stimulation of NK activation in tumor microenvironment may be a promising strategy to improve patient outcomes.
Purpose: In this work, we investigated how to prompt NK effector functions by modulating key Transcription Factors (TFs) to obtain transcriptional reprogrammed NKs with enhanced anti-tumor immunity.
Results: We explored the intracellular dynamics leading to NK activation elicited by BET inhibitors and applying RNA-Sequencing. Enrichment analysis of de-regulated genes (DEGs) revealed that immune-checkpoints (ICs) and other inhibitory receptors (KIRs) were the most down-regulated by the drugs. Top-scoring DEGs were integrated with data collected from our TNBC patients, as well as with established datasets of activated NKs. Gene promoters were in silico scanned with FIMO algorithm to predict upstream regulators. Our top-scoring TF was SMAD3, which we demonstrated to induce IC/KIR expression in NKs by the transcriptional cooperation with the epigenetic reader BRD4. Chromatin immunoprecipitation confirmed SMAD3/BRD4 enrichment on IC/KIR promoters and SMAD3/BRD4 inhibition was sufficient to reduce IC/KIR expression in NK92 and patient-derived NKs. Besides SMAD3, we identified other candidates TFs: VEZF1, RXRA, and MAZ. We further investigated these TF-dependent transcriptional programs merging our single-cell RNA-sequencing data with public datasets obtained from TNBC patients treated with IC inhibitors. We confirmed that the NK gene signature associated with our candidate TFs predicted a different immunotherapy response and NK activation is required to improve patient outcome.
Conclusions: Our results point out that the modulation of NK transcriptional programs can be a novel tool to potentiate NK effector functions toward TNBC. We identified several TFs that are putative master regulators of NK activation, opening novel opportunities to exploit them in the design of NK adoptive cell therapies in solid tumors.

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2235 – WS72.5

PD-L1 ligation enhances NK cell anti-tumor function by inducing a metabolic shift

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Programmed death ligand 1 (PD-L1), abundantly expressed on tumor cells, is a pivotal target for immunotherapy investigations. PD-L1 blockade is efficacious in a broad range of malignancies, intriguingly, including PD-L1neg tumors. Natural killer (NK) cells are well understood to be a vital mediator of innate immunity against viral infection and malignancy and have recently been shown to express PD-L1 upon activation. Here, we elucidate the functional role of PD-L1 on circulating and tumor-infiltrating NK cells in patients with various hematologic and solid malignancies, as well as in murine tumor models. PD-L1+ NK cells can engage with therapeutic anti-PD-L1 antibody atezolizumab, soluble PD-1 or PD-1 expressed by T cells or tumor cells, and its ligation significantly enhances NK cell-mediated tumor clearance. Surprisingly, the increased anti-tumor immunity is not the result of increased NK cell degranulation, but due to improved migration into tumors via the CXCR3 chemokine pathway and cytoskeletal dynamics, enabling better synapse formation with tumor cells. Furthermore, PD-L1 ligation in NK cells induces a metabolic shift from glycolysis toward fatty acid oxidation with increased expression of CPT1A and fatty acids uptake. This metabolic shift was essential for the atezolizumab-mediated effect, as tumor-bearing mice with the NK cell-specific deletion of CPT1A failed to respond to treatment. This feature is specifically advantageous in glucose-deprived tumor microenvironments (TME) of highly glycolytic tumors, where T cell function is compromised, as we showed using the tumor model overexpressing the GLUT1 glucose receptor. Taken together, our work demonstrates that PD-L1 ligation not only enhances NK cell cytotoxic function but also increases tumor infiltration and helps overcome challenging TME conditions, resulting in a more effective anti-tumor function. These findings underscore the potential of targeting PD-L1 to augment NK cell-mediated anti-tumor responses. By enhancing NK cell function and infiltration, PD-L1-directed strategies offer promising avenues for improving cancer immunotherapy outcomes.

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886 – WS72.6

ERAP1 controls NK cell responses by regulating peptide-dependent interactions between HLA class I and NK cell inhibitory receptors in hematological malignancies

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Background: Endoplasmic Reticulum Aminopeptidase 1 (ERAP1) plays a crucial role in trimming antigenic peptides for presentation by HLA class I molecules. It is highly expressed in many cancers, including leukemia and lymphoma. Inhibition of ERAP1, either genetically or pharmacologically, activates NK cells by disrupting the engagement of NK cell inhibitory receptors with their HLA ligands. Particularly sensitive to ERAP1 inhibition is the HLA-Bw4 B51:01 allele, which is recognized by KIR3DL1.
Purpose: This study aims to investigate whether inhibition of ERAP1 affects interactions with other HLA-NK inhibitory receptors pairs, potentially offering a novel approach for NK-cell-based cancer therapy.
Methods: A panel of leukemia and lymphoma cell lines, which express both ERAP1 and HLA-Bw4 alleles, was selected for ERAP1 silencing. Western blotting analysis confirmed the generation of ERAP1 knockout cell lines. The response of NK cells to ERAP1 depletion was assessed by functional killing and degranulation assays using primary NK cells (from 10 healthy donors) and established NK cell lines. The interaction between ERAP1-KO cells and NK cell inhibitory receptors was also examined through the binding capability of fusion proteins.
Results: ERAP1 depletion did not significantly alter the expression of HLA class I molecules on the cell surface. However, it led to increased killing and degranulation of NK cells. KIR^+ NK cells showed strong and consistent degranulation in response to ERAP1-KO targets in all healthy donors. Furthermore, the binding of NK inhibitory receptor-Fc fusion proteins to ERAP1-KO cells was weaker.
Conclusion: Inhibition of ERAP1 regulates interactions between NK cell inhibitory receptors and HLA class I molecules on target cells. This altered recognition between receptor and its ligand increases NK cell cytotoxicity reactions. Overall, these findings suggest that ERAP1 inhibition could be a promising therapeutic approach in hematological malignancies.

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WS73 – VIRAL IMMUNITY II

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515 – WS73.1

Developing intracellular antibodies that harness innate antiviral mechanisms to prevent HIV-1 infection

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The innate immune system consists of various sensors and mechanisms to detect invading viruses. This includes intracellular receptors that recognize and degrade the incoming virus. TRIM21 is an intracellular antibody receptor involved in the intracellular immune response. The TRIM21 protein interacts with an antibody-coated virus and targets it for proteosomal degradation involving the E3 ubiquitin ligase complex. This highly specific antibody-TRIM21 technology holds great promise for the engineering of therapeutic antibodies to prevent viral infections via targeting of host or viral proteins. Here we investigated the potential of this TRIM21 technology in order to develop intracellular immunity against HIV-1 infection.
We designed nanobodies, based on llama antibodies, targeting the HIV-1 capsid protein p24, fused to the human IgG1 Fc domain. These p24 nanobody-Fc constructs were synthesized, and produced by a mammalian expression system. Next, they were characterized by flow cytometry and tested for their functionality to bind p24 through an ELISA-based binding assay. Lastly, the p24 nanobody-Fc constructs were stably expressed intracellularly in the U87-CD4-CCR5 cell line
Our data show that these Fc-modified nanobodies recognize intact HIV-1 capsid and have a functional Fc domain. Notably, HIV-1 p24 capsid was neither detected intracellularly nor extracellularly after HIV-1 exposure in the p24 nanobody-Fc expressing cell-line, even though HIV-1 had integrated in the genome at levels comparable to control cells. These data strongly suggest that the Fc-modified p24 nanobody degrades p24 and thereby prevents virus production.
To conclude, we have developed a novel p24 nanobody construct that efficiently prevents HIV-1 infection. Intracellular production of Fc-modified p24 nanobodies highly reduce p24 production levels in U87-CD4-CCR5 cells upon infection with HIV-1. This technology has a wide variety of potential applications in providing TRIM21-mediated intracellular immunity against virus infections.

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358 – WS73.2

ULBP2-expressing HCMV mutants affect NK cell function and activation

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Purpose: HCMV infection elicits protective T and NK cell responses. Patients with inherited or acquired T/NK cell impairments, e.g., recipients of organ transplants under immunosuppression, are at high risk of HCMV infection. A protective HCMV vaccine – that is currently not available – would not only greatly reduce the risk for HCMV reactivation and disease in those patients, but also lower the incidence of graft rejection and is therefore of top priority. We hypothesize that a live-attenuated vaccine will elicit a broader and therefore more efficacious immune response. In this work, we aim to achieve immune cell activation as well as viral attenuation by generating HCMV strains expressing the NKG2D ligand ULBP2.
Methods: ULBP2-expressing HCMV mutants were constructed using either the TB40 (ΔUS2-US6) or theTB40R containing the immune-evasins US2-US6 and replace the UL16 region by ULBP2 which is expressed under a weak or strong promotor. Fibroblasts were infected with the respective virus mutants and analyzed by flow cytometry regarding ULBP2 and NK ligand expression. Cocultures of infected HFF with NK cells revealed immune cell activation and viral dissemination. Moreover, cytotoxic function of NK cells was analyzed by degranulation assays via CD107a expression.
Results: Fine-tuned, increased surface expression of ULBP2 was observed for the weak- and strong ULBP2-expressing mutants, but not in parental strains due to UL16-mediated retention. High amounts of soluble ULBP2 (sULBP2) were shed into supernatants also reflecting the promoter strength of ULBP2. However, sULPB2 did not affect NK cell cytotoxicity. In viral dissemination assays, trends became observable that NK cells exhibit improved control regarding virus transmission upon contact to ULBP2-expressing infected cells. Furthermore, ULBP2 expression in HCMV-infected HFF resulted in a fine-tuned NKG2D downregulation depending on ULBP2 cell surface expression levels, confirming graded interaction between NKG2D and ULBP2. Moreover, NK cells get activated by the ULBP2-expressing mutants as evidenced by the upregulation of CD69. Moreover, the expression of ULBP2 significantly enhanced NK cell cytotoxicity indicating a beneficial effect of ULBP2 expression regarding NK cell function.
Conclusion: Overall, expression of NKG2D ligands like ULBP2 to achieve NK cell attenuation represents a promising approach for a live-attenuated HCMV vaccine development.

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573 – WS73.3

Antiviral protective signature of Vδ2 T cells in pediatric patients after αβ/CD19-depleted hematopoietic stem cell transplantation

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Background: γδ T-cells represent key players in the immune-surveillance in the first months after TCRαβ/CD19-Depleted Haploidentical Stem Cell Transplantation (haplo-HSCT). Although encouraging data are available about the impact of Vδ2-targeting-therapy in improving haplo-HSCT clinical outcome, no data about the antimicrobial effectiveness of Vδ2 T-cells are still available.
Purpose: Aim of this study was to explore the potential direct and bystander antiviral activities of Vδ2 T cells after αβ- and B-cell depleted HSCT.
Methods: We performed a prospective study to compare overtime the phenotypic and functional signature of γδ T cells in patients experienced or not CMV reactivation (CMV-R and No-CMV respectively). The area under the curve (AUC) of the CMV-DNA was calculated for each patient. The immunological characterization of γδ T-cells was performed before (on the graft) and after haplo-HSCT pediatric patients (n=20) after 30, 60 and 120 days following HSCT by flow-cytometry. The antiviral activity of Vδ2 T-cells was tested on CMV-infected fibroblasts and the bystander effects on CMV-specific αβ T-cell immunity was analysed by flow-cytometry.
Results: Early after haplo-HSCT, the frequency of Vδ2 T-cells was significantly higher in No-CMV than in CMV-R patients, both at T30 and at T60. These cells were directly correlated with the plasmatic IL-15 and inversely correlated with the CMV-DNA AUC. Interestingly, this difference was already present in the grafts infused in No-CMV and CMV-R patients. The clustering analysis identified a key signature of protective Vδ2 T-cells, expressing CD16, CD45RA, NKG2D and CD107a. Stimulated Vδ2 T-cells from haplo-HSCT produced IFN-γ/TNF-α, increased CD86/HLA-DR expression on B cells and monocytes, and improved the αβ CMV-specific T-cell response. Finally, soluble factors released by activated Vδ2 T-cells inhibit CMV replication.
Summary/Conclusion: Altogether, these results identify an antiviral protective profile of Vδ2 T-cells early after haplo-HSCT and define their bystander activity on antigen presenting cell maturation and on αβ-specific T-cell immunity. These data open a new application of Vδ2-targeting immunotherapy after haplo-HSCT, adding the antiviral to the antitumor potential.

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329 – WS73.4

CD4+ T-cell tissue lymphopenia in human-papillomavirus-driven anal dysplasia in people living with human immunodeficiency virus

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Purpose: People living with human immunodeficiency virus (HIV) face a significant risk of human papillomavirus (HPV)-driven malignancies including cervical cancer and anal cancer (AC), despite receiving antiretroviral therapy (ART). Men who have sex with men (MSM) display poor HPV clearance and frequently develop high-grade squamous intraepithelial lesions (HSILs), precursors to AC. AC incidence continues to rise among HIV+MSM, with a 19-fold increased risk despite reconstituted peripheral CD4+ T-cell counts. The immune mechanisms underlying HSIL transition to AC remain poorly understood. Here we quantified major tissue T-cell subsets according to HPV16 and HIV infection status. We then investigated the local immune transcriptome associated with 1) HSIL clearance (regression) and 2) HSIL persistence in HPV16+HIV+ MSM before malignant transformation.
Methods: T-cell proportions and infiltrative capacity were examined by multiplex-spectral microscopy on 66 anal biopsies from MSM. Using spatial transcriptomics, differences in signalling pathways between dysplastic and peri-lesion regions were assessed in regressive or non-regressive (persistent disease) biopsies from HPV16+HIV+MSM.
Results: Despite ART and reconstituted peripheral CD4+ T-cell counts, total CD4+ T-cell numbers in local tissue were significantly lower (P=0.0135) in HIV+MSM (~300 cells/mm2) compared to HIV-MSM (~700 cells/mm2), with the lowest numbers present in HIV+HPV16+ MSM. This trend was also evident within tissue-resident memory (CD103+T_{RM}) and peripheral (CD103-) CD4+ T-cell subsets. Similar CD8+ T-cell numbers were observed across groups. When assessing T-cell infiltration from the stroma:dysplastic border into dysplastic regions, CD4+ and CD8+ T-cells displayed significantly higher infiltration in HIV+MSM than HIV-MSM (P=0.0205). Spatial transcriptomics revealed divergent molecular signalling pathways. Non-regressive HSILs were characterized by MHC class I/II and positive immune regulation pathways, while regressive HSILs displayed homeostasis signals.
Conclusion: HIV+ MSM exhibit diminished anal mucosal CD4+ T-cell counts, despite heightened infiltration. CD4+ T-cell tissue lymphopenia may be linked to poor cytotoxic T-cell function, resulting in inadequate HPV clearance and AC progression. The transcriptomes of non-regressive HSILs are suggestive of ongoing HPV and/or HIV viral replication. Given current disease burdens in the ART era, understanding cellular and molecular mediators of HPV-related regression is crucial, and may lead to the development of host (e.g. checkpoint-inhibitor) or viral (e.g. siRNA) next-generation therapies.

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974 – WS73.5

Increased levels of intestinal CD103+ cells and plasma IgG/IgA ratio are associated to spontaneous HIV control

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Purpose: Gut is an important tissue compartment of HIV reservoir and one of the main sources of the chronic inflammation found in people with HIV (PWH). HIV elite controllers (EC), are able to control HIV in the absence of antiretroviral therapy (ART). However, specific immune mechanisms associated with this spontaneous HIV control is not completely understood. Therefore, the purpose of the study was to elucidate which characteristics of cellular and humoral immunity are associated to spontaneous HIV control both in peripheral blood and gut.
Methods: Ileum and caecum biopsies and peripheral blood samples from EC (n=3), PWH on ART (ART; n=4) and HIV seronegative people (HD; n=4) were analyzed by multiparametric flow cytometry. Dendritic (DC), natural killer (NK) and T cell markers were measured, including gut homing markers (CD103, integrin β7 (β7+)). Plasma inflammatory markers as β2-microglobulin (β2M) and D-dimer levels were determined by immunoturbidimetric assay and an automated latex enhanced immunoassay, respectively. Lastly, total and HIV-specific IgG, IgA and IgM plasma levels were determined by ELISA in EC (n=20), ART (n=19) and HD (n=13).
Results: EC showed a higher frequency of CD103+ and CD103+β7+ myeloid DCs (mDCs), comparing with ART, in gut. We observed a higher CD103 expressing CD4+/CD8+ T cell ratio in EC, than in ART, in gut and blood. Taking to account all the participants, we found that different mDC subsets were directly correlated to CD4+ T and NK cell frequencies in gut. CD103+ mDCs were also associated to CD103+ CD4+/CD8+ T cell ratio in ileum. In addition, CD103+β7+ mDCs and T cells were inversely associated to plasma inflammatory markers (β2M and D-dimer) and monocyte frequencies in caecum and peripheral blood of EC and HD, but not in ART. Lastly, higher total and HIV-specific IgG/IgA ratio was observed in plasma of EC compared with ART; this ratio was correlated with the frequency of specific DC, NK cell and T cell subsets in gut and blood.
Conclusion: Increased frequencies of specific mDC and T cell subsets in gut, and higher plasma IgG/IgA ratio are associated to spontaneous HIV control and lower inflammatory status in EC.

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448 – WS73.6

CD4+T cells license Kupffer cells to revert the CD8+T cell dysfunction induced by hepatocellular priming

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Efficient priming of CD8$^+$T cell responses against non-cytolytic pathogens like HBV is believed to rely on CD4$^+$T cell help within secondary lymphoid organs, as the immunological dogma would dictate. This hypothesis is supported by an observation in experimentally infected chimpanzees, where CD4$^+$T cell depletion prior infection prevents CD8$^+$T cell priming and leads to persistent infection.
However, where when and how this issue occurs has never been described or mechanistically elucidated.
We took advantage of unique HBV transgenic mouse models, in which we have demonstrated that adoptive transferred HBV-specific CD8$^+$T cells that recognize hepatocellular viral antigens undergo activation and proliferation but fail to differentiate into antiviral effector cells. To understand the extent to which antigen-specific CD4$^+$T cells can help intrahepatic CD8$^+$T cell differentiation, we generated HBV-specific CD4$^+$ TCR transgenic mice (Env126), where all CD4$^+$T cells recognize an I-Ab-restricted T cell epitope of the HBV envelope protein.
Here, we show that the adoptive transfer of Th1-like Env126 effector CD4$^+$T cells in HBV-transgenic mice counteracts the CD8$^+$T cell dysfunction induced by hepatocellular priming, boost their proliferation and stimulate their production of IFN-γ, TNF-α, and Granzyme-B. This enhances CD8$^+$T cell-mediated liver immunopathology and suppresses HBV replication. Surprisingly, we found that dendritic cells are dispensable for the observed effect while on the contrary, Kupffer cells' (KCs) cross-presenting capacity is enhanced after adoptive transfer of Env126 effector CD4$^+$T cells. With multiphoton intravital microscopy we indeed revealed that HBV-specific CD4$^+$ and CD8$^+$ T cells simultaneously interact with individual KCs within the liver, and as such, the restorative process of Env126 T effector CD4$^+$T cells help is impeded upon KCs depletion. We finally identify CD40-CD40L signaling pathway engagement as the key for Env126 T cells-mediated KCs licensing and IL-27 as a potential novel mediator of dysfunctional CD8$^+$T cell rescue.
Our findings underscore the crucial role of antigen-specific CD4$^+$T cells in mitigating the CD8$^+$T cell dysfunction induced by intrahepatic priming through the extra-lymphoid licensing of KCs, revealing a hitherto unexplored dynamic. The described molecular and cellular mechanism behind this effect could lead to the development of innovative immunotherapeutic strategies to eradicate chronic hepatic infections and their life-threatening complications.

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WS74 – TRIGGERS OF AUTOIMMUNE AND INFLAMMATORY DISEASE

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European Journal of Immunology Clinical-Basic-Translational

1700 – WS74.1

Tlr7 bi-allelism defines a functionally distinct B cell subset and drives systemic autoimmunity

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The incidence of autoimmune diseases, like Systemic Lupus Erythematosus (SLE) is markedly increased in women and in 47 XXY Klinefelter syndrome (KS) men, as compared to 46 XY men, possibly in relationship with differences in X chromosome dosage. While in women, one of the two X chromosomes is inactivated (XCI), some genes can escape XCI, leading to increased expression of these genes in female cells. *Tlr7* gene localized on the X chromosome encodes an endosomal TLR recognizing ssRNA from self and non-self, potentially leading to inflammation or autoimmunity. Indeed, expressed at double dose, *Tlr7* is sufficient to trigger systemic autoimmunity in mice. We have recently demonstrated that *TLR7* evades X silencing in an important proportion of immune cells from healthy women and KS men, resulting in enhanced TLR7 protein expression in female PBMCs. TLR7 bi-allelic B cells exhibited enhanced capacity for class switching into IgG+ B cells and plasmablasts in response to TLR7-specific ligands. This observation supports the notion that TLR7 overexpression through biallelism is a candidate contributor to the higher risk of SLE, in women and KS men. To address the causal relationship between *Tlr7* expression from the inactive X chromosome and the development of dysregulated immune responses involved in disease pathogenesis in SLE, we generated an original dual reporter mouse model in order to track *Tlr7* biallelic cells referred as BiA7 B cells in vivo. We will present evidence that BiA7 B cells represent a functionally distinct subset of B cells with enhanced capacity to differentiate into antibody secreting cells and PC. We also developed and validated a genetic model to restrict Tlr7-driven cellular mosaicism, thereby reducing considerably the frequency of B cells with Tlr7 bi-allelic potential and enforcing *Tlr7* mono-allelism in vivo. Using this model in a pristane-induced model of lupus in C57BL/6 background we provide evidence that lack of Tlr7 bi-allelism protects from systemic autoimmunity and the development of anti-Sm/RNP autoantibodies. The results may provide insights into the nature of the mechanism underlying the enhanced female susceptibility to systemic B cell autoimmunity.

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290 – WS74.2

Biological effect of cytokine specific auto-antibodies on immune responses in a healthy population

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The presence and pathological effect of cytokine specific auto-antibodies (C-aAb) has been observed in autoimmune diseases (*Graudal et al., Ann Rheum Dis. 2002; Bradford et al., Cell Rep Med.2023*) and more recently in COVID-19 cases (*Bastard et al., Science 2020*). However, their presence in the plasma of healthy individuals raises questions about their biological effect and impact during different immune responses.
To address this question, we measured levels of c-aAb against IFNα, IFNg, CSF2, IL-1α, IL-6, and IL-10 in the plasma of 1,000 healthy donors of the Milieu Interieur (MI) cohort. Using TruCulture® whole blood stimulations, we observed a significant and strong association of IL-1α c-aAb with LPS induced IL-1α protein levels. LPS and *E. Coli* stimulation conditions showed differential gene expression associated with IL-6 c-aAb, while gene expression after Poly:IC stimulation was associated with CSF2, IFNa and IFNg c-aAb plasma titers. To further investigate the ability of healthy individuals' high-titer c-aAb to impact immune function, we conducted whole blood stimulations from 6 healthy donors in TruCulture tubes pre-loaded with LPS, PolyI:C, R848 as well as a Null control in the presence or absence of plasma containing high titer C-aAb against IFNα, IL-1α, IL-6, or IL-10. We observed widespread association of anti-IFNα and anti-IL-6 c-aAb with both baseline and induced gene expression. This experimental effect on c-aAb gene expressions was cross validated for certain genes in the MI cohort.
Our results demonstrate a biological effect of C-aAb on immune responses of healthy donors, at low concentrations previously thought to be non-neutralizing. Interestingly, their presence strongly impacted the regulation of immune gene expression even in the Null condition. Further investigation of the prevalence of C-aAb and their neutralizing activity in population-based cohorts will provide a better understanding of their role in shaping immune response variability between individuals.

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424 – WS74.3

Exploratory study of the utility of the detection of microRNAs in urine of patients with immune-mediated inflammatory diseases

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Purpose: Micro-RNAs (miRNAs) are non-coding molecules (19-25 nucleotides) that regulate gene expression at the post-transcriptional level. They are detectable in serum, plasma and urine and proposed as diagnostic/prognostic biomarkers of diseases. We have described that miR-19b, miR-26b, miR-19a and miR-143 expression in serum from patients with immune-mediated inflammatory diseases (IMIDs) can be diagnostic or severity biomarkers. However, serial serum measurement is an invasive technique, thus we aim to determine whether it is possible to detect these miRNAs in urine.
Methods: Urine from healthy donors (HD) and urine and serum from patients with IMIDs (early arthritis [EA] and spondyloarthritis [SPA]) were collected. For RNA extraction, 250µl of urine was centrifuged and the supernatant was processed with the miRNeasy-Micro kit (Qiagen), a mixture of the controls UniSp2, UniSp4 and UniSp5 and the carrier MS2. We tested 2µl, 4µl and 6µl of extracted purified RNA for retrotranscription that was performed with miRCURY-LNA RT kit and UniSp6 as control (Qiagen). To determinate the optimal concentration of cDNA different dilutions (1:20, 1:30 and 1:40) were used for quantification of miRNA by qPCR, being performed in triplicate with the miRCURY-LNA-SYBR-Green PCR kit and primers UniSp2, UniSp5, UniSp6, hsa-miR-19a-3p, hsa-miR-19b-3p, hsa-miR-26b-5p and hsa-miR-143-3p. The CT values were analysed with the Bio-Rad CFX and normalised according to $2^{\Delta CT}$. Differences between groups was performed by Mann-Whitney test.
Results: No differences in the efficiency of retrotranscription of UniSp controls were observed with different concentrations, so the 2µl RNA extract was chosen. The cDNA dilution factor does not affect UniSp expression values, however, the 1:20 dilution was chosen trying to compensate the lower RNA extract. miRNA expression was 25-50 times higher in serum versus urine. However, miRNA expression in urine from IMIDs patients was higher than in HD. The expression of miR-19b, miR-19a and miR-26b was higher in EA and miR-19a and miR-143 in SPA compared to HD ($p<0.05$).
Conclusion: The presence of miR-19b, miR-26b, miR-19a and miR-143 has been detected in urine, indicating that their study as biomarkers in IMIDs is possible. This offers the advantage of using a non-invasive sample type for the screening/monitoring of IMIDs.

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1182 – WS74.4

Presence and Characterization of Circulating Endothelial Cells in Individuals-at-Risk and patients with Rheumatoid Arthritis

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Purpose: Rheumatoid Arthritis (RA) is known to be associated with an increased risk of cardiovascular comorbidity which is characterized by endothelial dysfunction resulting in circulating endothelial cells (CECs) within the vasculature. Though previous literature has identified the presence of CECs in RA circulation, little is known regarding their phenotypic profile and role in RA pathogenesis. We aim to identify and characterize CECs in the circulation in healthy controls (HC), RA, and in 'individuals-at-risk' (IAR) of developing RA.
Methods: Blood was obtained from HC, IAR, and RA patients and multiparametric flow-cytometry analysis (23-color panel) was performed. RA patients were further stratified between ACPA+ vs ACPA. CECs were identified as CD45-PDPN-CD146-CD31+ and subsequent analysis of phenotypic markers was quantified by flow-cytometry.
Results: CECs had a significantly higher frequency in RA patients compared to HC ($p<0.05$) with a similar increase observed in IAR, with no difference demonstrated between ACPA+ vs ACPA-. Expression of adhesion and cell-growth markers ICAM-1,-CD34, and-YAP had a trending increase in RA compared to HC. IAR demonstrated significantly higher expression of ICAM-1 compared to HC ($p<0.01$). A stepwise increase in expression of CD34 and YAP was observed from HC>IAR>RA. Stratification of RA patients demonstrated significantly higher frequency/expression of YAP in ACPA+ vs ACPA- ($p<0.05$). Frequency/expression of immunoregulatory markers CD55,-HLA-DR,-and CD200R1 were all higher in RA compared to HC ($p<0.05$ for CD200R1). A stepwise increase in these markers was seen from HC>IAR>RA ($p<0.05$ for CD200R1). Stratification of RA patients demonstrated increased frequency of CD55 ($p<0.05$),-HLA-DR,-and CD200R1 ($p<0.05$) in ACPA+ compared to ACPA-. These phenotypic changes were paralleled by a shift in the metabolic-profile of the CECs where the frequency of a key metabolic mediator pAKT was demonstrated from HC>IAR>RA ($p<0.05$) with pAKT expression increased in ACPA+ vs ACPA- ($p=0.06$). Interestingly, a significant stepwise decrease was observed for PDGFRa expression from HC>IAR>RA ($p<0.01$), with PDGFRa expression also lower in ACPA+ vs ACPA-.
Conclusions: CECs could act as a potential biomarker in IAR and RA patients of disease onset and progression but may also have implications for greater risk of developing a cardiovascular comorbidity, further contributing to disease pathogenesis.

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1189 – WS74.5

Deciphering the impact of epigenetic imprinting for T cell differentiation in humans using an induced pluripotent stem cell (iPSC)-based in vitro model

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Human induced pluripotent stem cells (hiPSCs) can be generated from any somatic cell, yielding a defined monoclonal cell line with limitless expansion capacity and the ability to be differentiated into any given cell type, including T lymphocytes. However, differentiation efficiency varies greatly among different hiPSC lines and there is no consensus how hiPSC differentiation capabilities are affected by retained genetic and epigenetic "imprinting" from their cellular origin.
In our research, we have successfully reprogrammed human pro-inflammatory CD4+ and CD8+ T cells into stable hiPSC lines (T-hiPSCs) and established a differentiation protocol that mimics thymic T cell development via an hematopoietic stem cell (HSC) intermediate step up to the CD4+CD8+ double positive (DP) stage. Using this differentiation assay, we observed a distinct phenotype when T-hiPSCs were used as starting material compared to other stem cells, such as the early expression of TCR/CD3 and the absence of unwanted "innate-like" CD8αα cells. To investigate the cause of this seemingly superior T cell differentiation potential, we analyze the epigenome of several hiPSC lines as well as sorted intermediary populations from the differentiation model and compare these profiles to the ones generated from their *ex vivo* isolated counterparts (e.g. HSCs from umbilical cord blood, various precursor subsets from human thymus samples). As one important contributing factor, we identified a heterogeneity among generated hiPSC-HSCs with a subset of HSCs displaying enhanced T cell differentiation potential, which was accompanied by a distinct epigenetic imprinting on the DNA methylation level.
Taken together, we generated a large epigenetic dataset, which allows to assess the impact of epi-/genetic imprinting on human T cell development in an hiPSC-based in vitro model. These insights will not only improve our understanding of successful T cell generation in humans, but will also be instrumental to progress towards the hiPSC-derived manufacturing of clinical T cell products for adoptive cellular therapies.

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967 – WS74.6

Interleukin-17-induced granulopoesis alters neuroautoimmune susceptibility in a mouse model of multiple sclerosis

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Despite a 20-year known importance of interleukin-17 (IL-17)-mediated immunity in multiple sclerosis (MS), the decisive pathogenic mechanism of this cytokine remains elusive and under debate. Previous data from our lab suggests a peripheral role of IL-17 early in the disease course. This project investigates the effects of peripherally-mediated autoimmune mechanisms by IL-17 in a mouse model of MS, experimental autoimmune encephalomyelitis (EAE), using multidimensional flow cytometry. In the earliest stages of disease, priming of pathogenic T cells remains unaffected in mice lacking IL-17 (IL-17$^{-/-}$). Additionally, pathogenic functionality is not lost as IL-17$^{-/-}$ T cells initiate disease in wild-type mice using an adoptive transfer model of EAE. Just before disease onset, EAE immunized IL-17$^{-/-}$ mice show an expanded CD4+ T cell compartment in the spleen with increased production of pathogenic cytokines GM-CSF and IFNγ. To investigate whether CD4+ T cells were lacking in their migratory capacity, Rag1$^{-/-}$ mice, which lack functional T and B cells, were reconstituted with IL-17$^{-/-}$ and control CD4+ T cells and susceptibility towards EAE was analyzed. Mice receiving both groups of T cells developed disease, indicating a redundancy of IL-17$^{-/-}$ signaling for T cell migration. Though the IL-17$^{-/-}$ CD4+ T cell compartment mimics EAE-susceptible controls, the myeloid compartment of the spleen and dural meninges is significantly changed in IL-17$^{-/-}$ mice. Monocytes, macrophages, and dendritic cells are increased in the IL-17-deficient environment. Additionally, a shift in neutrophils towards more immature, anti-inflammatory signatures occurs in the IL-17$^{-/-}$ meninges, whereas a more mature, resident neutrophil population is observed in the spleen. Stromal cells have previously been recognized as a potential target of IL-17 signaling in the recruitment of inflammatory myeloid cells. Upon tamoxifen-induced deletion of the IL-17-specific receptor (IL-17RA) on PDGFRb+ stromal cells, EAE incidence and severity is significantly reduced. This phenotype was not observed upon deletion of IL-17RA on a wide range of immune cell subsets. This data suggests that IL-17 signaling promotes the recruitment and maturation of myeloid cells, potentially through stromal signaling, which is significantly alters the susceptibility towards development of EAE.

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POSTER SESSION 1

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P1.01 ADOPTIVE CELL THERAPY

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154 – P1.01.01

Both NK92 cells stably transfected with CD16 and expanded primary NK cells exert potent antibody-dependent cytotoxicity against multiple myeloma cells in vitro and in vivo

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NK92 cells were stably transfected with CD16 allowing for the exertion of antibody dependent cell mediated cytotoxicity (ADCC), hereupon labeled NK92-CD16 cells. Alongside, we also optimized a protocol to activate and expand NK cells obtained from PBMC of healthy human donors, generating expanded NK cells (eNK) with high cytotoxic potential. We tested both these effector cells against a panel of multiple myeloma (MM) human cell lines, alone and in combination with the anti-CD38 mAbs daratumumab or isatuximab, approved for MM treatment. We demonstrated a potent cytotoxic activity on the majority of the cell lines, especially when effector cells were combined with the therapeutic antibodies. The increase in ADCC activity was dependent on the level of CD38 expression in each MM cell line. No difference between daratumumab or isatuximab was detected when using NK92-CD16 cells. In specific eNK cells, the effect of isatuximab was more pronounced so we will study CD16 polymorphisms in these donors. Finally, we performed an in vivo experiment using MM1.S cells transfected with luciferase xenotransplanted in immunodeficient NSG mice. Once MM began to develop, these mice were treated with NK92-CD16 cells in the presence or absence of daratumumab. MM development was significantly inhibited by the combination of NK92-CD16 cells with the therapeutic antibody. These results indicate that the combination of highly cytotoxic NK cells with anti-CD38 antibodies is a plausible therapeutic approach for MM treatment.

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423 – P1.01.02

Tolerogenic dendritic cells manifest strong cellular respiration as evidenced by oxygen consumption and lactate production

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A promising treatment approach for autoimmune diseases is to re-induce tolerance using tolerogenic dendritic cells (tolDCs). While the potential of tolDCs to downmodulate pro-inflammatory pathogenic responses in multiple sclerosis (MS) has been recognized, little is known about their mode of action and optimal culture conditions: Since metabolic pathways have been assigned a crucial role in shaping immune responses, we hypothesize that the immunoregulatory effect of tolDCs can be shaped by adjusting the microenvironment during the culture process. In doing so, we aim to increase the conversion rate of monocytes into tolDCs and optimize the manufacturing procedure.
Human conventional DCs (convDCs) and tolDCs are generated from monocytes in the presence of IL-4 and GM-CSF and stimulated by adding the pro-inflammatory cytokines IL-1-beta, TNF-alpha, and PGE2. The tolerogenic phenotype of tolDCs is induced by 1,25(OH)2 vitamin D3 and confirmed by allo-MLR and flow cytometry. Next, we monitor metabolically relevant parameters, including oxygen (O2) consumption and lactate production, and assess the effect of different culture conditions such as O2 deprivation and daily media change on the cells' characteristics.
Our data show that tolDCs generated in the presence of 1,25(OH)2 vitamin D3 demonstrate reduced expression of the maturation markers CD80, CD83, and CD86 as compared to convDCs, and induce T-cell hyporesponsiveness in an allo-MLR. Additionally, tolDCs manifest significantly increased lactate production and O2 consumption as compared with convDCs. Furthermore, hypoxic culture conditions significantly decrease the viability and conversion rate of the cells, elicit reduced expression of identity markers (CD209, HLA-DR) and maturation markers (CD83, CD80, CD86), and upregulate migration markers (CCR5, CCR7). Functional assays revealed a significantly enhanced migration capacity of tolDCs under hypoxia. Moreover, daily removal of acidic metabolites affects the semi-mature phenotype of tolDCs, but not their tolerogenic function.
Our observations may provide further insights into how tolDCs contribute to tolerance induction by metabolomic reprogramming. Furthermore, these findings might offer a deeper understanding of tolDC fate after patient administration, where O2 levels do not exceed 5%. In summary, a better understanding of tolDC metabolism could lead to new ways of optimizing tolDC-based vaccines by increasing their potential to fight autoimmune diseases.

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485 – P1.01.03

Coculture with human umbilical cord-derived mesenchymal stromal cells facilitates ex vivo expansion of human regulatory T cell subpopulations

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Purpose: Ex vivo-expanded regulatory T cells (Treg) are a promising immunomodulatory therapy but challenges exist in identifying and efficiently manufacturing highly potent Treg for specific applications. Mesenchymal stromal cell (MSCs) are known to promote Treg survival and proliferation. In this study, the influence of co-culture with clinical–grade human umbilical cord-derived MSCs (hUC-MSCs) on yield and phenotype of three distinct Treg subpopulations was investigated.

Methods and Results: FACS-purified, $CD4^{+}/CD25^{hi}/CD127^{lo}$ total human Treg from n=7 healthy donor blood samples were expanded in 96-well plates with anti-CD3/anti-CD28 beads, IL-2 and rapamycin for 14-21 days with and without hUC-MSCs. Treg yield was 4.3±2.0 greater for hUC-MSC-co-cultured (MSC)-Treg than for control (CTRL)-Treg ($p<0.01$) while phenotypic marker expression (Foxp3/Helios/CD39/ICOS/CTLA-4/HLA-DR) and T effector cell (Teff) suppressive potency were similar. The $\%HLA\text{-}DR^{+ve}$ of expanded Treg correlated with %suppression of Teff proliferation. Therefore, three primary Treg subpopulations were FACS-purified from n=4 healthy donor blood samples: Treg-I($CD45RA^{+}/HLA\text{-}DR^{-}$), Treg-II($CD45RA^{-}/HLA\text{-}DR^{+}$), Treg-III($CD45RA^{-}/HLA\text{-}DR^{-}$) and each was culture-expanded in the presence or absence of hUC-MSCs. Under CTRL conditions, expansion was achieved for Treg-I(197.1±198.7-fold) and T-reg-III(26.8±29.6-fold) but not for Treg-II(0.7±0.6-fold). hUC-MSC co-culture resulted in significantly higher fold-expansion of all Treg subpopulations compared to CTRL, but the magnitude of the enhancing effect was substantially greater for Treg-II and Treg-III than for Treg-I. hUC-MSC co-culture also resulted in higher $\%HLA\text{-}DR^{+}$ for all Treg subpopulations, and in higher $\%FoxP3^{+}$ and $\%Helios^{+}$ for Treg-III. All three culture-expanded Treg subpopulations demonstrated potent suppression of $CD4^{+}$ and $CD8^{+}$ Teff proliferation and expression of other functionally-relevant surface markers (CD39/ICOS/CTLA-4) which were not modified by hUC-MSC co-culture.

Conclusion: Co-culture with clinical-grade hUC-MSCs substantially enhances the yields and preserves the potency of human Treg subpopulations in ex-vivo expansion cultures with greatest effect on non-naïve ($CD45RA^{-}$) subpopulations. This can facilitate the identification, functional characterisation and production of Treg subpopulations with distinct therapeutic benefits.

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532 – P1.01.04

The effect of adoptive cellular immunotherapy on solid tumors of a novel human JBT19 UPS sarcoma cell line

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Purpose: Adoptive cellular immunotherapy (ACI) is a promising modality of cancer treatment. However, it often fails in the treatment of solid tumors, primarily due to the inability of immune cells to infiltrate the tumor and effectively eliminate tumor cells. One example of such tumors with low immune infiltration is undifferentiated pleomorphic sarcoma (UPS), which is challenging to treat. This cell line is particularly useful for studying ACI's efficacy because it expresses PD-L1 and collagen. These are ligands for immune checkpoint receptors, PD-1 and LAIR-1, respectively. We have previously described a novel patient-derived UPS cell line, JBT-19. Therefore, we aimed to determine the effect of ACI on solid tumors of a novel human JBT19 UPS cell line.

Methods: We used nu/nu mice with xenografted JBT19 tumors and treated them with *ex vivo*-generated lymphocytes, whose reactivity and cytotoxicity to JBT-19 tumor cells were evaluated by flow cytometry analysis. The ACI efficacy was then evaluated by tumor growth. In addition, we modified the JBT-19 cell line to express GFP protein. Because UPS are highly metastatic tumors, we used GFP-expressing JBT19 cells and confocal microscopy to monitor the metastatic spread.

Results: Firstly, we compared different ways of ACI application and found out that whereas direct i.t. application of *ex vivo*-generated JBT19-reactive and -*in vitro* cytotoxic lymphocytes did not affect tumor growth, their i.v. application delayed tumor growth. However, none of these applications were sufficient to eradicate tumors at the applied dose. Furthermore, we found that JBT19 cells metastasized to lymph nodes independently on the ACI therapy.

Conclusions: Although ex vivo-generated lymphocytes were reactive to JBT-19 tumor cells and effectively killed them *in vitro*, their ability to kill JBT-19 tumor cells *in vivo* was negligible. And despite immunotherapy, the tumor metastasizes to lymph nodes. In conclusion, treating UPS with ex vivo-generated lymphocytes is a promising approach to affect tumor growth. However, the efficacy of this approach still requires optimization and/or additional therapeutic interventions.

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585 – P1.01.05

Tertiary lymphoid structure-related immune infiltrates in NSCLC tumor lesions correlate with low tumor-reactivity of TIL products

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Adoptive transfer of tumor infiltrating lymphocytes (TIL therapy) has proven highly effective for treating solid cancers, including non-small cell lung cancer (NSCLC). However, not all patients benefit from this therapy for yet unknown reasons. Defining markers that correlate with high tumor-reactivity of the autologous TIL products is thus key for achieving better tailored immunotherapies. We questioned whether the composition of immune cell infiltrates correlated with the tumor-reactivity of expanded TIL products. Unbiased flow cytometry analysis of immune cell infiltrates of NSCLC tumor lesions was used for correlations with the T cell differentiation and activation status, and with the expansion rate and anti-tumor response of generated TIL products. The composition of tumor immune infiltrates was highly variable between patients. Spearman's Rank Correlation revealed that high B cell infiltration negatively correlated with the tumor-reactivity of the patient's expanded TIL products, as defined by cytokine production upon exposure to autologous tumor digest. In-depth analysis revealed that tumor lesions with high B cell infiltrates contained tertiary lymphoid structure (TLS)-related immune infiltrates, including $BCL6^+$ antibody-secreting B cells, IgD^+BCL6^+ B cells and $CXCR5^+BLC6^+$ $CD4^+$ T cells, and higher percentages of naïve $CD8^+$ T cells. In conclusion, the composition of immune cell infiltrates in NSCLC tumors associates with the functionality of the expanded TIL product. Our findings may thus help improve patient selection for TIL therapy.

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691 – P1.01.06

Exploring the Impact of Immune Reconstitution on Clinical Outcomes Following Allogeneic Stem Cell Transplantation and CAR-T Cell Therapy

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Introduction/Background: Immune therapies such as allogeneic stem cell transplantation (allo-SCT) and chimeric antigen receptor T (CAR-T) cell therapy are proven treatments for patients whose blood cancers have not responded to chemotherapy. However, up to 50% of patients relapse. The recovery of the patient's immune system, particularly T-cells, significantly influences survival rates.

Materials and Methods: In this longitudinal study, we biobanked peripheral blood mononuclear cells from twenty patients undergoing allo-SCT and from ten patients who received CAR-T cell therapy. We utilised multicolour flow cytometry to analyse $CD4^+$ T-cells, $CD8^+$ T-cells, mucosal-associated invariant T (MAIT) cells, invariant natural killer T (iNKT) cells, $CD56^+$ T-cells, NK cells, monocytes, and subsets of γδ T-cells. In the CAR-T cell therapy cohort, we assessed circulating CD19 CAR-T cells and their exhaustion marker phenotype. Statistical analysis was conducted using R Studio (V4.2.3) and GraphPad (V10.2.0).

Results: Preliminary findings in the allo-SCT group linked slower T-cell recovery and poor outcomes, particularly $CD56^+$ T-cells, known for their potent anti-tumour activities. In the CAR-T cell therapy group, patients achieving a complete response (CR) showed higher numbers of CAR-T cells compared to those with persistent disease (PD). Conversely, the persistent disease (PD) group exhibited higher levels of T-cell exhaustion markers.

Conclusion: Our preliminary findings suggest that tracking immune profiles following allogeneic stem cell transplant could serve as valuable predictors of clinical outcomes. In the CAR-T cell therapy cohort, we observed a potential correlation between sustained CD19 CAR-T cell persistence and favourable outcomes, while the presence of exhaustion markers may signify poor clinical prognoses.

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698 – P1.01.07

Genome

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Chimeric antigen receptor (CAR) T-cell therapy has revolutionised the treatment of leukaemia and lymphoma, but the requirement for viral vectors to deliver the gene encoding the CAR into T-cells results in complex manufacturing procedures, is costly, and raises safety issues due to the risk of insertional mutagenesis. Non-viral delivery methods are therefore being actively explored but need to overcome the challenging nature of T-cells as being *hard-to-transfect* due to numerous biological barriers that limit transgene delivery and expression. The cell-penetrating peptide (CPP) RALA is an amphipathic peptide comprising 30 amino acids that delivers nucleic acids including plasmid DNA (pDNA), mRNA or siRNA into mammalian cells. The positive charge associated with RALA promotes condensation with negatively charged nucleic acids into self-assembling nanoparticles of <200nm that facilitate delivery into cells *via* an endosomal-based mechanism. This project is exploring whether RALA can deliver nucleic acids, such as CAR-encoding genes, into human T-cells and has shown that RALA can deliver both pDNA and mRNA encoding eGFP into the Jurkat leukaemic T-cell line with similar transfection efficiencies (TEs)of approximately 15-30%. Further studies have optimised transfection and culture conditions to increase TEs and cell viabilities. Interestingly, this work also demonstrated 3.53-fold differences in RALA-mediated transgene expression between independent stocks of Jurkat T-cells sourced from the same supplier but cultured in different labs, which provides a novel opportunity to identify and characterise the biological barriers that limit transgene delivery and expression in this cell type. Proteomic analysis of each Jurkat T-cell stock has highlighted notable differences in expression of proteins that influence endosomal trafficking and nuclear import, which suggests that they may limit transgene delivery and expression. Several of these proteins are being characterised in further studies. It is hypothesised that manipulating human T-cells to surmount inherent biological barriers may increase the efficiency of non-viral delivery of nucleic acids, including CAR genes, into human T-cells for clinical purposes.

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733 – P1.01.08

gd T cell therapy for post-transplant cytomegalovirus infection: preclinical proof-of-concept.

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Background: Human cytomegalovirus (CMV) disease remains a major challenge in hematopoietic stem cell transplant recipients (HSCTR) and solid organ transplant recipients (SOTR), associated with recurrence (15-30%) and antiviral drug resistance (5%). Given the limited efficacy of available antiviral drugs, there is a growing interest in developing anti-CMV adoptive cell therapies. Both αβ and Vδ2^{neg} γδ T cells are key effectors of the anti-CMV immune response. Taking into account the limitations of αβ T cell therapies in CMV-seropositive HSCTR receiving a graft from a CMV-seronegative donor (D-R+ HSCTR), and in D+R- SOTR, our goal was to explore a complementary Vδ2^{neg} γδ T cell-based immunotherapy.

Results: Using a clinical-grade protocol, we obtained a robust Vδ2^{neg} γδ T cell (DOT cells) amplification *in-vitro*, from both CMV-seropositive and CMV-seronegative healthy donors. Regardless of CMV serostatus, amplified γδ T cells displayed an activated central memory phenotype, low exhaustion markers, and an overt cytotoxic phenotype. In response to CMV-infected cells, amplified DOT cells degranulated and secreted IFNγ, and altogether controlled viral dissemination *in-vitro*. At the mechanistic level, CMV-induced reactivity was observed independently of the γδTCR, but required the co-stimulatory receptor LFA-1. To evaluate *in-vivo* efficacy, we further derived a mouse γδ T cell expansion protocol from the human protocol, recapitulating the phenotypic features of amplified human γδ T cells. Adoptive transfer of amplified mouse γδ T cells was protective against mouse CMV infection. Finally, CMV-reactive γδ T cells could also be amplified from kidney transplant recipients undergoing a refractory CMV infection, paving the way for an autologous adoptive cell therapy. Amplified γδ T cell maintained their viability and functionality in the presence of immunosuppressive drugs currently used in SOTR such as tacrolimus, ciclosporin, or everolimus.

Perspectives: Altogether, these data provide a proof-of-concept for a future clinical trial of amplified γδ T cells in refractory or resistant CMV disease in SOTR.

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968 – P1.01.09

Generating metabolically superior T cells as a novel immunotherapy to treat solid tumors.

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Harnessing the natural ability of T cells to target and eliminate cancer, adoptive T cell transfer therapy (ACT) has shown promise in inducing clinical responses in a subset of patients. However, the effectiveness is notably hindered when tackling solid tumors, mainly due to the challenging tumor microenvironment (TME). This environment is characterized by a reduction in nutrients, particularly glucose, which limits the availability for T cell function.

Therefore, our objective was to address the metabolic competition within the TME by engineering "metabolically superior T cells" (MSTCs). These MSTCs are modified cytotoxic T lymphocytes capable of utilizing trehalose as a carbon source instead of glucose. Trehalose, a disaccharide utilized by insect cells but not naturally metabolized by human cells, offers an alternative energy substrate. By introducing genes encoding the trehalose transporter (Tret1) and the trehalose-hydrolyzing enzyme (Trehalase-Treh1) from Drosophila melanogaster into T cells, we initiated a new metabolic process. MSTCs thus gain uncompetitive carbon and potentially improve the efficacy of ACT therapy against solid tumors.

We successfully cloned Treh1 and Tret1 genes into a retroviral vector and transduced them into human primary CD8+ T cells. Trehalose metabolic tracing analysis confirmed that MSTCs can utilize trehalose and integrate trehalose-derived carbons into the exact same metabolic pathways as glucose. Furthermore, in glucose-deprived conditions supplemented with trehalose, MSTCs exhibited enhanced survival, proliferation, and cytokine secretion, indicative of sustained effector functions crucial for antitumor immunity. Preliminary in vivo findings reveal a significant improved efficacy in CAR-T cell therapy against glioblastoma, underscoring the potency of this technology.

These promising results suggest that MSTCs engineered to utilize trehalose offering new avenues for enhanced cancer therapy.

Funding resource: This work was supported by grants from the Israel Science Foundation No.883/22 and No.2377/20, and the ICRF–CRI Immunotherapy Project Grant, a medical research grant from Israel Cancer Research Fund and Cancer Research Institute.

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971 – P1.01.10

Exploring DARPins to detect MHC class I restricted antigen presentation

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The global prevalence of Epstein-Barr virus (EBV) exceeds 90%, with significant implications for public health given its association with various malignancies including infectious mononucleosis, contributing to approximately 1.8% of cancer-related deaths. Despite its discovery over 60 years ago, critical knowledge gaps persist regarding the cellular components initiating immune responses against EBV, essential for vaccine and treatment development.

Current methods for detecting EBV epitopes primarily rely on indirect approaches, such as T cell clones, which are laborious, time-consuming, and dependent on epitope immunogenicity. Although investigations into direct detection methods utilizing immunoglobulins like T cell receptor (TCR)-like antibodies are underway, achieving sufficient target specificity remains a challenge.

In this study, we propose employing designed ankyrin repeat proteins (DARPins), engineered proteins with target-specific binding capabilities, as an alternative to immunoglobulins for detecting specific EBV peptides presented on HLA-B35.

As proof-of-concept, we target intracellular EBV antigens via the MHC-complex using DARPins, circumventing the TCR and thus bypassing reliance on the TCR repertoire. We characterize the peptide specificity of selected DARPins through alanine scanning. Compared with T cells reacting to the same peptide, these DARPins display different binding angles. Furthermore, we enhance the epitope binding capacity in a physiologically relevant setting by associating these DARPins with cell membranes using chimeric antigen-like receptors (CARs). Ultimately, we assess the avidity and killing capacity of DARPin-CARs relative to T cell receptors.

In conclusion, we aim to establish DARPin technology as a novel approach for specific detection, diagnosis, and elimination of EBV-infected cells. In addition, our study offers novel insights into T cell and antigen-presenting cell interactions, furthering understanding of immune responses to EBV.

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986 – P1.01.11

CAR-T cell therapy for solid tumors against a carbohydrate target

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Background and Objectives: Ca-10 is a carbohydrate antigen expressed on the murine Ehrlich tumor and recognized by the monoclonal antibody A10, which exhibits reactivity against various human solid tumors. Our prior research demonstrated that mice vaccinated with Ca10 generated specific antibodies to this antigen, leading to growth inhibition of transplanted tumors. In this study, we describe the antigen Ca10 as a feasible target for CAR-T cell immunotherapy against solid tumors.
Methods: The CAR was engineered based on the A10 antibody sequence. The single-chain variable fragment (ScFv) chain was inserted into the pcDNA3.1 plasmid and synthesized in a soluble form using transfected HEK 293T cells. This was done to verify its proper expression and antigen recognition ability, assessed through ELISA and flow cytometry. Jurkat cells and primary CD3+ cells from healthy blood donors were then genetically modified with a second-generation Ca10ScFvCAR construct (Ca10-CAR) via lentiviral transduction. Subsequently, their capability to identify Ca10+ cells was assessed *in vitro*.
Results: The soluble ScFv fragment exhibited precise recognition of the target antigen, both when in a soluble state and when expressed on the surface of Ca10+ tumor cells. Jurkat cells were effectively transduced with the Ca10-CAR construct and demonstrated selective activation when co-cultured with Ca10+ cells, as evidenced by PLCγ phosphorylation and CD69 expression. In human primary T cells, a CAR transduction efficiency of up to 60% was obtained. When co-cultured with Ca10+ cells, Ca10-CAR expressing T cells exhibited specific IFN-γ production and efficiently lysed target cells.
Conclusion: Our results suggest that the antigen Ca10 may be a specific and effective target for the treatment of solid tumors by CAR-T cells.

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988 – P1.01.12

Cytotoxicity of Vδ1 γδ T Cells Isolated from Healthy Donor PBMCs against Colorectal Cancer Cells.

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Purpose: γδ T-cells are associated with favourable patient prognosis in several cancer types and promising cytotoxic lymphocytes for next-generation cancer immunotherapies. γδ T-cells are MHC unrestricted, excellent for allogeneic adoptive cell therapy, and targets of immune checkpoint blockade in MHC-I deficient tumours. Clinical-grade protocols have been developed to selectively expand Vδ1 γδ T-cells *in vitro*. PBMCs, a source of Vδ1 T-cells, provide ease of access and large numbers of cells. This work aimed to expand γδ T-cells from PBMCs and assess cytotoxic tumour killing towards colorectal cancer (CRC) cell lines.
Methods: γδ T-cells from PBMCs were expanded from eight healthy donors. αβ T-cell depletion was followed by anti-CD3 selection. Isolated cells were cultured with IL-15, anti-CD2, and anti-CD3 for 21±1 days, to drive preferential Vδ1 T-cell proliferation. Cells were phenotyped by flow cytometry and donors with >0.5% αβ T-cells at day 21 excluded. Tumour killing assays with expanded immune cells (n=5) and CRC cell lines (HT-29) were undertaken (Effector:Target (E:T) ratios 0:1, 5:1, 10:1, and 20:1). Immune cell products were grouped based on high (n=3) and low (n=2) Vδ1 T-cell proportions at day 21 and high (n=2) and low (n=3) cell number fold increases in total Vδ1 T-cells between days 0 and 21. Tumour cell cytotoxicity between groups was compared.
Results: Expanded cells from seven donors increased from $1x10^6$ to $5x10^6$ cells, of these Vδ1 T-cells increased from 7% to 32%, Vδ2 T-cells decreased from 23% to 10% and αβ T-cells remained <0.5% by day 21. There was more HT-29 cytotoxicity at an ET ratio of 5:1 (25%), 10:1 (33%) and significantly more at 20:1 (38%, p=0.03) compared to control (0%). There were no significant differences in cytotoxicity between groups with high and low proportions and cell number fold changes in Vδ1 T-cells.
Conclusion: γδ T-cells from PBMCs of healthy blood donors that were treated to preferentially expand Vδ1 T-cells demonstrated anti-tumour cytotoxicity. This was not dependent on the proportions of Vδ1 T-cells present or the extent of Vδ1 T-cells expansion of cell number fold change over time.

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1048 – P1.01.13

Epigenetic editing for conventional to regulatory T cell reprogramming

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Regulatory T cells (Tregs) harbor an enormous therapeutic potential as their immuno-suppressive function may counteract overshooting immune responses in an antigen-specific manner. Adoptive transfer of Tregs is emerging as a promising therapeutic intervention to restore immune tolerance in situations of unwanted immune reactions such as autoimmune diseases, organ transplant rejection or graft-versus-host disease. To this end, patient-derived, *in vitro* expanded Tregs have been successfully tested in clinical trials. However, low Treg numbers in peripheral blood, limited expandability, lack of antigen-specific expansion protocols and loss of Treg lineage stability hamper their wide-spread application. Therefore, alternative cellular sources for immune-suppressive Tregs are of great interest.
The demethylated state of the Treg-specific demethylated region (TSDR) in the *FOXP3* gene is a key epigenetic switch to assure sustained expression of the Treg master transcription factor FOXP3. Our group has shown that activation of the TSDR by CRISPR-dCas9-based epigenetic editing induced physiological FOXP3 protein expression levels in human naïve and memory CD4+ T cells. However, this was not sufficient to convey a Treg phenotype. Here, we show that improved epigenetic editing using *in vitro* transcribed mRNA is highly efficient and that after prolonged culture, edited conventional T cells upregulate specific Treg markers and downregulate production of pro-inflammatory cytokines. Importantly, this is also the case in pro-inflammatory memory T cells, which were far believed to be resistant to FOXP3 induction and phenotype switching.
We here suggest mRNA-based epigenetic editing as a new viable tool for the targeted modification of T cell phenotypes, which might be also be exploited for clinical applications in the future.

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In vitro targeting of non-hematological tumour cells by immortalized NK cells engineered with a CD6-based chimeric antigen receptor

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Adoptive transfer of T cells engineered with chimeric antigen receptors (CARs) have shown good efficacy against haematological malignancies, but further developments and optimisations are still needed in the case of solid tumours. One challenge is the finding of the most suitable target, as tumour-specific antigens (TSA) may vary between patients and tumour-associated antigens (TAA) may lead to on-target off-tumour toxicities. CD6 is a lymphocyte receptor expressed by all T cells, and some B and NK cell subsets, whose reported ligands (CD166/ALCAM, CD318/CDCP-1) are overexpressed on malignant cells. Thus, we designed a CD6-CAR encompassing the whole extracellular region of CD6 and the cytoplasmic activation motifs of CD3zeta and CD137/41BB to target CD166-positive and/or CD318-positive tumour cells. In order to avoid some constraints imposed by autologous CAR-T cells (e.g., development of cytokine-release syndrome and immune-effector-cell-associated neurotoxicity syndrome), we explored the alternative use of allogeneic NK cells, which present milder toxicities. To that end, NK cell leukemia KHYG-1 cells were lentivirally transduced and selected for stable CD6-CAR expression. Co-culture assays with double CD166- and CD318-positive cells of ovary carcinoma (SKOV-3) or colon adenocarcinoma (DLD-1) origin showed superior cytotoxic effects for CD6-CAR KHYG-1 cells compared to untransduced (UT) KHYG-1 cells. The analysis of co-culture supernatants also showed higher secretion levels of INF-γ, CCL-4/MIP-1β and granzyme B for CD6-CAR KHYG-1 cells compared to UT ones. By contrast, when performing similar co-cultures with cells of lymphoblastoid (Raji, Daudi) and erythromyeloid (K562) origins, similar cytotoxicity levels were observed for both UT and CD6-CAR KHYG-1 cells. Interestingly, such leukemic cells differ from DLD-1 and SKOV-3 ones in that they show low/negative CD166 and CD318 expression levels as supported by transcriptomic data from the Human Protein Atlas platform. In conclusion, the results support the notion that CD6-CAR KHYG-1 cells will specifically target tumour cells expressing high CD166 and/or CD318 surface levels, which would represent a safety advantage when performing in vivo studies. Accordingly, preliminary cell transfer assays to immunodeficient NSG mice shows no toxic effects upon i.v. infusion with increasing amounts (1, 5 and 10 x10E6) of CD6-CAR KHYG-1 cells. Grants: PREP2022-000394 and PID2022-140932OB-I00 funded by MCIN/AEI/10.13039/501100011033.

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Eosinophil counts in anti-CD19 CAR-T cell therapy. Could they predict efficacy and prognosis?

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Purpose: Anti-CD19 CAR-T cell therapies have achieved complete response rates over 90% in B-cell acute lymphoblastic leukemia (B-ALL). However, one-third of these patients with an initial response, relapse within six months. Several studies have looked for a biomarker that can predict the efficacy of this therapy. Patients with non-Hodgkin lymphoma (NHL) with higher eosinophil counts before or after CAR-T cell infusion have shown better outcomes. However, its significance in B-ALL remains unknown. The aim of this study was to determine whether these findings were reproducible in our clinical trial cohort and to study their significance in ALL patients.
Methods: Thirty six patients in our phase I clinical trial (CART19-BE-01 NCT03144583) who received our own designed anti-CD19 academic CAR-T cell therapy (ARI-0001, varnimcabtagene autoleucel) were included (27 with B-ALL, 7 with NHL and 2 with chronic lymphocytic leukemia). All of them were resistant or refractory to treatment and had a prognosis of less than 2 years. We obtained clinical data of survival and determined eosinophil counts at baseline and one, two and three months after infusion.
Results: In our overall cohort, patients with eosinophil counts higher than the median (13/34) showed improved event-free survival (617.5 vs 349.5 days, p=0.03) and overall survival (783.8 vs 524.7 days, p=0.03). B-ALL patients event-free survival was higher in the group with basal eosinophil counts over the median (6/27) (694.5 vs 329.7 days, p=0.02). However, no differences in survival were observed between the NHL patients.
Conclusion: Eosinophil count could be a survival and prognostic biomarker in patients treated with anti-CD19 CAR-T cell therapy. However, further studies with larger cohorts are required.

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Study on the effect of corticosteroids on lung development with the SSEA1+ stem/progenitor cells derived lung organoid model

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Purpose: The immaturity of the lung is a leading cause of mortality in preterm infants with severe pulmonary complications, such as respiratory distress syndrome (RDS). Antenatal corticosteroids are widely used as a treatment for women at risk of preterm delivery. However, there is only limited evidence showing the detailed mechanisms of antenatal corticosteroids on fetal lung maturation, and the impact of corticosteroids on lung stem cells has not been clarified. Thus, our study aimed to investigate the influence and mechanism of corticosteroids on lung organoid model generated from pulmonary-derived SSEA-1$^+$ stem/progenitor cells, hoping to explore the effect and mechanisms of antenatal corticosteroids on fetal lung development.

Methods: To explore the effects of corticosteroids in fetal lung development, the pulmonary SSEA1$^+$ stem cells were isolated from neonatal mice and embedded in 50% Matrigel. SSEA1$^+$ cells were cultured for 14 days to generate the lung organoids, providing sustained dexamethasone stimulation throughout the process. The effect of steroids on epithelium development was evaluated by qRT-PCR, immunofluorescence, ELISA, and western blot.

Results: The sustained stimulation with dexamethasone did not affect organoid generation but resulted in a slight increase in organoid irregularity. We found that the gene expression of type 1 and type 2 alveolar cell markers increased under the conditions of 100 nM and 1000 nM dexamethasone treatment. In contrast, dexamethasone reduced the expression of *MUC5AC*, the goblet cell marker. However, the expression of basal, club, and ciliated cell markers showed no significant difference between control and dexamethasone stimulation. We will also further clarify the molecular mechanisms involved.

Conclusion: These results demonstrated that corticosteroids promote the differentiation of SSEA1$^+$ stem cells into alveolar-like lung organoids, indicating the beneficial impact of corticosteroids on pulmonary maturation. Consistent with the well-known function of corticosteroids in anti-inflammation, our data also suggested the decrease of mucin production in lung organoids. Despite our initial understanding of corticosteroids in lung development, further researches are required to clarify the long-term and potential side effects of corticosteroids on fetal lung development and health.

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1874 – P1.01.17

Galectin-1-deficient Foxp3+ regulatory T cells show limited long-term protection in experimental inflammatory bowel disease

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Cell therapy with regulatory T cells (Tregs) have been proposed in recent years thanks to their potential to maintain immune homeostasis and its capacity to control unwanted immune responses. Galectin-1 is a β-galactoside-binding protein lectin that exhibits broad anti-inflammatory and pro-resolving activities by targeting different subsets of immune cells. Our previous studies showed that galectin-1 is expressed by regulatory T cells and contributes to their immunosuppressive function. The aim of this study was to investigate the role that galectin-1 plays in the function Treg cells *in vivo*. Remarkably, while basal Treg cell properties and *in vitro* suppression capacity were similar, mice lacking galectin-1 showed enhanced inflammatory responses to DSS treatment compared to galectin-1 sufficient mice although the Tregs/T effector balance was increased in colitic *Lgals1*$^{-/-}$ mice. Additionally, cell therapy studies with Treg cells were conducted using an experimental model of chronic colitis induced by transfer of naïve CD4^{+} T cells in immunodeficient *Rag1*$^{-/-}$ mice. Our results demonstrate that galectin-1 is an effector molecule actively involved in the modulation of intestinal inflammatory responses mediated by effector T cells. Galectin-1 expression stabilizes the immunomodulatory phenotype of the Treg cells, as well as their persistence *in vivo* under inflammatory conditions thus improving the long-term efficacy of cell therapy with Treg cells. Taken together, these findings reveal that galectin-1 is critical for Foxp3^{+} Treg cell function and suggest that the endogenous expression of galectin-1 is required to prevent Treg cell plasticity toward a Th1-like Treg cell phenotype in experimental colitis. These evidences highlight that the expression of galectin-1 should be carefully evaluated in future cell therapy protocols with Treg cells in order to achieve efficient and stable long-term immunosuppression in patients with inflammatory bowel diseases.

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2088 – P1.01.18

Antibacterial activity of KHYG-1 cells stably expressing a lymphoid scavenger-like receptor-based chimeric antigen receptor

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Bacterial infections can lead to severe life-threatening situations (septic shock) mainly as a result of immune-debilitating disorders (e.g. diabetes mellitus, cancer, AIDS), aggressive medical/surgical interventions (e.g., organ transplantation, prosthesis replacements, immunosuppressive drugs), emergence of antimicrobial resistant (AMR) strains, and shortage of new antibiotics in the pipeline of the pharma industry. In this context, there is an urgent need of new strategies for treating severe bacterial infections regardless of their AMR status. Adoptive transfer of either normal or engineered immune cells offers the possibility of potentiating the immune response against infectious diseases. To this end, we have taken advantage of the broad-spectrum microbicidal activity of natural killer (NK) cells and the broad-bacterial recognition properties of CD6 -a lymphoid scavenger-like receptor - to generate NK cells expressing a CD6-based chimeric antigen receptor (CD6CAR). Such CD6CAR encompasses the whole extracellular region of CD6, the transmembrane region of CD8α and the cytoplasmic activation motifs of 4-1BB/CD137 and CD3ζ. Lentiviral particles coding for the CD6-CAR were used to transduce KHYG-1 cells (an immortalised leukemic NK cell line), which were further selected for stable and high CD6-CAR expression by cell sorting. Co-culture assays of CD6CAR$^+$ KHYG-1 cells with Gram-positive (*Staphylococcus aureus and Listeria monocytogenes*) and Gram-negative (*Escherichia coli, Klebsiella pneumoniae, Acinetobacter baumanii and Pseudomonas aeruginosa*) bacterial strains for 2h demonstrated a superior cytotoxic activity with regard to untransduced (UT) KHYG-1 cells, as evidenced by reduced bacterial colony counts and bacterial metabolic activity (XTT assays). These results constitute the first evidence on the prospect of developing an adoptive CD6CAR NK cell transfer immunotherapy against bacterial infection and warrants further *in vitro* and *in vivo* assays to better characterize its efficacy and safety. Accordingly, preliminary cell transfer assays to immunodeficient NSG mice show no toxic effects upon i.v. infusion with increasing amounts (1, 5 and 10 x10E6) of CD6-CAR KHYG-1 cells.

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2135 – P1.01.19

Search and find: Regulatory T cells

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Background and purpose of the study: Cellular immunotherapy with modified regulatory T cells (Tregs) represents a step towards an alternative therapeutic option for patients with autoimmune diseases that restores immune homeostasis and induces regeneration of damaged tissue. Tregs are a rare subset of CD4+ T cells with CD4+ CD25+ FoxP3+ phenotype. Optimization of the isolation and activation of Tregs for therapeutic purposes is required, especially in relevant animal models. Our work addresses the optimization of activation, expansion and phenotypic purity of ex-vivo Tregs.

Methods: Mouse spleens and lymph nodes were harvested from C57BL/6 mice. The Treg population was enriched from the total cells by magnetic separation. The cells were activated and expanded in diverse conditions and monitored. Cell expansion and immunophenotype were evaluated.
Results: The aim of this work was to determine the conditions that lead to a high number of viable cells, a high frequency of Treg cells and the most pronounced FoxP3+ CD25+ Treg phenotype.
Cell expansion peaked between day 4 and 8 and flow cytometry indicated successful Treg enrichment. The efficiency of Treg enrichment varied between conditions.

Conclusions: We have successfully established isolation and expansion protocols for murine CD25+ Foxp3+ regulatory T cells and comprehensive flow cytometry staining panels. During the expansion protocol, the proportion of Treg cells decreases while the proportion of contaminants increases. By comparing different cultivation conditions, we were able to find the condition that yields the highest percentage of Treg cells.

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2237 – P1.01.20

Novel A20 based therapeutic strategies to fight lung cancer

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Background: Lung cancer remains the leading cause of cancer-related mortality globally. Despite immune checkpoint blockade therapy emerging as the primary treatment for lung adenocarcinomas harboring non-targetable oncogenic KRAS mutations, patients still have poor prognosis. This underscores the urgency to enhance immunotherapy-based treatment approaches. Recently, we identified that the systemic downregulation of the anti-inflammatory protein A20, induces a tumor-suppressive microenvironment in mouse models of lung cancer. This identifies A20 as a promising target for immune modulation to improve the effectiveness of immune-based therapies.
Methods: Using mouse models of KRAS-driven lung tumorigenesis, we evaluated the response of the tumor immune microenvironment in response to partial systemic A20 downregulation. In vitro analyses of A20 heterozygous and wild type CD8+ T cells were carried out using flow cytometry and RT-qPCR. In vivo evaluations included survival assays, histological examinations, flow cytometry, and measurement of tumor size.
Results: Our findings revealed that systemic reduction in expression of the immune-modulatory enzyme A20, observed in A20 heterozygous mice, induced modifications in the tumor microenvironment. This led to the formation of an anti-tumorigenic milieu, promoting increased infiltration of $CD4^+$ and $CD8^+$ T lymphocytes, as well as antigen presenting cells. In our *in vitro* studies, we observed that decreased A20 expression in $CD8^+$ T cells enhanced their proliferation capacity and demonstrated greater anti-tumor activity in killing assays compared to wild-type $CD8^+$ T cells. Furthermore adoptive transfer of antigen-specific $CD8^+$ T cells resulted in a more significant reduction in tumor cell growth in A20 heterozygous recipients compared to wild-type recipients. This improved efficacy of T cell transfer can be attributed to the potent anti-tumorigenic microenvironment present in the A20 heterozygous mice.
Conclusion: Our preliminary results demonstrated that systemic downregulation of the anti-inflammatory protein A20 in immune cells of the stroma, enhances the anti-tumor capability of $CD8^+$ T cells and impedes tumor growth in mice. Additional experiments are required to investigate whether A20 heterozygosity in other immune cells boosts their anti-tumor potential. Additionally, we aim to investigate whether a combination therapy involving controlled A20 downregulation in immune cells alongside immune checkpoint blockade effectively limits tumor growth in KRAS-driven lung adenocarcinoma.

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P1.02 ALLERGY AND ASTHMA

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127 – P1.02.01

Quantitative transcriptome analysis of purified equine mast cells identifies a dominant mucosal mast cell population

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Introduction: Equine asthma is a prevalent respiratory disease in horses, presenting challenges to animal welfare and performance. Mast cells (MCs) are implicated in asthma pathogenesis, yet their specific role in equine asthma remains unclear. This study aimed to characterize MCs in equine asthma through transcriptome analysis of bronchoalveolar lavage fluid (BALF) from asthmatic horses.
Methods: BALF samples were collected from three horses with varying degrees of asthma severity. The study used a unique MACS Tyto sorting process to separate MCs from bronchoalveolar lavage fluid (BALF). Expression profiles of MC-specific markers, proteases, surface receptors, enzymes, transcription factors, and immune-related genes were analyzed.
Results: MCs isolated from BALF predominantly exhibited a mucosal MC (MMC) phenotype, expressing high levels of tryptase but lacking chymase and carboxypeptidase A3. The high-affinity IgE receptor (FcεRI) alpha and gamma chains were expressed, while the beta chain expression was low. MCs showed low expression of stem cell factor receptor (CD117/c-kit) and interleukin-33 receptor (ST2). Notably, MCs expressed high levels of MHC class II genes, complement components, suggesting involvement in immune responses. Expression of histamine receptor 4 and leukemia inhibitory factor indicated potential inflammatory functions.
Discussion: Transcriptome analysis revealed a distinct MMC population in the airways of asthmatic horses, characterized by unique expression profiles compared to classical connective tissue MCs. The absence of chymase and low beta chain expression in FcεRI suggests atypical features of equine MCs. High expression of MHC class II genes and immune-related molecules indicates potential roles in antigen presentation and immune modulation. The findings suggest that BALF MCs may contribute to inflammatory responses in equine asthma, emphasizing the need for further research on MC subtypes and their functions in equine respiratory diseases. Overall, this study provides valuable insights into the phenotype and functions of MCs in equine asthma, laying the groundwork for future investigations on MC-mediated mechanisms and therapeutic targets in equine respiratory disease

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137 – P1.02.02

Short Chain Fatty Acids and other microbiota metabolites in Lipid Transfer Proteins Allergy

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Purpose: Microbiota and their metabolites have been increasingly studied for their impact on human health. Short Chain Fatty Acids (SCFA) are small carboxylic acid molecules, with one to six carbon atoms, produced predominantly by gut microbiota, which have relevant biological activity. Furthermore, Ole e 7 and Pru p 3 are Lipid Transfer Proteins (LTP) from olive pollen (*Olea europaea*) and peach (*Prunus persica*), respectively. The prevalence of Pru p 3 allergy is extensively described in Europe and, likewise, Ole e 7 allergy is especially prevalent in Andalucía (Spain). The aim of this study was to elucidate the role of microbiota metabolites in the pathogenesis of allergy disease to LTPs.

Methods: A cohort of 92 allergic patient were recruited at Reina Sofía University Hospital (Córdoba, Andalucía, Spain) and classified into two groups: MONOLE or BISENSITISED, depending on whether they had positive specific IgE to Ole e 7 exclusively, or to both Ole e 7 and Pru p 3, respectively (ImmunoCAP 250; positivity threshold > 0.35 kU/L). Additionally, 31 non-allergic individuals were considered as negative controls. Faecal samples were collected and dehydrated to quantify microbiota metabolites (number of carbon atoms): acetate (C2), propionate (C3), butyrate (C4), valerate (C5), caprylate (C8), methionine (C5), formate (C1) and isobutyrate (C4), using proton nuclear magnetic resonance spectroscopy.

Results: No differences were found between groups regarding demographic variables (age, gender, body mass index, place of residence, pet cohabitation and adherence to Mediterranean diet). The analysis of microbiota metabolites revealed that caprylate was the only significantly different metabolite ($p<0.05$) when comparing the three groups independently, allergic versus non-allergic, and MONOLE versus BISENSITISED (caprylate: 0.0422 μmol/g (non-allergic controls) < 0.0673 μmol/g (MONOLE) < 0.1992 μmol/g (BISENSITISED)). Additionally, we found significant differences when we compared the three groups and allergic versus non-allergic, for both C2:C3 and C2:C4 ratios (C2:C3: 3.26 (non-allergic controls) > 2.65 (MONOLE) > 2.43 (BISENSITISED); C2:C4: 4.62 (non-allergic controls) > 3.18 MONOLE ≈ 3.07 (BISENSITISED)).

Conclusion: These results suggested that microbiota metabolites could be involved in the pathogenesis of LTP allergy, and could be associated with the direction of the allergic march, from non-allergic to bisensitised patients.

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148 – P1.02.03

PLAUR alternative splice pattern analysis in patients with bronchial asthma and rheumatoid arthritis

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Purpose: The *PLAUR* gene encodes uPAR (CD87), a multifunctional protein involved in fibrinolysis, blood coagulation, bradykinin production, and cell adhesion and migration regulation. *PLAUR* undergoes alternative splicing in exons 5 and 6, resulting in shortened uPAR variants. The impact of these splicing variants on immunopathology remains unexplored.

Aim: This study aimed to define *PLAUR* expression and splicing patterns in monocytes, M0 macrophages, and M1 macrophages from patients with bronchial asthma (BA) and rheumatoid arthritis (RA).

Methods: The study included:

- 39 healthy adult controls
- 30 RA patients: 15 with low disease activity (DAS28_CRP ≤ 3.2) and 15 with moderate and high disease activity (DAS28_CRP > 3.2)
- 31 BA patients: 16 with well-controlled asthma (BA-M) and 15 with poorly controlled asthma (BA-S)

Monocytes were isolated from peripheral blood by magnetic separation and activated with M-CSF and IFNγ to promote differentiation into M1 macrophages. RNA from these cells was reverse-transcribed to cDNA. RT-PCR quantified the total *PLAUR* transcripts, and fragment analysis of specific PCR amplicons covering the alternatively spliced exons determined the frequency of splicing isoforms.

Results: Compared to healthy volunteers, patients with poorly controlled BA (BA-S) displayed a statistically significant increase in the ΔE6 T7b variant across all cell types (monocytes, M0, and M1 macrophages). Additionally, M0 and M1 macrophages from BA-S patients showed a significant increase in the ΔE5 T7a variant frequency compared to healthy controls. Significant changes in ΔE6 T7b and ΔE5 T7a splice variant frequencies were observed between BA-M and BA-S M0 macrophages. Both BA-M and BA-S groups exhibited changes in transcript frequency during macrophage differentiation.

In RA patients, significant changes in splice variant frequency were only observed during differentiation (M0 to M1) and not between disease activity groups.

Conclusion: Our findings suggest a role for alternative PLAUR splicing in macrophage differentiation. Additionally, the observed changes in BA patients point towards a potential role of uPAR variants in the pathogenesis of various immunopathological diseases.

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153 – P1.02.04

Lymphocyte transformation test in assessing allergic causality and cross reactivities to glycopeptides and lipoglycopeptides in non-severe cutaneous adverse reactions

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Purpose: Lymphocyte transformation test (LTT) has been reported as a reliable tool to determine vancomycin sensitization in DRESS cases. Cross-reactivity between glycopeptides is controversial and has only been immunologically confirmed by in-vitro testing in a small subset of vancomycin-induced DRESS patients. Our objective is to evaluate the usefulness of LTT in identifying the specific drug causing the adverse drug reaction (ADR) in non-Severe Cutaneous Adverse Reactions and analyze glycopeptide cross-reactivity.

Methods: We performed a retrospective, cross-sectional, descriptive study in 20 patients with delayed adverse reactions in a tertiary hospital (La Paz University Hospital, Madrid, Spain) between 2015 and 2024. Clinical histories were reviewed to determine the results of allergy studies, including skin testing (prick and intradermal with delayed reading and/or patch tests). Skin testing was not performed in cases of organ-specific reactions. Probability scores were assigned using the Spanish pharmacovigilance system algorithm and the Roussel Uclaf causality assessment method (RUCAM) algorithm for the causality evaluation in cases of hepatitis.

Results: 45% of patients (9/20) were positive to glycopeptides or dalbavancin and 40% (8/20) to another drug; hence in 85% of patients an immune causality was proven by LTT.

Regarding patients with exanthematous reactions (10/20), 9 skin tests were made with only 1 positive result, while 50% of LTT were positive. Four patients were positive to glycopeptides or dalbavancin, and 4 patients to other drugs; so LTT allowed to demonstrate the immune causality in 80% of patients.

As for specific-organ reactions (7/10), immune causality was proven in 85.7% of patients (6/7): 3 to glycopeptides and/or dalbavancin and 3 to other drugs.

Cross-reactivity was defined as the positivity of the LTT to at least two drugs despite exposure to only one. Following this criteria, 4 cases were detected: 3 cross reactivities between vancomycin and teicoplanin, and 2 cross reactivities between vancomycin and dalbavancin.

Conclusions: LTT proved to be useful in identifying immune-mediated ADRs to glycopeptides. Cross-reactivity was observed, particularly between vancomycin and teicoplanin, and LTT allowed to identify them. LTT was a valuable instrument to guide physicians in uncertain cases, with unclear clinical manifestations or inconclusive tests.

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167 – P1.02.05

Recombinant Blomia tropicalis allergens for precise differential diagnosis of allergic sensitization

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Background: Around 30% of the general population is affected by house dust mite (HDM) allergy and *Blomia tropicalis* (Blo t) is an important HDM species in tropical and sub-tropical areas. Our goal was the isolation and characterization of allergens from this mite species and to use them for component resolved diagnosis, to distinguish between genuine IgE sensitization to Blo t and *Dermatophagoides pteronyssinus* (Der p).

Methods: We produced wild-type-like recombinant allergens (Blo t 1, Blo t 2, Blo t 5, Blo t 8, Blo t 10, Blo t 13, Blo t 21) by expression in *Escherichia coli* and characterized them by mass spectrometry, circular dichroism analysis and patients' IgE reactivity. Microarrays were produced containing these recombinant Blo t allergens as well as the most important Der p allergens. Sera from HDM allergic patients from the tropics (n=15) and from a temperate climate zone (n=33) were tested for IgE reactivity on the microarray chip.

Results: We found that Blo t 2, Blo t 5 and Blo t 21 were the most important allergens for Blo t sensitized patients. Patients from the temperate climate zone without exposure to Blo t showed strong IgE reactivity to Der p allergens and low IgE reactivity to Blo t allergens, which was inhibited by preincubation with Der p allergens. Patients from the tropics were identified as either Blo t monosensitized or Blo t/Der p cosensitized.

Conclusion: Our results demonstrate that microarrays containing a panel of the most important Blo t and Der p allergens are able to identify the disease-eliciting HDM species, which is a prerequisite for the selection of the allergen-specific forms of treatment for the patients.

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426 – P1.02.06

Impact of crosstalk between Th2 cells, ILC2s and cDC2s on lung eosinophilia in a mouse model of allergic bronchopulmonary aspergillosis.

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Allergic bronchopulmonary aspergillosis (ABPA), a severe form of eosinophilic, allergic asthma is caused by the airborne fungal mold *Aspergillus fumigatus* (*Af*). The predominant immune response to this disease is type 2 immunity-driven, especially T helper 2 (Th2) cells are crucial for the ABPA pathology. However, the contribution of other type 2 immune cells, like ILC2s and cDC2s remains to be investigated. We have established a mouse model that mimics ABPA, which is based on repeated intranasal treatment with a low dose of living *Af* conidia. This model revealed that type 2 innate lymphoid cells (ILC2s) and two distinct type 2 conventional dendritic cell (cDC2) populations are increased in an *Af*-challenged lung. ILC2s appeared to be critical for induction of lung eosinophilia but they were not sufficient because eosinophilia was blunted in Rag1$^{-/-}$ mice despite significant increase of ILC2s. Moreover, deletion of IL-4 and IL-13 from Th2 cells completely ablated lung eosinophilia while deletion of both cytokines in ILC2s had no effect. Unexpectedly, mice with selective deletion of cDC2s showed not only an impaired Th2 and eosinophil response but also reduced numbers of ILC2s in the ABPA lung. We conclude that cDC2s induce both Th2 cells as well as ILC2s, with both cell types being critical for the strong eosinophilia observed in ABPA.

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449 – P1.02.07

Skin mast cells require integrin β1 for their perivascular alignment and induction of contact hypersensitivity

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Purpose: Allergic contact dermatitis (ACD) is a T-cell mediated chronic inflammatory skin disease affecting 20% of Europe's population over their lifetime. As crucial sentinel cells, mast cells (MCs) have critical pro-inflammatory functions in innate and adaptive immune responses due to the immediate release of active mediators, including TNF and histamine. Notably, perivascular MCs exhibit essential functionality for the onset and kinetics of inflammation by the directional release of mediators into the bloodstream. However, the mechanism underlying MC attachment to vessel walls, a prerequisite for the intraluminal degranulation, remains to be fully elucidated.

Methods: Here, we studied the role of the adhesion molecule integrin β1 (Itgb1) on perivascular MC vessel attachment and intravascular degranulation *in vivo*, using a mouse model of conditional Itgb1 deletion in connective tissue-type MCs (CTMCs). Utilizing advanced quantitative high-throughput image analysis techniques to fluorescence microscopy images, we investigated MC morphology and distribution in the ear skin. Furthermore, using the contact hypersensitivity (CHS) mouse model for ACD, we examined the functional significance of Itgb1 in MCs in the context of skin inflammation.

Results: Fluorescence microscopy of Itgb1-deficient mice ($MC^{\Delta Itgb1}$) revealed that Itgb1 is crucial for the spatial distribution of MCs in the ear skin. In detail, the spindle-like morphology, homogeneous distribution, and perivascular alignment of MCs in the perivascular niche along the blood vessels is impaired in $MC^{\Delta Itgb1}$ mice, particularly at arterioles. Moreover, we observed a reduced capacity to degranulate into the blood vessels. Upon CHS, $MC^{\Delta Itgb1}$ mice showed a drastically diminished ear swelling, accompanied by a significant reduction of leukocyte infiltration. In particular, the challenged ear skin exhibited a reduction in the number of neutrophils, macrophages and $CD8^+$ T cells. Furthermore, the lack of Itgb1 in MCs resulted in an impaired degranulation efficiency *in vitro*.

Conclusion: Our findings reveal the pivotal role of Itgb1 in MC distribution, morphology, and perivascular alignment along the dermal blood vessels. Additionally, we highlight its significance in MC degranulation capacity and pro-inflammatory functions in CHS responses.

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500 – P1.02.08

Characterisation of Furin deletion in myeloid cells during allergic inflammation.

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Furin is an important member of the proprotein convertases enzymes family, which modulate the activation of several immune mediators such as TNF, TGF-β and IL-1β. Studies in mice have revealed the importance of Furin in T cell tolerance and homeostasis. Furthermore, it has been reported that mice with Furin deficiency in myeloid cells (LysMCre-fur) have important biological differences in macrophages function during inflammatory responses. Nonetheless, little is understood regarding the implications of Furin deletion within allergic contexts. Therefore, we utilize the LysMCre-Fur mice to induce papain-dependent allergic inflammation characterized by elevated IgE titers. Through comprehensive flow cytometry analysis, we aim to identify the cellular pathways within the myeloid and lymphoid cell compartments in this setting. Finally, using *in vivo* and *ex vivo* functional assays we further characterize myeloid cells in their metabolism, phagocytosis, and migration capabilities. Due to its role in immune modulatory processes, the identification of cellular pathways associated with Furin deficiency in myeloid cells might unravel new therapeutic targets in inflammatory diseases, including allergies and severe cases of viral infections.

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647 – P1.02.10

Effect of diesel exhaust particles on immune response of primary human nasal epithelial cells in HDM-allergic rhinitis patients

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Purpose: House dust mite (HDM) has been known as an allergenic stimulant that affects the skin, upper and lower airways, and the eyes, causing atopic sensitization and allergic symptoms in HDM-allergic patients. The purpose of this study was to investigate whether the immune function was disrupted by diesel exhaust particles (DEPs) in primary nasal epithelial cells from HDM-allergic rhinitis patients.

Methods: PHNE cells from individual subjects with HDM allergy (HDM+) or without HDM allergy (HDM-) were cultured at an air–liquid interface (ALI) to create a fully differentiated, in vivo-like model of the epithelium. Subsequently, they were exposed to DEPs and/or HDM at the apical side of the PHNE during ALI days 10 to 14. The formation and integrity of tight junctions were monitored by measuring transepithelial electric resistance (TEER). The levels of tight junction proteins and epithelial immune molecules were assessed through confocal microscopy and biochemical assays.

Results: When the PHNE were exposed to DEPs and/or HDM at the apical side on ALI day 10 for 24h, 48h, 72h, and 96h, TEER significantly decreased in PHNE of HDM+ subjects after 96h. A similar trend was observed in PHNE of HDM- subjects; however, there was no statistical significance. No difference in pattern was observed between HDM- and HDM+ PHNE in cell viability when the cells were exposed to 50 μg cm^{-2} DEPs and/or 30 μg ml^{-1} HDM for 24h, 48h, 72h, and 96h, although cell viability significantly decreased in response to DEPs but not HDM exposure for 48h, 72h, and 96h in both groups. The expression levels of cytokines expressed in PHNE, such as IL-8, IL-25 and IL-17RC, were significantly changed by exposure to DEPs or HDM for 96h in both HDM- and HDM+ PHNE on ALI day 10, but the pattern of differentially expressed immune molecules differed between HDM- and HDM+ PHNE.

Conclusion: The sensitivity of immune molecules expressed in PHNE in response to DEPs or HDM differed between the HDM- and HDM+ groups. This difference may induce barrier and immune dysfunction of PHNE against environmental pollutants such as DEPs and HDM in HDM+ rhinitis, resulting in nasal symptoms.

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661 – P1.02.11

Specific immunoglobulin E binding patterns to wheat salt-soluble proteins in Thai patients with wheat-induced anaphylaxis

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Purpose: Wheat is recognized as one of the primary eight allergens, contributing to a substantial public health concern worldwide, particularly in Thailand. Symptoms associated with wheat allergy can vary widely, ranging from mild to severe manifestations, including anaphylaxis. Cases of wheat-induced anaphylaxis (WA) have been documented in Thai patients. However, while several studies have linked salt-insoluble proteins, such as gluten, to anaphylaxis, fewer investigations have examined the role of salt-soluble (SS) proteins in this regard. Therefore, the identification of wheat SS allergens could greatly enhance the diagnostic capabilities for WA among sensitized patients in Thailand. Here, we examined the binding patterns of SS proteins in sera from WA patients and performed allergenic determination using immunoblotting and basophil degranulation assays.

Methods: Patient sera were collected from 10 individuals diagnosed with WA, and levels of wheat-specific IgE (sIgE) were quantified using ImmunoCap. SS proteins were extracted from durum wheat flour using a buffer containing salt. Immunoblotting was performed using the patient sera to determine the binding pattern of sIgE to wheat SS protein extract. Major SS proteins were purified using the electro-elution method before allergenic determination by immunoblotting and basophil degranulation assays.

Results: The ImmunoCap results revealed varying levels of sIgE ranging from 3 to 486 kUA/L in the patients. Analysis of the sIgE profiles of wheat SS proteins demonstrated IgE binding to a 40 kDa SS protein in 9 out of 10 profiles, while 8 out of 10 profiles exhibited IgE binding to either a 45 kDa or 38 kDa SS protein. Additionally, the 38 kDa SS protein (SS38) induced more than 60% degranulation of IgE-primed basophils at a concentration of 1 ng/ml.

Conclusion: The wheat SS protein involved in Thai WA patients is localized at 38 kDa (SS38) and is capable of inducing basophil degranulation, indicating an allergic reaction. SS38 may serve as a significant marker for WA in Thai patients, although further experimentation with a larger population of WA patients is necessary to confirm this observation. Further characterization of the SS38 is also warranted for future studies.

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761 – P1.02.12

GM-CSF-primed eosinophils enhance mast cell activation

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Purpose: The Allergic Effector Unit (AEU), consisting of mast cells (MCs) and eosinophils (Eos), has been shown to contribute to chronic allergic inflammation. However, the detailed mechanisms underlying the cellular interaction have not been fully elucidated. In this study, we investigated whether soluble mediators derived from MCs or Eos or physical cell contact can activate them and thus affect their effector status, which may help to understand diseases besides allergies in which both effector cells are involved.

Methods: MCs isolated from human breast skin were activated with anti-IgE or cortistatin-14. MC activation was assessed by β-hexosaminidase and histamine release as well as CD63 expression. MCs or their culture supernatants were added to Eos from peripheral blood of healthy volunteers and Eos activation was assessed by expression of CD69 using flow cytometry. Cytokine-blocking antibodies were used to assess the contribution of MC cytokines to Eos activation. To simulate Eos infiltration into the skin, Eos from peripheral blood of healthy individuals or chronic spontaneous urticaria (CSU) patients were injected into skin explants from healthy donors. Histamine levels were analyzed in the interstitial fluid extracted with hollow microneedles.

Results: Supernatants from MCs activated by anti-IgE or cortistatin significantly upregulated CD69 on Eos. Blocking of GM-CSF significantly reduced CD69, while IL-5 or IL-3 blockade had no effect. MCs treated with GM-CSF-activated Eos supernatants, showed significantly enhanced degranulation, particularly at low anti-IgE stimulation strength. Peripheral Eos from chronic spontaneous urticaria patients significantly enhanced MC degranulation *in vitro* and after injection in *ex vivo* skin resulting in higher histamine release compared to healthy Eos.

Conclusions: Our findings suggest that the bidirectional interaction between MCs and Eos (AEU) could lead to a reciprocal activation cycle driven by physical contact and the release of GM-CSF by MCs. This promotes chronic activation of these two cell types and contributes to disease pathology. Therefore, disruption of the AEU, e.g., by targeting GM-CSF or its receptor, could be a valuable approach for the treatment of chronic allergic inflammation.

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765 – P1.02.13

Global associations of macronutrient supply and asthma disease burden

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Objectives: Global age-standardized prevalence of asthma has decreased over time, in parallel with which, the gross domestic product (GDP) per capita and nutritional landscapes also changed at a global level. Both socioeconomic status and nutritional factors are critical confounder for asthma, but most studies so far neglected to interrogate their correlations and interactions comprehensively. Hence, we aim to systematically investigate the relationship between nutrient supply, a good proxy of food environment, socioeconomic status and asthma disease burden at a global level over time.

Methods: Asthma disease burden, macronutrient (protein, carbohydrate and fat) supply and GDP data covering more than 150 countries around the globe from 1990 to 2018 was collated. Various multi-response generalized additive mixed models (GAMMs) were used to analyze the effects of macronutrient supplies and GDP over time on asthma disease burden.

Results: A model considering the interactions between macronutrient supplies and GDP, with an additive effect of time was favoured. Modelling results showed that carbohydrate supply was most strongly associated with increase of asthma disease burden, while fat supply had an opposite effect, and protein supply conferred less influences.

Conclusions: Globally, carbohydrate supply seems to play a driving role for asthma disease burden while fat supply might be the opposite. This is supported by previous studies about the amelioration of established asthma by ketogenic diet and might be linked to the diet quality. Further in-depth studies are warranted, which will be critical for future clinical research and practice and public health intervention.

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769 – P1.02.14

How nutritional landscapes impact food allergy

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Food allergy is an emerging public health concern and characterizing the propensity towards food allergy is one of the "holy grails" in allergy research. Foods, nutrient environments and host biology are critical determinants for food allergy development. Previous studies mainly focused on the macroenvironments (hygiene hypothesis), the hosts' biological characteristics and specific allergenic proteins. Some important aspects like the intrinsic properties of common trigger foods and their extrinsic interactions with other foods, nutrients and the dietary environments have been neglected.

Leveraging unprecedented epidemiological and nutritional data, we concentrated the overall dietary environment of common food allergens and their intrinsic nutrient compositions. We found that dietary environments, reflected in the supplies of allergenic foods and the general macronutrients, minimally influenced food allergy. Interestingly, lower protein and glycine contents in trigger foods were correlated with reduced allergy.

This work represents the first report to our knowledge to comprehensively investigate nutritional factors impacting food allergies based on the epidemiological and nutritional landscapes. We believe that our findings are of interests to allergists and immunologists. It will inform future allergy studies and clinical management for food allergy and might influence public health dietary recommendations.

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979 – P1.02.18

Peanut-allergic young children after 1 year of oral immunotherapy show changes in their gut microbiota composition and functional potential

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Background: In recent years, oral immunotherapy (OIT) has shown promising results in the treatment of peanut allergy, especially in young children. However, which mechanisms are involved in the OIT and how tolerance is promoted remain unclear.

Purpose: In this study, our aim was to assess whether OIT, already after 1 year, affects the gut microbiota of peanut-allergic young children, and if differences are observed compared to similarly aged children that continue to avoid peanut.

Methods: Fecal samples were collected from 30 peanut-allergic children (1-3 years old), 15 of which received peanut OIT while 15 avoided peanut for a period of 1 year, as part of our larger study named SmaChO (Small Children OIT). Microbial DNA was isolated from fecal samples, and 16S rRNA sequencing was performed using the Illumina NextSeq P1 platform. Fecal water was also extracted and used to stimulate healthy PBMCs, from which the cytokine production was measured by ELISA. For all of the analyses, comparisons at 1 year follow-up between the two groups of children, as well as between baseline and 1 year, within each group, were performed.

Results: After 1 year, the children treated with OIT showed a distinct gut microbiota profile as compared to their untreated counterpart, with an increased richness and more abundant Clostridia bacteria, including *Faecalibacterium* and *Lachnoclostridium*. The trajectory of numerous microbial taxa over time also differed between the two groups, and in general, less changes from baseline were observed in case of no treatment. Furthermore, fecal water from children undergoing OIT prompted a type 1/regulatory cytokine secretion from healthy PBMCs, while fecal water from the peanut avoidance group elicited a type 2 cytokine skew.

Conclusion: One year of OIT in young peanut-allergic children has an impact on their gut microbiota, both in terms of composition and potential function. These observed alterations may be linked with the development of peanut tolerance, while the untreated children continue the path of allergy development.

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1014 – P1.02.19

Comparison of two multiplex arrays for in vitro allergy diagnosis with the reference singleplex method

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Purpose: The aim of this study was to evaluate the analytical performance of the recently commercialized ALEX$^{2®}$ microarray (MacroArray Diagnostics, 117 extracts and 183 molecular components) for *in vitro* allergy diagnosis, to compare it with ISAC® (ThermoFisher, 112 molecular components), the most commonly used array, and with the reference singleplex ImmunoCAP® (ThermoFisher).

Methods: Serum samples of 40 patients from La Princesa Hospital Allergy Unit (Madrid, Spain) with previous ISAC® analysis were tested with ALEX$^{2®}$. Cohen´s kappa (κ), positive percentage agreement (PPA) and negative percentage agreement (NPA) of ALEX$^{2®}$ results compared to ISAC® (as a non-reference standard) were calculated for the 102 common molecular allergens. In 29 of these samples ImmunoCAP® specific IgE (sIgE) results for allergen extracts and components were also available and were compared to ALEX$^{2®}$ in a similar manner. Clinical records and skin prick tests (SPT) data were used to evaluate the in vitro sensitization results.

Results: An overall agreement (OA) of 98% (κ=0,88) was observed between ALEX$^{2®}$ and ISAC®, with 91% PPA and 99% NPA. Similar agreements were found when ALEX$^{2®}$ and ISAC® molecular components were compared with ImmunoCAP® (OA: 95% (κ=0,89), PPA: 94%, NPA: 95% and OA:96% (κ=0,92), PPA: 95%, NPA: 97% respectively). However, OA for extracts between ALEX® and ImmunoCAP® was barely 51% (κ=0,21) with 33% PPA and 100% NPA. The discrepancies were due to plant-food and pollen extracts. All cases had positive SPT and ImmunoCAP® results, together with clinical records suggestive of an allergic reaction. The possibility of cross-reactive carbohydrate determinants (CCD) was considered with only 2 out of 11 discrepant sera (18%) showing a positive CCD result using ISAC®.

Conclusion: Our results show a substantial agreement for molecular components between ALEX$^{2®}$ and ISAC® and between both microarrays and the singleplex ImmunoCAP®. However, a low concordance was found for extracts when ALEX$^{2®}$ and ImmunoCAP® were compared, mainly plant-food and pollen extracts. These findings highlight the need for further evaluation of ALEX$^{2®}$ extracts performance before using this multiplex platform on a routine basis.

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1043 – P1.02.20

Oral tolerance to walnut and peanut evaluated through basophil degranulation

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Purpose: The utility of basophil activation test (BAT) has been proved in the diagnosis of allergic diseases. Furthermore, it has been demonstrated to be useful distinguishing between sensitization and true allergy to peanut and also transient desensitization to sustained unresponsiveness. The aim of this study was to evaluate the predictive capacity of BAT in achieving oral tolerance to walnut and/or peanut.

Methods: In this descriptive prospective study, a total of 14 paediatric patients were included. All of them exhibited allergy symptoms to walnut and/or peanut and underwent oral tolerance between 2021 and 2023. BAT using walnut and/or peanut extracts (ALK Abelló y LETI Prick-Test) at different concentrations (0.1-1,000 ng/mL) was performed, together with serum sIgE to whole extract and the molecular available components (Jug r 1, Jug r 3, Ara h 1, Ara h 2, Ara h 3, Ara h 6, Ara h 8, y Ara h 9; ImmunoCAP250) quantification. All measures were performed before starting immunotherapy and one year later.

Results: Among all, 14/14 (100%) patients exhibited cutaneous symptoms, 2/14 (14.3%) respiratory symptoms and 1/14 (7.1%) digestive symptoms and 2/14 (14.3%) needed adrenaline implementation. Mean age was 10.5±3.3. The 78.6% were men. Of all, 42.9% exhibited allergy to peanut, whereas 64.3% to walnut. sIgE to Ara h 9 increased after one year of immunotherapy (1.8±4.4 kU/L vs. 5.3±12.4 kU/L; p = 0.068), whereas BAT against peanut at all analysed concentrations decreased, but only when using 1 ng/mL of whole extract, it followed a statistical tendency (71.7% vs. 52.4%; p = 0.075). Regarding walnut allergy, these tendencies were not observed. Currently, all patients tolerate nuts.

Conclusions: BAT could be a candidate biomarker predictor of tolerance to peanut, even though more studies with larger cohorts are needed to confirm this hypothesis and extrapolate it to other food allergies.

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1074 – P1.02.22

Development of atopic diseases among offspring of mothers belonging to gestational diabetes mellitus (GDM) risk group

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Objectives: The main aim was to evaluate if the diagnoses of atopic dermatitis, asthma and allergic rhinitis at 1-, 2- and 5- years of age in children were related to maternal GDM. The second aim was to analyse the impact of maternal health characteristics on the development of the abovementioned skin or respiratory disorders in the offspring.

Study design: The follow- up study group of Gestational Diabetes Study (GDS) conducted at Tartu University Hospital, Estonia, between 2014-2020 comprised of 223 mother- children diads. All women taking part in the GDS had at least one risk factor for developing GDM. Information about the diagnoses of atopic dermatitis, asthma, and allergic rhinitis in children at 1-, 2-, and 5- years of age was obtained from Electronic Health Records.

Results: Our results show that maternal pre-pregnancy obesity (BMI> 30) and pathological weight gain during pregnancy play an important role in the development of atopic dermatitis, asthma and allergic rhinitis among the offspring of women belonging to the GDM risk group. Only children of women with GDM needing antidiabetic medications were more prone to the development of respiratory disorders at 2 years of age.

Conclusion: Among pregnant women at risk for GDM, regardless of the GDM diagnosis itself, maternal weight related factors have a significant impact on the development of offspring's atopic diseases. Therefore, besides GDM mothers, greater attention must be paid to women at risk, but not developing GDM- their children seem to be even more at risk of atopic diseases as these women do not receive necessary interventions during pregnancy.

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1224 – P1.02.23

Interleukin-1β signaling is critical to urban particulate matter-induced exacerbation of airway allergic inflammation.

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Purpose: Warnings on health impacts of air pollutants had been reported in clinical surveys worldwide. Past researches showed that the aerosol particles induced aggravation of allergic airway inflammation through exerting type 2 helper T cells (Th2)-biased response and accumulation of oxidative stress. However, underlying mechanisms between lowering the threshold of disease initiation and immunomodulation are not fully understood. In this study, we investigated the particulate matter 2.5 ($PM_{2.5}$)-induced effect on IL-1β and inflammasome-related pathway in asthma setting due to their established role sensing the environmental cues.

Methods: We set up a $PM_{2.5}$ exposure model in addition to murine model of ovalbumin (OVA)-induced allergic airway inflammation without Alum adjuvant. The OVA model included a sensitization phase with subcutaneously OVA injection and a challenge phase through intranasal route. According to grouping, the pollutant particles (collected in March 2019, Kaohsiung, Taiwan) were given weekly during the sensitization phase. Blockade of IL-1β pathway were conducted by neutralizing antibody in both phases. Further *ex vivo* experiments were done to track the cellular source of IL-1β in activated immune cells.

Results: In disease setting, exposure to the particles enhanced lung infiltrations and resulted in more severe eosinophilic inflammation and induction of neutrophilic inflammation. In the circulation, the particles suppressed OVA-specific IgG2a and generated a Th2-biased microenvironment. However, the particles alone had no significant effect on the airway function and histopathology in naive mice. Neutralizing of IL-1β in the OVA model would decrease the lung infiltrations and suppress OVA-specific IgG1 and IgE. Following the results, neutrophils were identified as the potential source of $PM_{2.5}$-enhanced IL-1β in both inflammasome-dependent and independent manner.

Conclusion: Collectively, these data suggested the contribution of IL-1β pathway in $PM_{2.5}$-induced adjuvant effect on airway allergic inflammation. The $PM_{2.5}$-enhaced IL-1β could be secreted by the infiltrating neutrophils in both inflammasome-dependent and independent manner. Blockade of the pathway by neutralizing antibody partially ameliorate the particle-induced impact. Thus, providing a novel coping strategy on pollutant control and further understanding on etiology of asthma.

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1275 – P1.02.24

In vitro characterisation of the role of human macrophage migration inhibitory factor in driving house dust mite mediated trained immunity

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Purpose: Trained immunity (TI) describes a phenomenon by which innate immune cells such as macrophages, acquire an immunological memory resulting in a heightened response following exposure to a non-specific secondary pathogenic stimulus. Triggered by metabolic changes and subsequent chromatin remodeling, TI leads to elevated levels of pro-inflammatory cytokines. TI may be harmful in the context of asthma as the surge in cytokine production could further drive exacerbations in patients. Recent literature shows that increased expression of the pro-inflammatory cytokine, macrophage migration inhibitory factor (MIF) enhances this trained response in macrophages from mice containing the high expressing human MIF polymorphism, in response to the allergen house dust mite (HDM). This study is the first to investigate the molecular mechanisms behind why high human MIF expression leads to an enhanced trained response in HDM-mediated trained immunity *in vitro*.

Methods: Naive bone marrow-derived macrophages (BMDMs) from novel humanized MIF mice with either the low-expressing MIF promoter polymorphism ($CATT_5$) or the high-expressing MIF promoter polymorphism ($CATT_7$) or wild type (WT) litter mate controls, were trained with HDM *in vitro*, prior to being exposed to a secondary heterologous stimulus on day 7. The MIF inhibitor SCD-19 was used to show a MIF-specific role, MTA and HAT inhibitors show epigenetic remodeling occurs, Seahorse extracellular flux analysis shows an altered metabolic phenotype. To translate these findings to a clinical setting, monocyte derived macrophages (MDMs) isolated from serum from both healthy and severe asthma patients were investigated for trained immunity.

Results: HDM-trained $CATT_7$ BMDMs show significantly enhanced production of proinflammatory cytokines such as IL-6, IL1β and TNFα in response to secondary insults with LPS in comparison to both $CATT_5$ and WT BMDMs. Similar results were seen in patient samples, with increased MIF expression correlating to amplified trained responses. As well as having increased pro-inflammatory cytokine production, trained macrophages have an altered metabolic phenotype post HDM-stimulation.

Conclusion: High human MIF expression correlates to an enhanced HDM-induced trained response which may drive further exacerbations in asthmatic patients.

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1312 – P1.02.25

Development of monoclonal antibodies against cat allergen Fel d 3 and their application for the analysis of cat allergen extracts

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Cats rank among the most popularly owned companions globally. They are a major source of allergens and their continuous exposure to allergic individuals leads to the development of allergic rhinitis and asthma. Presently, allergen extracts of low precision, consisting of naturally occurring mixed allergen components and other biomolecules, remain commonly employed for diagnosing allergy and administering immunotherapy. The principal challenges associated with these extracts include inconsistencies in composition between different manufacturers and even production lots, and a deficiency in standardization. The quantification of specific allergen components within these extracts could be achieved through immunoassays utilizing allergen-specific monoclonal antibodies (MAbs). Such thorough examination of allergen extracts could serve as a foundation for standardization and subsequent enhancement of the product.

In response to the growing demand for the standardization of allergen extracts, cat allergen Fel d 3 fused with maltose-binding protein (MBP) was synthesized in *E. coli*. The allergen was purified using affinity chromatography of MBP-passenger proteins. Then the hydrolysis reaction was optimized to detach MBP and recombinant Fel d 3 was purified using two-step affinity Ni-NTA and ion exchange chromatography. The antigenic similarity of recombinant Fel d 3 with a native allergen was confirmed by its reactivity with the IgE from blood serum specimens of patients with diagnosed cat allergy by indirect ELISA. Then Fel d 3-specific murine MAbs were generated by hybridoma technology. They were purified by protein A affinity chromatography, characterized by immunochemical methods and a highly specific sandwich ELISA for the quantification of Fel d 3 was developed. Lastly, sandwich ELISA was used to measure the precise concentration of Fel d 3 in cat allergen extracts from different manufacturers and source materials – epithelium, hair and dander. The lowest content of Fel d 3 was in the extracts from epithelium material. Our study demonstrates the significant differences between allergen extracts from different manufacturers or source materials and demonstrates the MAbs being an applicable tool for the analysis of allergen extracts which leads to better standardization opportunities.

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1359 – P1.02.26

Macropinocytosis is the principal uptake mechanism of antigen-presenting cells for allergen-specific virus-like nanoparticles

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Purpose: Virus-like nanoparticles (VNP) are regarded as efficient vaccination platforms and have proven to be useful for the non-anaphylactogenic delivery of allergen-specific immunotherapy in preclinical models. In fact, we previously showed that *Moloney* murine leukemia virus-derived VNP, containing a shielded version of the major mugwort pollen allergen, were hypoallergenic and induced immunotolerance. The ability of antigen-presenting cells (APCs) to take up VNP has been a curiosity of substantial practical significance, however, the physiological principles governing particle uptake remained elusive as of yet.

Methods: Herein we sought to determine the mode of VNP uptake by APCs. Accordingly, we screened a collection of substances known to inhibit different uptake pathways by APCs. The human leukemia monocytic cell line THP-1 and the murine dendritic cell line DC 2.4 were examined for the uptake of fluorescently labelled VNP in the presence or absence of inhibitors. The inhibitory effect of candidate substances that blocked VNP uptake in APC lines was subsequently evaluated in studies with primary APCs present in splenocyte and lung cell homogenates *in vitro*, and their impact on allergen-specific T-cell activation was investigated upon intratracheal application of VNP *in vivo*.

Results: Uptake of allergen-specific VNP *in vitro* and *in vivo* was mainly observed by macrophages and $CD103^+$ dendritic cells and was sensitive to inhibitors that block macropinocytosis, such as hyperosmolarity induced by sucrose or the polyphenol compound Rottlerin at low micromolar concentrations, but not by other inhibitors. Also, T-cell proliferation induced by allergen-specific VNP was significantly reduced by both substances. In contrast, substances that stimulate macropinocytosis, such as Heparin and phorbol myristate acetate (PMA), increased VNP-uptake and may thus help modulate allergen-specific T-cell responses.

Conclusion: We have identified macropinocytosis as the principal uptake mechanism of APCs for allergen-specific VNP *in vitro* and *in vivo*, paving the way for further improvement of VNP-based therapies, especially those that can be used for tolerance induction in allergy in the future.

Funding: This work has been supported by the Federal State of Lower Austria under the Danube Allergy Research Cluster (Danube-ARC) grant (project no. 10) and the Medical University of Vienna.

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1380 – P1.02.27

TCR- and HLA-transgenic humanized mouse model for birch pollen allergy

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Purpose: Sensitization to birch (*Betula verrucosa*) pollen is one of the main causes of pollinosis in the Northern hemisphere. Bet v 1 represents the major birch pollen allergen, to which over 95% of birch pollen allergic patients are sensitized. Bet v $1_{142\text{-}153}$ had been previously identified as the immunodominant T-cell epitope in birch pollen allergic individuals and its contribution to cross-reactivities with other PR-10 proteins was elucidated.

Methods: A transgenic mouse model expressing a human Bet v $1_{142\text{-}153}$-specific T cell receptor (TCR), human CD4, as co-receptor, and the associated human leukocyte antigen (HLA)-DR7 was generated, to investigate the processes of allergic sensitization and specific organ manifestations upon natural allergen exposure *via* the airways. Primary and secondary lymphoid organs of hCD4-TCR-HLA-DR7 transgenic mice were analyzed by multiparameter flow cytometry and compared with wildtype C57BL/6 mice. Functionality of T cells derived from spleens of allergen-naïve hCD4-TCR-HLA-DR7 transgenic mice was evaluated in splenocyte proliferation assays and specific cytokine secretion was studied.

Results: Of the $CD3^+CD4^+$ T cells 90.1±8.4% co-expressed hCD4, and 61.6±9.8% of those $CD3^+mCD4^+hCD4^+$ cells co-expressed the human Bet v $1_{142\text{-}153}$-specific TCR. Of $CD19^+$ B cells 60.1±8.0% co-expressed HLA-DR7. *In vitro*, specific T cell proliferation to Bet v 1 or Bet v $1_{142\text{-}153}$ peptide but not to Art v 1 or Art v $1_{23\text{-}36}$ peptide (representing the unrelated major allergen/immunodominant T cell peptide of mugwort pollen) could be induced. Bet v 1-specific T cells secreted both Th1 and Th2 cytokines into cell culture supernatants. Results of *in vivo* experiments upon exposure of allergy mice to birch pollen extract will be presented.

Conclusion: This is the first preclinical TCR- and HLA-transgenic humanized mouse model for birch pollen allergy which is responsive to the immunodominant Bet v $1_{142\text{-}153}$ peptide recognized by allergic patients. This model will be useful to investigate mechanisms of birch pollen allergy and to develop preventive and therapeutic strategies.

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1429 – P1.02.28

Clinical relevance of molecular diagnosis in patients sensitized to olive tree (Olea europaea) pollen in a cohort of patients from the province of Cádiz

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Purpose: Olive tree (Olea europaea) pollen is one of the most important causes of pollinosis in the Mediterranean basin. Twelve allergens (Ole e 1 to Ole e 12) have been purified and characterized from it, and patients can be sensitized to many different combinations of them. The aim of this study is to describe the sensitization profile to olive tree pollen and to assess molecular diagnosis to predict the efficacy of allergen-specific immunotherapy (AIT).

Methods: A cohort of 823 patients from the province of Cádiz was selected based on pneumoallergen screening performed during 2023. In this study, levels of total immunoglobulin E (tIgE) and specific immunoglobulin E (sIgE) against the complete extract (CE) of olive tree pollen and against specific components (SC) -major allergen Ole e 1 and minor allergens Ole e 7 (LTP) & Ole e 9- were analyzed. The ratio 1 (sIgE-CE/tIgE) and ratio 2 (sIgE-SC/sIgE-CE) of these components was determined.

Results: 73.4% (604) of patients with positive pneumoallergen screening were sensitized to the CE of olive tree pollen. 98% (592) of sensitized patients tested positive for Ole e 1, with a mean of 20 kU/L (Q1-Q3: 1.7-24.1); 28.3% (171) tested positive for Ole e 7, with a mean of 24.4 kU/L (Q1-Q3: 0.6-38.3); and 23.5% (142) tested positive for Ole e 9, with a mean of 31.8 kU/L (Q1-Q3: 2.2-54.1). 6.5% of patients sensitized to the CE of olive tree pollen presented sIgE levels lower than 0.35 IU/L against the studied components.

35.6% patients (61) were sensitized to Ole e 7 as the major allergen according to ratio 2.

Conclusion: In our population, the allergen Ole e 1 behaves as the major allergen of olive tree pollen, while allergens Ole e 7 and Ole e 9 behave as minor allergens. Patients monosensitized to Ole e 7 (9) along with patients who, according to ratio 2, exhibited predominant sensitization to Ole e 7 (61), might have less effective immunotherapy since Ole e 7 is not quantified in most commercial extracts. Therefore, the use of ratio 2 could be clinically useful in predicting the efficacy of immunotherapy.

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1508 – P1.02.29

More severe symptoms are associated to specific IgE detection of a higher number of LTPs using microarray technology.

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Purpose: The diagnosis of polysensitised patients in allergology presents challenges due to multiple reactions and difficulty in identifying triggers. In this regard, molecular diagnosis by microarrays has shown promise in identifying the allergens involved and cross-reactivity relationships. This study analysed the detection of specific IgE in patients sensitised to multiple allergens, using two microarray techniques (ImmunoCAP ISAC and ALEX). Specific IgE profiles, concordance of results and their relationship with symptomatology were analysed to determine whether there is an association between LTP-specific IgE levels and allergic symptoms.

Methods: A prospective longitudinal observational study was conducted in a cohort of 39 polysensitised allergic patients in whom symptoms were studied after fruit ingestion and specific IgE was analysed using two microarray techniques. For statistical analysis, nine allergenic components of the LTP families were selected, non-parametric tests were used and a significance level of $p<0.05$ was established.

Results: More than 80% of the patients tested positive for six or more allergens, and the LTP families had a high detection rate of positive allergen components. With a Pearson correlation value of 0.81, the ISAC and ALEX approaches showed a high degree of correlation for LTPs sensitization values. Additionally, sensitivity to more LTPs was found to be correlated with severe symptoms when compared to no symptoms ($p=0.008$ for ISAC and $p=0.028$ for ALEX). High average values of these positive LTPs were likewise found to be associated with severe symptoms ($p=0.046$ for ISAC and $p=0.027$ for ALEX). Severe symptoms were also linked to sensitization to higher numbers of LTPs and high average values of positive LTPs when diverse groups of LTP allergens were created, based on the sequence homology of LTP proteins, the route of allergen entrance, or their prevalence in our cohort.

Conclusion: The detection of specific IgE using microarray methods is useful for the diagnosis and treatment of polysensitised patients. Patients who are sensitised to more LTPs and who have higher values of specific IgE against LTP allergens are more prone to experience severe symptoms.

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1555 – P1.02.30

Performance characteristics of a novel, fully automated immunoassay microarray for the qualitative serological detection of specific IgE directed against Bet v 1

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Purpose: Bet v 1 (main allergen from the Birch tree pollen) is an important contributor to IgE-mediated allergic disorders. We report the performance characteristics of a novel, single-use, microarray immunoassay (MosaiQ Allergy Bet v 1, AliveDx, Eysins, Switzerland) (Bet-v-1-Microarray) when used with the fully automated MosaiQ® system, for the qualitative detection of specific IgE (sIgE) directed against Bet v 1, compared with ImmunoCAP™ Specific IgE (Phadia AB). The assay's reproducibility and repeatability were also assessed.

Methods: Bet-v-1-Microarrays, sized 40 mm x 10 mm x 8 mm, were prepared by printing Bet v 1 allergens onto functionalized glass chips and assembled into magazines for automatic processing on the MosaiQ 125 instrument. 163 anonymized, residual serum samples, characterized by the comparator method as reactive (n=63) or as non-reactive (n=100) were tested with the investigational device. Magazine lot reproducibility was assessed over five days, on three magazine lots, across two instruments; instrument reproducibility was evaluated over five days, on one magazine lot, across three instruments; repeatability was assessed on one instrument and one magazine lot, two runs per day, over five days. Reproducibility panels were composed of three samples (non-reactive, low-reactive, high-reactive).

Results: In the method comparison evaluation, after the exclusion of 1 reactive sample as per protocol, the investigational device identified as reactive 60 out of 62 characterized reactive samples by the comparator and all 100 non-reactive samples; for a positive, negative and overall agreement of 96.8% (95%CI: 88.8%, 99.6%), 100% (95%CI: 96.4%, 100%) and 98.8% (95%CI: 95.6%, 99.9%), respectively. Agreement of the investigational device with expected results in the evaluations of reproducibility by lot (869 data points) and by instrument (435 data points) as well as in the assessment of repeatability across days and runs (291 data points) were all 100%.

Conclusion: Bet-v-1-Microarray showed high concordance with the compared device for the qualitative detection of Bet v 1 sIgE. Additionally, Bet-v-1-Microarray demonstrated a high degree of precision in the reproducibility and repeatability evaluations. This device/platform has the potential to multiplex and contribute to the comprehensive assessment of allergic disorders. Further ongoing steps include the addition of other allergens to the microarray.

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1557 – P1.02.31

LPS in combination with amoxicillin increases basophil activation and can be used to improve basophil activation test for IgE-mediated allergy

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Purpose: Basophils are among the most important effector cells in allergy. In the context of allergy to drugs, like antibiotics, the infectious context may have some role in basophil-related allergic response that should be considered in *in-vitro* tests. We investigated whether the addition of TLR ligands (TLR-L) that emulate interactions with infectious components, could increase basophil activation and degranulation.

Methods: Twenty-nine amoxicillin (AX)-confirmed allergic and 20 tolerant exposed subjects were analysed by basophil activation test (BAT), using AX alone or in combination with TLR-L 1–8, using CD63 or CD203c basophil activation markers.

Results: Among all TLR-L tested, LPS in combination with AX gave the maximum effect, increasing CD63 expression in 51.7% of AX-allergic compared to 41.4% in the absence of LPS, without compromising specificity. Importantly, using LPS+AX we were able to detect 47% of patients who would have been otherwise reported as negative by conventional BAT (using AX alone). Additionally, combining strategies based on LPS+AX and different activation markers, an increased sensitivity (54.8%) and a specificity of 100% were achieved compared to conventional BAT.

Conclusion: There are differences between upregulation of CD63 and CD203c basophil-activation markers in response to pathogen receptor stimulation. The inclusion of LPS in BAT could be a diagnostic-wise strategy for beta lactam allergies.

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1634 – P1.02.32

The crucial role of lipid receptors on pathogenic T helper cells during house dust mite allergic airway inflammation

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Purpose: Asthma is a an inflammatory airway disorder that affects millions worldwide, with increasing incidence also attributed to climatic changes. CD4+ T helper type 2 (Th2) cells are pivotal in orchestrating the inflammation within the lung tissue. Recent studies have highlighted the involvement of lipid receptors on T cells as key regulators of immune responses in asthmatic lungs, which show an altered lipid composition compared to healthy lungs. Elucidating the mechanisms underlying lipid receptor-mediated T cell differentiation and communication with the airway epithelial barrier promises novel immunotherapeutic avenues.

Methods: To investigate the role of lipid receptors on CD4+ T cells in vivo, we applied acute and chronic house dust mite (HDM)-induced allergic airway inflammation models in wildtype and CD4-specific lipid receptor knockout mice. We examined lungs, mediastinal lymph nodes, and serum of the mice by flow cytometry, ELISA, histological stains, and single-cell RNA sequencing

Results: Significant differences in HDM-specific serum IgE levels and cytokine profiles were observed in acute and chronic asthma models (unpaired Student's t test). Our data further showed that lipid receptors are crucial for the generation T follicular helper cells, which are known as precursors of T helper 2 cells, and are in particular important for the generation of tissue resident memory CD4+ T cells that maintain allergen-specific immunity in the asthmatic lung.

Conclusion: The results underline, that lipid signaling plays an essential role in the pathogenesis of asthma by regulating the immune response and contributing to airway inflammation. Further research is mandatory to find a promising strategy for asthma treatment.

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1687 – P1.02.33

Interactions of metal allergens with T cell receptors

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Metal allergens are the most prevalent chemical sensitizers. Upon diagnostic patch testing, 11%, 3% and 2% of the general population react to nickel, cobalt or chromium, respectively. Sensitization may lead to allergic contact dermatitis, a T cell mediated disease, or implant failure. Despite these real-live data, it is currently unknown how metal ions interact with T cells and activate them. Available regulatory tests predict only a moderate potency for metal allergens, possibly because T cell assessment is lacking.

To include T cells in a test system, we applied a CD154 (CD40L)-based activation-induced marker (AIM) assay. Peripheral blood mononuclear cells were incubated with $NiSO_4$, $CoCl_2$, $PdCl_2$ or $CrCl_3$ in the presence of a CD40 blocking antibody, enabling CD154 surface staining. Cells were analyzed by flow cytometry and reactive CD154+CD4+ memory T cells were sorted for RNA- and unique molecular identifier-based full-length αβ T cell receptor (TCR) sequencing.

Using buffy coats and fresh blood donations (allergic and non-allergic individuals), we identified high frequencies of reactive T cells peaking at ~400 µM before toxic effects became dominant. Most frequent were Pd^{2+}-reactive cells (mean 3.0%, n = 17), followed by Cr^{3+}-, $Co^{2+}$and Ni^{2+}-specific cells (0.64%, n = 6; 0.51%, n = 10 and 0.27%, n = 10).

Among TCR repertoires, individual gene segments were overrepresented for certain metal-specific TCRs, e.g., TRAV9-2 for Ni^{2+}-specific TCRs (28% of TCRs vs. 6% of random TCRs) or the TRBV4 family for Pd^{2+}-specific TCRs (21% of TCRs vs. 6% of random TCRs). As a second, independent feature, Ni^{2+}-, Co^{2+}- and Pd^{2+}- but not Cr^{3+}-specific TCR showed an increased use of the amino acid histidine in the a- and b-chain complementarity determining region 3 (CDR3).

In conclusion, we provide direct quantitative data on metal-specific T cells and identify metal ion binding points to the TCR. The engagement of TCRs via conserved features is reminiscent of superantigens and the strong T cell reactivity may contribute to the high prevalence of metal allergies.

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1792 – P1.02.34

A novel alveolar macrophage depletion mouse model identifies IRF4 as a master regulator of alveolar macrophage effector function during type 2 lung inflammation

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Background: Alveolar macrophages (AMs) represent the predominant immune cell population within healthy pulmonary tissue and have been implicated in exerting an immunoregulatory function within the lung milieu. However, their precise involvement in allergic conditions has been subject to extensive debate, primarily due to the limited availability of techniques capable of selectively depleting AMs and distinguishing between the functional contribution of resident embryonic-derived AMs versus recruited bone marrow-derived counterparts.

Methods and Results: Intranasal administration of interleukin 33 (IL33) and house dust mite (HDM) extract, established *in vivo* models of allergic asthma, result in an early recruitment of eosinophils, neutrophils, and type 2 innate lymphoid cells (ILC2s) into the bronchoalveolar lavage fluid (BALF). To elucidate the underlying mechanisms orchestrating inflammatory cell influx in the BALF, single-cell RNA profiling of IL33-treated animals was conducted, revealing substantial transcriptional alterations within resident AMs, concomitant with the synthesis of type 2-associated chemokines, notably CCL17 and CCL24. Subsequent ATAC sequencing unveiled an enrichment of IRF4 motifs in resident AMs following IL33 exposure, accompanied by an upregulation of IRF4 expression with intracellular staining. To test the impact of resident AMs on BALF cellular influx *in vivo,* we engineered a novel transgenic mouse model (Specific AM Deleter mice – or SPAM deleter) enabling selective depletion of AMs through diphtheria toxin administration. Leveraging this system, intratracheal adoptive transfer of IRF4-deficient resident AMs into SPAM deleter mice post-AM depletion revealed a reduction in cellular infiltrate within the BALF, albeit not in the pulmonary parenchyma, upon exposure to IL33 or a model of HDM-induced asthma. This reduction correlated with diminished levels of BALF eotaxin (CCL11, CCL24) and CCL17. Additionally, we demonstrated the dependence of IRF4 expression on ILC2-derived IL13, rather than IL33, through adoptive transfers involving *Il4ra*-/- and *Il1rl1*-/- AMs in SPAM deleter animals and utilizing ILC2-deficient transgenic mice.

Conclusion: We generated a novel mouse model allowing for specific depletion of AMs and found that resident AMs promote cellular influx in the BALF but not in the lung parenchyma during models of experimental asthma. Mechanistically, ILC2-derived IL13 drives IRF4 upregulation in AMs, thereby inducing the expression of type 2-associated chemokines.

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2043 – P1.02.35

Serum levels of TSLP and CD14 are lower in children with atopic dermatitis and food allergy to cow's milk or a combination of cow's milk and eggs

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Introduction: Atopic dermatitis (AD) is a chronic inflammatory skin disease characterized by the interplay of genetic, environmental, and immunological factors, frequently associated with food allergies (FA). Both AD and FA are associated with penetration of allergens and microorganisms through the disrupted skin or gut barriers. In this study, we search for serum biomarkers associated with barrier integrity (TSLP) and immune response to bacteria (CD14 and lipopolysaccharide binding protein (LBP)) in patients with AD and FA to milk, eggs or its combination.

Methods: We collected blood serum from 165 infants or toddlers (aged 3-12 months) with manifested forms of AD. Part of these patients had AD associated with FA (mainly to cow milk and egg). Serum levels of TSLP, CD14 and LBP markers were measured by ELISA and comparative analysis for each marker was performed in patients with and without FA to milk; patients with and without FA to eggs; and patients with and without combined milk and eggs FA.

Results: We found significantly lower levels of serum TSLP in AD patients with FA to milk ($p=0.036$), and with combined milk and eggs FA ($p=0.038$), but not with FA to eggs only ($p=0.084$) in comparison to AD patients without FA. The serum levels of CD14 were also significantly lower in patients with AD and FA to milk ($p=0.0089$), and to milk and eggs ($p=0.023$), but not to eggs only ($p=0.077$). The serum levels of LBP weren't significantly different in all groups tested compared to AD patients without FA

Conclusions: Biomarkers related to barrier integrity and immune response to bacteria are lower in patients with AD and FA to milk or milk and eggs in combination. Further, we will link these data with the results of the microbiota analysis and identify the combination of markers that could predict the onset and progression of the disease in AD and FA patients.

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2158 – P1.02.36

Molecular insights into shrimp allergy

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Background: Crustacean allergy, particularly to shrimp, is one of the eight major food allergens that account for approximately 90% of severe allergic reactions. Given its typical association with a type I hypersensitivity reaction, quantitative determination of specific IgE stands as the most reliable diagnostic approach.

Purpose: Our aim was to assess the seroprevalence of specific IgE antibodies, specifically targeting molecular recombinant Tropomyosin rPen a1, in individuals exhibiting clinical symptoms suggestive of shrimp allergy.

Methods: We conducted a retrospective study spanning a 39-month period (01/01/2021-01/04/2024). Sera from 25 patients were analyzed for shrimp specific IgE (F24) and/or their major molecular recombinants (F351) (Tropomyosin rPen a1) using a fluoro-enzyme immunoassay (FEIA).

Results: Among the 25 samples analyzed from individuals with clinical symptoms suggestive of shrimp allergy, 14 (56%) tested positive for shrimp specific IgE. The mean age of the participants was 19.5 years. Five out of the 14 (35,7%) patients who tested positive for f24 experienced a severe allergic reaction in the form of Quincke's edema while 6 (42.8%) manifested urticaria. Out of the participants, only 9 (64.3%) sought additional testing for Tropomyosin rPen a1. In 77.7% of these cases, the results were positive, indicating a true allergy to shrimp. Cross-reactivity was detected in 11 of the 14 f24-positive patients, which represents 78.6% of the group. Among them, 27,2% had food allergy and 36,3% had respiratory allergy.

Conclusion: The high prevalence of shrimp allergy in our series, along with the notable risk of anaphylaxis associated with Tropomyosin rPen a1, highlights the importance of molecular antigens in evaluating prognosis and improving the management of allergic patients. A larger cohort study is needed to confirm these findings.

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2196 – P1.02.37

Component-resolved allergy diagnostics in Honeybee venom allergy

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The purpose of our study is to perform component resolved diagnosis (CRD) of honeybee venom allergy (HBV) using specific recombinant allergen molecules.

Patients and Methods: 30 patients (21 male and 9 females, aged 11-69 years) were included in this study. The included criteria were history of the systemic allergic reaction after bee stings and positive reaction after allergy skin test with allergen extract of bee venom (papule size > 3 mm, erythema > 3 mm). The following molecules were used to perform CRD: rApi m1 (Phospholipase A2) (i208); rApi m2 (Hyaluronidase) (i214) and major allergen of HBV - Api m 10 (Icarapin) (i217).

The serum concentration of specific IgE (sIgE) against natural bee venom extract (i1) as well as against recombinant allergen molecules of bee venom were measured by FEIA and values ≥0.35 kU/l were considered positive.

Results: The elevated >0.35 kU/l serum sIgE levels against natural bee venom extract (i1) was observed in all participants (100%). The results of positive sIgE against recombinant allergen molecules were as follows: 43% Api m 10 (Icarapin), 70% rApi m1 Phospholipase A2 (i208) and 60% rApi m2 Hyaluronidase (i214). Noteworthy, in 7 (23%) of the participants we observed positive sIgE levels against all three specific recombinant allergen molecules.

Conclusion: Api m1, Api m2 and Api m10 are suitable for identifying specific sensitization to HBV. Taking into consideration that CRD is particularly important for the elucidation of double sensitization, the commercial availability of a panel of marker allergens can be considered highly valuable for adequate diagnostics.

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Abstracts

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2209 – P1.02.38

Obesity-induced gut dysbiosis is associated with lung endothelial glycocalyx shedding and exacerbation of asthma

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Purpose: The hallmark of obese asthma is the infiltration of neutrophils and Th17 cells to the lungs. This asthma phenotype confers more resistance to corticosteroid treatment. Endothelial glycocalyx (EG) is a network of proteoglycans and glycoproteins that regulates leukocyte-endothelium interaction. EG shedding induced by heparanase increases leukocyte migration. We hypothesized that obesity-induced gut dysbiosis promotes EG shedding and increase pulmonary neutrophil and Th17 cell influx, resulting in asthma exacerbation.

Methods: Female C57BL/6 mice were fed with high-fat diet (HFD) for 12 weeks following exposure to ovalbumin (OVA) for the induction of experimental asthma. Bronchoalveolar lavage fluid (BALF) and lungs were collected to evaluate pulmonary inflammation by flow cytometry and histopathologic analyze. Airway resistance was measured by FlexVent. Lung EG integrity was evaluated by electron microscopy. EG shedding was evaluated in serum samples from asthmatic with obesity and lean asthmatic subjects by ELISA. Intestinal permeability was measured by FITC-dextran assay. Gut microbiota was characterized by 16S RNA sequencing. Lungs and spleen homogenates were incubated under aerobic or anaerobic conditions to evaluate bacterial translocation.

Results: Mice with obesity exposed to allergen (HFD+OVA) showed increased neutrophils in the BALF, Th17 cells and mucus production in the lungs compared to lean mice exposed to OVA (LFD+OVA). HFD+OVA also exhibited increased airway resistance compared to LFD+OVA. We observed reduction in lung EG thickness in HFD+OVA compared to HFD and LFD+OVA groups. Asthmatic patients with obesity exhibited increase of serum heparanase compared to lean asthmatic subjects. HFD+OVA exhibited an increase of *Romboutsia* and reduction of *Parabacteroides* in the feces compared to LFD+OVA. These bacteria are related to endothelial function and heparanase inhibition, respectively. HFD+OVA displayed an increase of intestinal permeability and consequent bacterial translocation to spleen. In the lungs of HFD group, aerobic bacteria were predominant with loss of anaerobic bacteria growth observed in LFD mice, independent of OVA exposure.

Conclusion: Obesity promotes bacterial translocation that is probably inducing EG shedding after the allergen exposure. EG shedding increases neutrophil and Th17 cell influx to the lungs and exacerbates asthma. EG might represent therapeutic target to reduce pulmonary inflammation during the comorbidity obesity and asthma.

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Lung Microbiota analysis of asthmatics reveals pathogenic microbial blooms corresponding to lung inflammation and reduced microbial diversity

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Background: The existence of the lung microbiota has in recent years become to be appreciated. The lung microbiome by the nature of the lung function and mucociliary clearance is believed to be dynamic but diversified. We sought to characterise the lung microbiota in severe and non-severe asthmatics and investigate if the microbiota provided an insight into the pathology of severe asthma.

Methods: Bronchoalveolar lavage (BAL) samples from 44 patients with a known diagnosis of asthma were collected. BAL cell counts and cytokine levels in both the lavage sample and matched serum samples were analyzed. Following DNA extraction, the microbial community of the BAL was analyzed using whole genome sequencing followed by analysis using the OneCodex platform and confirmation by qPCR.

Results: The lung microbiome is dynamic but does have consistent microbial species such as Micromonospora and Prevotella (90 % of all samples) with an average Shannon diversity of 3.5. Severe asthmatics who smoke and have reduced FEV-1 displayed less abundance of *Prevotella pallens* and those with a Body mass Index (BMI) > 30 had reduced presence of Micromonospora species. Several samples displayed the presence of reduced microbial diversity and pathogenic blooms of *Haemophilus influenzae*, *H. parainflunezae*, *Staphylococcus sp. HMSC057B0* and *Streptococcus pneumoniae*. These blooms correlated with increased BAL neutrophils, lymphocytes and raised BAL IL1b, IL-8, IL-17 and TNFa and serum neutrophils.

Conclusion: The lung microbiota is reflective of asthmatic severity and screening for decreased microbial diversity and microbial blooms could provide a useful tool for asthma stratification but also targeted therapeutics during episodes of exacerbation.

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Airway and systemic immunoglobulin profiling and immune response in adult asthma

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Introduction: Immunoglobulins play a vital role in host immune response and in the pathogenesis of conditions like asthma. Therapeutic agents such as monoclonal antibodies target specific elements of the asthmatic inflammatory cascade. Decisions to utilize these medications are often based on systemic inflammatory profiling without direct insight into the airway inflammatory profile. We sought to investigate the relationship between immunoglobulin and cytokine profiles in the airway and systemic immune compartments of adult asthmatics.

Methods: Blood sampling and bronchoscopy with bronchoalveolar lavage (BAL) were performed in 76 well-defined adult asthmatics. Antibody and cytokine profiles were measured in both BAL and serum using ELISA and quantibody arrays.

Results: There was no relationship between BAL and serum levels of IgE which is of significance in an asthma population. Significant correlations were found between BAL and serum levels for IgM (P<0.0001), IgA (P<0.001), IgG2 (P<0.02) and IgG4 (P<0.0001) and also BAL and plasma levels of IL-10, IL-13, IL-4, TNF-α (P <0.0001), IL-1β (P=0.04), IL-5 (P=0.001) and IL-6 (P =0.002).

Conclusions: This study highlights the importance of sample site when investigating the roles of immunoglobulins and cytokines in disease pathogenesis and suggests that both localized and systemic immune responses are at play. The prescription of asthma monoclonal therapy is generally based on systemic evaluation of cytokine and immunoglobulin levels. Our research suggests that this approach may not fully reflect the pathophysiology of the disease and may provide insight into why some patients respond to these targeted therapies while others do not.

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P1.03 ANTIGEN PRESENTATION

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111 – P1.03.01

Antigen presenting cells in melanoma – role in tumor regression

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Purpose: We aimed to evaluate the presence of antigen presenting cells (APC) in various areas of cutaneous melanoma, with regression and non-regression features in order to correlate with the local immune response.

Methods: 102 parrafin embeded cutaneous melanoma samples (Ethical Commitee approval and individual informed consent) were investigated for the presence of Langerhans cells (LCs) and other immune cells that infiltrate the regressed and non-regressed areas. 62 superficial spreading melanoma (SSM), 31 nodular melanoma (NM) and 9 acral-lentiginous melanoma (ALM) were investigated. Immunohistochemistry: S-100, HLA-DR, LCA, CD-1, Langerin (CD207), CD4, CD5, CD8, CD 14, CD 16, CD22. Melanoma regression was established according to the pathology guidelines.

Results: Out of the 102 samples, reggresion was identified in 39 SSM and 7 ALM, statistically significant in stage I melanomas localized on the trunk. Within the regressed areas LCs were found frequently with a nodular distribution. Regression is associated with statistically significant presence of tumor infiltrating lymphocytes (TIL), in almost all stage I melanoma cases (84.3%). TIL has mainly Th cells, reduced number of B cells and macrophages; CD4: CD8 ratio is 4:1 with a large interval from 1.3 to 19. TILs were found in thin and superficial melanomas with a high density in deep dermis. Dendritic cells (DC) S-100+ were identified in deep dermis in all samples at a density of 55±6/mm2, more frequent in *in situ* melanomas as compared to profound and invasive melanomas. LC:DC S-100 + ratio was identified in the 5.5 to 15.5 interval. LC residing in the melanoma epidermis have a lower proportion of S100+ expression in the majority of melanomas (80%) in comparison to the normal adjacent epidermis ($p < 0.0005$), while around 50% of the samples had a significant reduction of LC CD1+ in the tumor epidermis compared to the normal epidermis ($p<0.0005$).

Conclusion: The presence of APCs in the regressed areas of cutaneous melanomas brings new information on the active immune response within the tumor.

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136 – P1.03.02

Targeting the unconventional

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Since 2004 we know that proteasomes can ligate peptide fragments through a process called peptide splicing and few examples of their immunogenicity in the context of cancer have been published. We developed several pipelines to identify and predict these unconventional epitopes and tested their immunogenicity and potential for therapeutical applications mediated by CD8+ T cell cytotoxic activity.

The immunological relevance of proteasome-generated spliced epitopes is still unclear and their identification requires specific know-how and tools, which have been developed by my lab and collaborators, putting us at the cutting edge of this research field. The potential role of these targets for the next generation immunotherapies still needs to be defined, for example using these tools.

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359 – P1.03.03

Celiac disease defined by over-sensitivity to gliadin activation and superior antigen presentation of dendritic cells

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The autoimmune condition, Celiac Disease (CeD), displays broad clinical symptoms due to gluten exposure. Its genetic association with DQ variants in the human leukocyte antigen (HLA system has been recognised. Monocyte-derived mature dendritic cells (MoDCs) present gluten peptides through HLA-DQ and co-stimulatory molecules to T lymphocytes, eliciting a cytokine-rich microenvironment. Having access to CeD associated families prevalent in the Czech Republic, this study utilised an in vitro model to investigate their differential monocyte profile. The higher monocyte yields isolated from PBMCs of CeD patients versus control individuals also reflected the greater proportion of dendritic cells derived from these sources following lipopolysaccharide (LPS)/ peptictryptic-gliadin (PTG) fragment stimulation. Cell surface markers of CeD monocytes and MoDCs were subsequently profiled. This foremost study identified a novel bio-profile characterised by elevated CD64 and reduced CD33 levels, unique to CD14++ monocytes of CeD patients. Normalisation to LPS stimulation revealed the increased sensitivity of CeD-MoDCs to PTG, as shown by CD86 and HLA-DQ flow cytometric readouts. Enhanced CD86 and HLA-DQ expression in CeD-MoDCs were revealed by confocal microscopy. Analysis highlighted their dominance at the CeD-MoDC membrane in comparison to controls, reflective of superior antigen presentation ability. In conclusion, this investigative study deciphered the monocytes and MoDCs of CeD patients with the identification of a novel bio-profile marker of potential diagnostic value for clinical interpretation. Herein, the characterisation of CD86 and HLA-DQ as activators to stimulants, along with robust membrane assembly reflective of efficient antigen presentation, offers CeD targeted therapeutic avenues worth further exploration.

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Screening and Application of hepatocellular carcinoma-related Antigen AFP Specific T cell Epitope Spectrum

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Purpose: To identify the predominant T cell epitope spectrum of AFP presented by 45 predominant HLA-A/B/ C molecules in Chinese and East Asian populations, and to establish a broad-spectrum T cell epitope peptide library that not only conforms to the HLA polymorphism of the vast majority of populations in this region, but also fully reflects the richness of T cell epitopes in HCC antigen. Combined with ELISpot technology, a multi-level universal detection system for HCC patient-specific T cell immune function was established.

Methods: We used multiple databases to predict and screen the candidate epitopes of AFP, and then used two experimental methods to verify the immunogenicity of the candidate epitopes. First, we collectc PBMCs from peripheral blood of HCC patients and confirm HLA genotyping .then we utilized the matching HLA-A/B/C molecular candidate epitope and PBMCs co-culture for 6 hours, and the activation frequency of $CD8^+$T/INF-γ+ was detected by ICS with FCM. Second, the competitive binding experiment was performed between the candidate epitopes and HMy2. CIR cell lines which expressing different HLA molecules.

Results: 68 candidate epitopes could be cross-represented by different HLA-A/B/C molecules, and aslo could induce strong AFP-specific $CD8^+$T responses in vitro by Elispot experiment with PBMCs of HCC patients.Compared to the negative group the activation frequency of $CD8^+$T/INF-γ+ increase by more than 5 multiple.

Conclusion: 68 AFP epitopes are AFP specific $CD8^+$T cell epitopes, which can be used for the universal detection system of specific T cell immune function in HCC patients and the study of tumor vaccine.

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990 – P1.03.05

Exploring the role of T cell epitopes in the development of non-specific Lipid Transfer Proteins Allergy

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Purpose: Type I hypersensitivity is mediated by specific immunoglobulin E (sIgE), produced by B cells. T cells are less studied, although important, due to Human Leukocyte Antigen (HLA) class II-mediated peptide presentation by dendritic cells. Allergen-specific responses of CD4+ T-cells contribute to sensitisation and promotes their differentiation towards Th2. The purpose of this study was to elucidate the role of HLA-class II in the development of allergy to non-specific Lipid Transfer Proteins (nsLTPs), specifically to Pru p 3, the peach nsLTP, and to Ole e 7, the olive pollen nsLTP.

Methods: Allergic patients to Ole e 7 and/or Pru p 3 were selected at the Reina Sofía University Hospital (Córdoba, Spain) according to sIgE levels (ImmunoCAP 250; positivity threshold>0.35 kUI/L) and classified into three groups: MONOLE (Ole e 7-monosensitised; n=14), MONPRU (Pru p 3-monosensitised; n=15) or BI (bisensitised; n=13). Their HLA class II (HLA-DRB1, -DQB1, -DPB1) was sequenced using Luminex® xMAP® technology. Prediction of T-cell epitopes was conducted by Immune Epitope Database Analysis Resource, using Uniprot code Q9LED1 for Pru p 3 and Ole e 7 sequence from literature.

Results: MONOLE patients mainly exhibited rhinoconjuntivitis (92.9%) and asthma (85.7%), but not oral-allergy symptoms, in contrast to MONPRU patients, who described Rosaceae symptoms (73.3%), and less often, respiratory symptoms (60.0% rhinoconjuntivitis and 26.7% asthma). Additionally, BI patients manifested oral-allergy and respiratory symptoms. HLA-class II frequency analysis in all patients showed the prevalence of HLA-DRB1*04, –DQB1*03, including serologic DQ7, DQ8 and DQ9, and DPB1*04 (23.8%, 42.9%, and 50%, respectively). Statistical analysis revealed significant differences in DR11-DQ7 frequency between the three groups (MONOLE=0/14; MONPRU=10/15; BI=4/13; for DR11 and MONOLE=1/14; MONPRU=12/15; BI=4/13; for DQ7; $p<0.05$) and a tendency for DR4 (MONOLE=10/14; MONPRU=5/15; BI=4/13; $p=0.054$). Furthermore, T-cell epitope analysis predicted a high binding capacity between Pru p 3 epitopes and DR11, and also, between Ole e 7 epitopes and DR4. DQ3 epitope binding showed good scores with both nsLTPs.

Conclusion: Certain HLA class II alleles could promote presentation of specific nsLTPs epitopes to T-cells. The difference in the binding ability among epitopes and HLA molecules could underlie the predisposition of some individuals to nsLTPs allergy.

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1772 – P1.03.06

A new look on the antigens involved in multiple sclerosis: using immunopeptidomics to identify naturally processed myelin epitopes

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Purpose: Multiple Sclerosis (MS) is a chronic disease affecting 2,8 million people worldwide. Current treatment options include immunosuppressive agents. However, these therapies heighten the risk of opportunistic infections and malignancies. Therefore, antigen-specific therapies are being developed. Crucial to the development of these therapies is the knowledge of which epitopes are involved in MS. In this project, we have made use of a mass spectrometric-based approach called immunopeptidomics to identify epitopes that have been processed from myelin proteins by dendritic cells (DCs).
Methods: Mature, stimulatory DCs (mDCs) and tolerance-inducing DCs (tolDCs) were differentiated from monocytes. mDCs and tolDCs were electroporated with myelin oligodendrocyte glycoprotein (MOG) and myelin basic protein (MBP) mRNA. Tolerogenicity of tolDCs was determined before and after electroporation by making use of allogeneic mixed lymphocyte reaction and immunophenotyping. The expression of MOG protein in electroporated tolDCs was studied with intracellular staining. Antigen presentation in electroporated mDCs was assessed by measuring the activation of MBP-specific T cells in a DC-T cell coculture with interferon (IFN)-γ ELISA. Immunopeptidomic analysis was performed on electroporated mDCs and tolDCs to identify the presented myelin-derived antigens.
Results: Electroporation did not change the tolerogenic phenotype of tolDCs as evidenced by reduced expression of the costimulatory markers CD80/CD83/CD86 and a reduced T cell-stimulatory activity compared to mDCs. MOG protein expression was detected after mRNA electroporation. mRNA-electroporated mDCs were able to activate T cells in an antigen-specific manner as seen in the higher secretion of IFN-γ in the cocultures with mRNA-electroporated mDCs compared to those with non-electroporated mDCs. MOG- and MBP-derived epitopes were detected on mRNA-electroporated mDCs and tolDCs by immunopeptidomic analysis.
Conclusion: We have shown here that immunopeptidomic analysis on DCs loaded with myelin proteins by electroporation can be used to identify naturally processed myelin epitopes. Interestingly, one of the identified epitopes had not yet been described in literature. Furthermore, we were able to show that electroporation was able to induce antigen presentation to T cells. In further experiments, we will also perform immunopeptidomics on DCs loaded with myelin lysate by phagocytosis. This further approaches the in vivo processes involved in antigen presentation.

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1845 – P1.03.07

Closer Peptide Repertoire Similarity of HLA-B14:03 and HLA-B27:05 Sheds Light on Ankylosing Spondylitis Susceptibility

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The human major histocompatibility complex class I (HLA-I) gene HLA-B*27 is the main risk factor for the rheumatic disease ankylosing spondylitis (AS) through an unknown mechanism. However, in African populations, where B*27 is rare, the B*14:03 allotype is strongly associated with AS, while B*14:02, which only differs from B*14:03 at one residue (L156R), is not associated with the disease. Here, we have used large-scale mass spectrometry-based peptide sequencing to analyze the peptidomes of HLA-B*14:03, HLA-B*14:02, and HLA-B*27:05, obtaining more than 2000 peptide ligands for each molecule. Remarkably, we found 1011 ligands shared by the AS-associated HLA-B*27:05 and -B*14:03 alleles and not by the non-AS-associated B*14:02 allele. Surprisingly, although B*14:03 and B*27:05 differ by 15 amino acids in their peptide binding domain, they show a large overlap of their ligands (64 and 43% respectively), while B*14:03 and B*14:02, which differ by only one residue, show a lower degree of overlap (33-35%). Furthermore, the B*14:03 peptide repertoire most resembles that of B*27:05 at the P1, P2, and P5 positions of the peptides, and differs most at the C-terminal position, where B*14:03 is more restrictive than B*27:05. We have modeled how the change at residue 156 of the two B*14 alleles could have a long-range indirect effect on residues P1, P2, and P5 of the peptide ligands, explaining the different amino acid distribution observed between the two B*14 subtypes at those positions. Most of the 1011 specific ligands of B*14:03 and B*27:05 presented R/K/A/G at P1, R at P2, and L/F at the C-terminal position. Furthermore, we found that 10 of these 1011 peptides are common with the specific ligands of the most frequent HLA B*27 alleles associated with AS and have not been identified in the ligandomes of the B27 subtypes not associated with the disease, and may act as arthritogenic self-peptides.

In summary, our results show that the two AS-associated allotypes B*14:03 and B*27:05, but not the non-AS-associated allotype B*14:02, share very similar peptide repertoires and binding characteristics, supporting specific common peptide ligands of HLA-B*27:05 and B*14:03 as a mechanism to explain the development of ankylosing spondylitis.

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1903 – P1.03.08

Unlocking HLA diversity: innovative CRISPR strategies for peptide repertoire analysis

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The human leukocyte antigen (HLA) system plays a crucial role presenting peptides to T lymphocytes during infectious, tumoral and autoimmune processes. However, identifying these peptides poses challenges due to the high polymorphism, polygeny, and limited availability of specific antibodies for most HLA alleles. Traditionally, transfection methods have been used to express HLA molecules of interest, but these techniques have significant limitations in terms of expression regulation and availability of suitable cell types.

To address these limitations, we propose an innovative strategy employing CRISPR technology to selectively eliminate undesired class I HLA molecules, followed by an analysis of the peptide repertoire via immunoprecipitation with a pan-specific HLA antibody. In JY cells expressing HLA-A*02:01, B*07:02, and C*07:01, we deleted HLA-B and -C molecules and examined the peptide repertoire of the HLA-A*02:01 molecule using mass spectrometry. Subsequently, we immunoprecipitated HLA molecules with PA2.1 and the pan-specific antibody W6/32 in both wild-type (WT) clones and clones lacking HLA-B and -C ($BC^{-/-}$). To validate our approach, we compared our methodology with the use of a specific antibody (PA2.1).

Notably, the overlap observed between the peptides identified in $BC^{-/-}$ clones using the pan-specific W6/32 antibody and those identified using the HLA-A*02-specific PA2.1 antibody was comparable to the overlap observed between the peptides identified using pan-specific W6/32 from $BC^{-/-}$ clones and those identified using HLA-A*02-specific PA2.1 from WT clones. On average, our analysis revealed approximately 2000 peptides presented by HLA-A*02:01 in both settings. These results support the feasibility of our methodology for identifying the peptide repertoire of other HLA molecules of interest for which specific antibodies do not exist.

Furthermore, we deleted HLA-A*02:01 in the BC-/- clones to explore the ligands presented by non-classical class I HLA molecules. Following immunoprecipitation with W6/32, we identified over three hundred peptides presented in the absence of HLA-A, -B, and -C by mass spectrometry.

These findings highlight the potential of our approach in delineating the peptide repertoires of other HLA molecules lacking specific antibodies, thus opening new avenues for investigating antigen presentation mechanisms in various pathophysiological contexts.

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1991 – P1.03.09

Optimization of dendritic cell activation via HIV-1 infection under xeno-free conditions

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Human platelet lysate (hPL) as a medium constituent for immune cells has been investigated and improved over decades and with the rising qualities in its production, the replacement of fetal calf serum (FCS) for various cell lines gains feasibility and urgency. To achieve this, specific qualitative criteria need to be met in order to identify successful replacement, namely benchmarks such as viability, differentiation, activation and functionality of the immune cells.

In this study, the quality and quantity of human monocyte-derived dendritic cells (DCs), cultured in (hPL)-based or (FCS)-containing media when infected with HIV-1 or stimulated with lipopolysaccharide (LPS), were monitored. The DC phenotype of immature, mature and HIV-infected DCs as well as cell viability, DC differentiation and maturation status and infection levels were studied in detail using multi-parameter flow cytometry. Confocal microscopy was applied as a qualitative and quantitative tool for virus particles measurements and DC phenotype and productive infection of DCs was quantified by ELISA against the viral p24 protein.

Confocal imaging and p24 ELISA as quantification tool for infection levels illustrated dendritic cells cultured in FCS superior compared to hPL-cultured DCs. Unspecific activation was higher in FCS-grown immature DCs. Calculations based on the viability of dendritic cells throughout the procedure relative to the seeded amount and the differentiation and maturation rates showed elevated yields of LPS-matured cells grown in hPL, while HIV-C infection led to significantly higher yields of matured cells in the FCS setting.

So far, we found that hPL presents as a viable substituent for the differentiation and activation of dendritic cells through LPS application. Since for HIV-infection as well as in immune-competent *in vitro* models, an immature DC phenotype is essential, to date FCS cannot be replaced from the differentiation protocol so far and thus, this process needs further optimization.

The project was funded by the Austrian Sciene Fund (FWF-project P33510-B13 to Professor Doris Wilflingseder) and supported by the intramural funding program of the Medical University Innsbruck Ph.D. Research Training Groups, Project 2022-1-2 “CONNECT”

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2058 – P1.03.10

The hidden power of anti-inflammatory macrophages: unveiling their role as cross-presenters in the activation of cytotoxic T cells

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Cross-presentation is the process by which antigen-presenting cells (APC) present antigens derived from extracellular sources on their major histocompatibility complex class I (MHC-I) molecules. Cross-priming is the process by which APCs activate naïve CD8+ T cells via cross-presentation in secondary lymphoid organs. Consequently, cross-presentation and cross-priming are essential for the elimination of infected or malignantly transformed cells.

Traditionally, dendritic cells have been recognized as the primary cell type responsible for cross-priming, with other APCs like macrophages often considered incapable of this process. However, this view has recently been challenged. Using mRNA transfection of autologous cytolytic T cells and newly engineered antigen specific T-cell lines, we were able to contrast the cross-presenting and cross-priming capabilities of pro-inflammatory and anti-inflammatory macrophages and also link these with their effector phenotypes.

Contrary to our initial expectations, we discovered that anti-inflammatory macrophages are very good cross-presenters. Moreover, we found that, although they are poor activators of naïve CD8+ T cells, they are highly capable of reactivating memory CD8+ T cells. These findings suggest a significant role for anti-inflammatory macrophages in combating recurring infections.

Our research expands the already versatile roles of macrophages, as it suggests that they are not only involved in tissue homeostasis, wound healing, and acute and chronic innate immune responses, but also in battling infections by the reactivating cytotoxic T lymphocytes from the adaptive immune system.

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2153 – P1.03.11

Increasing contribution of mitochondria-derived peptides to the HLA-DR immunopeptidome on different T1D stages

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The trigger behind b-cell destruction in human Type 1 Diabetes (T1D) is unknown. Changes in their protein content are sure to alter the MHC-peptide repertoire of APCs and have an impact in autoreactive T-cell activation. Thus, the analysis of these variations might help identifying key processes behind T1D initiation or maintenance.

To assess the changes in pancreas proteome along T1D progression, human pancreas tissue homogenates from Pre-diabetic, Early and Long term T1D patients were digested with trypsin and analysed by mass spectrometry. Nearly 6000 proteins were identified in all samples, with quantitative and qualitative differences between disease stages. Roughly 100 proteins were differentially expressed across samples and interestingly each of them contained at least 200 unique proteins. The loss of insulin and the reduction of hallmark endocrine proteins confirmed the expected characteristics across samples. The same homogenates were used for pulsing moDCs expressing the T1D-related HLA-DR4 allele. MHC-II-peptide complexes were immunoprecipitated and the eluted peptides analysed by mass spectrometry. The identifications met the characteristics of MHC-II-presented peptides, and a large percentage of their parental proteins reflected the differences across their corresponding proteomes. Peptides derived from potentially relevant parental proteins were detected across samples including the EEF1A1, an autoantibody target in T1D patients, and Reg1A and Reg1B, described T-cell autoantigens in NOD mice. Furthermore, our previous data demonstrated that 25% of these parental proteins were identified in lysosomal fractions from a human b-cell line, including 10 of the parental proteins deemed relevant and 50 mitochondrial proteins. Noteworthy, the mitochondrial proteins showed a progressive decrease in overlapping peptides from Pre-diabetic to T1D stages. Interestingly, both diabetic stages tended to present longer peptides which harboured a higher number of HLA-DR4-binding cores. This could represent a greater number of stable MHC-peptide complexes, widening the array of peptides potentially recognized by the immune system.

These results point at changes in protein content in the pancreas throughout diabetes development and highlight their effect in APCs' MHC-peptide repertoire. The changes in peptide characteristics and the presence of their parental proteins in b-cell lysosomes may indicate a doorway for these proteins into the APCs processing and presentation machinery.

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P1.04 ANTIGENS

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165 – P1.04.01

Genetic and Immunological Analysis of PfEBA175 Gene and Antibody Responses in Malaria Patients from Different Transmission Settings in Ghana

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Malaria remains a significant public health challenge in Ghana, with varying transmission intensities across different regions. Understanding the genetic diversity of malaria parasites and host immune responses is crucial for developing effective control strategies. In this study, we investigated the genetic and immunological characteristics of the *Pf*EBA175 gene and antibody responses in malaria patients from various transmission settings in Ghana.

Dried blood spot samples (n=125) were collected from individuals residing in areas with very high (VH), high (H), low/medium (L/M), and very low (VL) malaria transmission settings. *P. falciparum* DNA and serum were extracted using the Chelex extraction method. Serum samples were analyzed using ELISA to assess natural antibody immune responses against EBA175 and the gametocyte stage antigen Pfs230. *P. falciparum* DNA was amplified using PCR, followed by gel electrophoresis and sequencing using the Illumina platform. Multiple sequence alignment and maximum likelihood tree construction were performed to assess genetic relatedness.

Our results revealed no significant differences in immune responses against EBA175 and Pfs230 across different transmission settings and age groups. Moreover, despite the identification of variations in *Pf*EBA175 gene sequences compared to the reference strain *P. falciparum* 3D7, phylogenetic analysis did not identify a clear genetic distinction between parasites from the different transmission settings. This finding suggests potential gene flow or genetic exchange between parasites across different transmission zones, challenging the notion of geographical isolation in malaria parasite populations.

These findings provide insights into the genetic diversity of malaria parasites and host immune responses in diverse transmission settings in Ghana, which is essential for informing malaria control interventions and vaccine development efforts.

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420 – P1.04.02

Proteomes of gut commensal bacterial extracellular vesicles: search for conserved surface epitopes

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Purpose: Gut microbiota produces extracellular vesicles (EVs) loaded with proteins, polysaccharides, nucleic acids and metabolites, which modulate intestinal host-microbe interactions and systemic immunity. Recent studies imply that maternal microbial EVs may also be transferred to the fetus. Targeted studies of microbial EVs in host tissues is challenging due to lack of specific reagents distinguishing host and microbial EVs. We analyzed proteomes of mammalian gut commensal EVs, in order to generate antibodies against widely conserved surface epitopes.

Methods: We cultured 15 common bacterial species of the mammalian intestinal microbiota, broadly representing the known phylogenetic diversity, extracted EVs using ultracentrifugation and size exclusion chromatography, characterized the EVs using nanoparticle tracking and electron microscopy, and analyzed the EV proteomes by LC-MS/MS. In addition, we retrieved previously published commensal microbiota EV proteomics datasets. We characterize functional protein groups using the EggNOG mapper and UniProt, and search for optimal immunogenic surface epitopes using the EpitoCore pipeline.

Results and conclusion: The EV proteomic profiles of gram-positive and gram-negative bacteria were expectedly different. The observed proteomes varied in terms of both composition and richness. The most abundant EV proteins tended to be associated with cell surface (such as ABC transporters and porins) but were usually different between bacterial species. The most widely distributed proteins were generally slow-evolving ones with cytosolic localization (such as ribosomal proteins, translation initiation factors and chaperones).

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431 – P1.04.03

Selection of high affinity recombinant antibodies against human complement protein C3

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Introduction: C3 is the key protein in the activation of the Complement system and it is a contributor for the maintenance of an effectively functioning immune system. C3 also appears to be a target for autoantibodies during the development of autoimmune diseases such as Systemic Lupus Erythematosus (SLE). In order to study the molecular aspects of C3 autoantigenicity we aimed to select a high affinity anti-C3 antibodies from the "Griffin 1" phage display library expressing human scFv antibodies.

Materials and methods: We screened the "Griffin 1" phage library in four rounds of selection for anti-human C3 scFv antibodies. We applied a new approach with decreasing the amount of antigen twice in every successive round which resulted in selection of high-affinity recombinant antibodies. The selected anti-C3 phages were used to transfect a non-suppressor *E.coli* HB2151 cells followed by induction and expression of soluble monoclonal anti-C3 scFv antibodies. A quantitative analysis by ELISA was used to assess the scFv clones as low or high affinity ones. A qualitative analysis by immunoblot determined the epitope specificity of the selected clones against C3 and its smaller fragments C3b, C3c and C3d.

Results: Four rounds of phage selection yielded a phage suspension with a titer of 2.76 x 10^4 pfu/ml. Forty clones of recombinant antibodies recognized C3 molecule and five clones, designated B11, F8, F9, F10 and F11, were found to have the highest binding affinity to the tested antigen. Four clones B11, F8, F10 and F11 recognized C3, C3b and C3c, but not C3d.

Conclusion: Antibody phage display is a successful approach to overcome the low immunogenicity of evolutionary conservative proteins like C3. Additionally, the selection approach with decreasing amounts of antigen resulted in four high affinity monoclonal anti-C3 scFv antibodies with established epitope specificity within C3c, suitable for further experimental work.

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439 – P1.04.04

Uncovering untapped potential: noncanonical neoepitopes and T-cell metabolism in paediatric acute lymphoblastic leukaemia

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Tumour-specific antigens, also termed neoepitopes, are ideal targets for cancer precision therapies due to their exclusive presence on cancerous cells and frequent implication in the malignant transformation process, rendering them not easily repressible by the tumour via antigen loss. Nevertheless, presentation of neoantigens is often limited by their capability to efficiently undergo the human leukocyte antigen class I presenting pathway due to possible sequence restrictions. To overcome this, our project harnesses noncanonical neoepitopes, consequently obtaining more potentially antigenic and immunogenic neoepitope sequences. Due to the high prevalence of tumour-specific fusion proteins in paediatric acute lymphoblastic leukaemia, noncanonical neoepitopes could be a promising target for immunotherapy. Using our *in vitro-in silico* pipeline we have already identified potential neoepitopes, which we are currently validating via distinct *ex vivo* approaches, including exploring their ability to induce anti-cancer T-cell responses. However, to ultimately harness the most promising targets for immunotherapy, it is crucial to gain a better understanding of the functional profile of patients' T-cells, including differentiation, effector functions and exhaustion levels. The latter are inherently linked to the T-cells' metabolic profile, an area that still lacks comprehensive exploration in children and will be a primary focus of our project. In conclusion, our study aims to improve adoptive cell therapies through identification of novel immunogenic cancer-specific neoantigens and the strengthening of T-cell responses tailored to our patients.

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755 – P1.04.05

Potential cross-reactivity between tisular transglutaminase neo-epitope antibodies and Helicobacter pylori antibodies in coeliac disease diagnosis

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The processing of gliadin peptide by the tissue transglutaminase (tTG) produces deamidation and cross-linking reactions resulting in deamidated gliadin peptide (DGP) and tTG-neo epitope complexes that become immunogenic and may lead in coeliac disease (CD) in genetically predisposed indivduals. Detection of tTG neo-epitope antibodies is increasingly used for CD screening due to its high sensitivity because of the conformational epitope recognition. However, some patients show high titers of tTG neo-epitope that not correlates with levels of IgA and IgG against tTG, DGP or IgG against endomysium. Once known this, we observed that some of these patients were positive for *H. pylori* IgG antibody suggesting a possible cross-reactivity between *H. pylori* antibodies and tTG neo-epitope antibodies. To look into this phenomenon, we analyzed the *H. pylori* IgG antibody levels from the sera of 33 patients with tTG neo-epitope positive test for IgA and IgG antibodies using ELISA. Results show that 11 patients (30%) tested positive for *H. pylori* IgG, and only 2 of them were tested and resulted positive for *H. pylori* through endoscopic examination, suggesting that antibodies against *H. pylori* might cross-react with neoepitope assays of tTG, potentially interfering in CD screening. Moreover, given the possible cross-reaction with other antibodies, intestinal diseases and other infections should be considered in cases where tTG neo-epitopes antibodies are positive and result negative for *H. pylori*. Further studies are needed to clarify the clinical significance of this potential cross-reactivity.

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764 – P1.04.06

Expansion of CD8 T-cells recognizing tumor-shared antigens is correlated to the clinical outcomes of hepatocellular carcinoma patients treated with atezolizumab and bevacizumab

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Purpose: Hepatocellular carcinoma (HCC) is the most common type of liver cancer detected late and therefore associated with poor prognosis. Development of immunotherapy allowed improvement of survival in advanced HCC. However, objective responses occur only in a third of patients and the identification of biomarkers to predict therapeutic efficacy or patient relapse remains a major health issue.

Methods: CD8 specific immune responses of various antigens (telomerase, survivin, NY-ESO-1, mage (A1 and A3) and glypican) were evaluated by IFN_{γ} ELISpot assays in 10 healthy volunteers (HD) and 20 patients with advanced HCC treated by atezolizumab and bevacizumab in first-line setting included in ITHER study (NCT02840058). Peripheral blood mononuclear cells were analyzed before and after 3 months of atezolizumab and bevacizumab. The correlation between biological and clinicopathological parameters were analyzed.

Results: The frequencies of mage-A1, mage-A3 and glypican specific CD8 T-cell responses were higher in HCC patients than HD (5/19 vs 1/10 for mage-A1, 3/19 vs 1/10 for mage-A3 and 5/18 vs 1/10 for glypican). In addition, the frequency of telomerase specific CD8 T-cell responses were higher in responder HCC patients before and after treatment than non-responder HCC patients. Interestingly, the survivin and NY-ESO-1 specific CD8 T-cell responses were only observed in responder HCC patients at baseline; and after immunotherapy, the frequency of these specific responses were lower in non-responders compared to responders HCC patients in particular for survivn (1/9 vs 5/10). Moreover, mage and glypican specific CD8 T-cell responses were only observed in responder HCC patients after atezolizumab and bevacizumab treatment. At baseline, responder HCC patients had a higher frequency of mage-A1 specific CD8 T-cell responses than non-responder (4/10 vs 1/8).

Conclusion: These results validated the immunogenicity of these antigens derived peptides in advanced HCC patients treated by immunotherapy. Moreover, HCC patients with no survivin and NY-ESO-1 specific CD8 T-cell responses at baseline, and no mage and glypican specific CD8 T-cell responses after treatment had a poor prognosis. Thus, our results suggest a correlation of specific CD8 T-cell responses signature with atezolizumab and bevacizumab-mediated clinical outcomes. Our results also provide the rational for the immune-monitoring of HCC patients.

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776 – P1.04.07

A SARS-CoV-2 nucleocapsid protein-derived peptide influences primary and memory cellular responses by affecting the signal 1 and 2 during T cell activation

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Purpose: Cytotoxic $CD8^+$ T cells play an important role in vaccination and protection against SARS-CoV-2. In the attempt of identifying SARS-CoV-2 antigen-derived epitopes, we observed that an HLA-A2-restricted peptide from the nucleocapsid protein ($N_{219-227}$, LALLLLDRL, herein referred to as "LAL") possesses the ability to hinder $CD8^+$ T cell responses specific for other SARS-CoV-2 portions. Consequently, the aim of the present study was to determine the effects of the LAL peptide on epitope-specific naive and memory T cells. Moreover, we sought to explore the underlying molecular mechanisms involving both innate and adaptive immunity.

Methods:

PBMCs from Healthy donors with any history of melanoma and of SARS-CoV-2 infection or vaccination were used to investigate the impact of the LAL peptide on the priming naive $CD8^+$ T cells specific for the melanoma-derived EV10 through an *in vitro* approach. Subsequently, we examined the effect of this peptide on memory antigen-specific responses (elicited by a pool of commonly recognized viral epitopes) and upon general TCR stimulation (via aCD3 and aCD28) by Intracellular Cytokine Staining and by assessing proliferation as well as the expression of other functional markers. Concurrently, we measured the influence of the LAL peptide on the expression and presentation of MHC class I (on T2 and LCL cell lines) and on innate immune cell numbers and functionality.

Results: The LAL peptide negatively affected primary responses decreasing the expansion of primed naive $CD8^+$ T cells specific to EV10. Furthermore, it caused a significant reduction in cytokine production upon stimulation with a pool of memory viral peptide epitopes and upon general TCR ligation via aCD3 and aCD28 in $CD8^+$ T cells. These effects were connected with a reduction of the expression of MHC class I and of the co-stimulatory molecule CD86.

Conclusion: The LAL peptide hampered both the signals 1 (MHC class I expression) and 2 (co-stimulatory molecule) required for T cell activation. This accounted for a reduction in primary and memory epitope specific $CD8^+$ T cell responses, suggesting that this nucleocapsid-derived sequence could exert immunoevasion mechanisms during SARS-CoV-2 infection and indicating the importance of its targeting for vaccine rational design.

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1311 – P1.04.10

Chimeric virus-like particles as an efficient carrier for generating monoclonal antibodies against conserved epitopes of SARS-CoV-2 spike protein

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Diagnostics and prevention of the coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are essential for controlling the virus spread and reducing ongoing mortality rates. Even though the developed vaccines helped to control the pandemic and prevented severe cases of the disease, the virus still circulates with novel variants constantly emerging, hence SARS-CoV-2 molecular tests are still relevant. Given that the SARS-CoV-2 spike (S) protein is susceptible to mutations, the conserved spike epitopes hold great promise for future virus research.

Yeast-expressed chimeric virus-like particles (VLPs) formed by major capsid protein VP1 of hamster polyomavirus, exposing the target sequences on its surface are successfully applied as an effective immunogen. In this study, eleven 10-11 aa-long S protein peptides (at positions 129-137, 247-256, 278-287, 364-373, 420-430, 457-467, 491-500, 554-564, 815-825, 863-873 aa of ancestor S protein and 491-500 aa of Omicron S protein) were chosen based on the conservative aa sequence, surface localization and interaction with ACE2 receptor and were used for the generation of monoclonal antibodies (MAbs) by hybridoma technology. The results of the indirect ELISA proved that only five peptides were highly immunogenic. Overall, thirteen hybridoma cell lines secreting high affinity and specificity mouse IgG MAbs against six different SARS-CoV-2 S protein epitopes including positions 247-256, 591-500, 554-564, 815-825, 863-873 aa of ancestor and 491-500 aa of Omicron S protein were developed. Purified MAbs recognized recombinant S proteins by ELISA and Western blot. In addition, all of the generated MAbs were reactive with recombinant S proteins of different SARS-CoV-2 variants, such as B.1.1.7, B.1.351, B.1.617.1, B.1.617.2 and B.1.1529 which confirm that recognized epitopes are likely to be conservative. To analyze the ability of the MAbs to recognize natural SARS-CoV-2, indirect immunofluorescence analysis was used, which showed that six generated MAbs clones reacted with S protein in coronavirus-infected cells. The obtained results demonstrate that recombinant chimeric VPLs harboring target epitopes are specialized tools for development of targeted MAbs. The generated MAbs could be used for further research of the antigenic structure of SARS-CoV-2, also for developing MAbs-based immunoassays.

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1473 – P1.04.11

Identification of the novel HLA-C*01:65:02 allele

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Purpose: A novel HLA-C allele was identified in a liver transplant donor during our routine HLA typing analysis using nextgeneration sequencing (NGS).

Methods: Genomic DNA was isolated from splenic lymphocytes using Maxwell 16 Blood purification kit (Promega), HLA loci were sequenced on on an Illumina based GenDx NGSgo-MX6-1 platform (GenDx, Netherlands) and analyzed with NGSengine v2.31 (GenDx). Informed consent was obtained to perform genetic study.

Results: During HLA-C sequence analysis, a novel allele was shown as result, now named HLA-C*01:65:02. Subsequent analysis indicated that our sequence was identical to HLA-C*01:65:01 except for the codon 174, in exon 3. This change consists in a single nucleotide change from AAT in C*01:65:01 to AAC in C*01:65:02, which is a synonymous substitution for the amino acid asparagine. The amino acid sequence of C*01:65:01 is identical to HLA-C*01:02:01, except for the codon 197 in exon 2. This change consists in a single amino acid substitution from GAG of C*01:02:01 to AAG in C*01:65:01, which leads to a change in the amino acid sequence from glutamic acid to lysine. The full HLA typing of the individual expressing this novel allele was found to be HLA-A*02:01:01, -; -B*18:01:01, 56:01:01; -C*01:65:02, 05:01:01; -DRB1*03:01:01, 11:01:01; -DQB1*02:01:01, 03:01:01; -DPB1*04:01:01, 11:01:01.

Conclusion: The sequence was first submitted to GenBank and then to the IPD-IMGT/HLA Database. The name C*01:65:02 has been officially assigned by the WHO Nomenclature Committee for Factors of the HLA System in January 2024. This follows the agreed policy that, subject to the conditions stated in the most recent Nomenclature Report; names will be assigned to new sequences as they are identified.

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1590 – P1.04.12

Anti-MPO and anti-PR3 doble-positive: more is less

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Purpose: Anti-neutrophil cytoplasmic antibodies (ANCA) are mainly direct against myeloperoxidase (MPO) or proteinase-3 (PR3). Anti-MPO or anti-PR3 single positivity are closely related to ANCA-mediated vasculitis (AAV), although they have also been associated less frequently with other autoimmune diseases such as inflammatory bowel disease (IBD). These autoantibodies are rarely found to be concurrently present in patients, but in clinical practice some patients present MPO+PR3+ double-positivity. Currently, few studies have characterized the clinical features of these patients. The aim of this study was to compare the characteristics of patients with double-positive ANCA versus single-positive.

Methods: Retrospective study of 105 serum samples MPO+PR3+ (DP), MPO+PR3- (MPO+) or MPO-PR3+ (PR3+) analyzed by CLIA. Clinical data of ANCA-associated vasculitis (AAV), non-AAV autoimmune disease, inflammatory bowel disease (IBD) and organ involvement were collected and compared between DP and MPO+ or PR3+ patients.

Results: DP patients show lower anti-MPO values than MPO+ (43 CU (IQR 30-96) vs 153 CU (IQR 62-434); p<0.001) and lower anti-PR3 values than PR3+ (52 CU (IQR 34-176) vs 166 CU (IQR 52-605); p=0.004). AAV diagnosis is more frequent in both MPO+ (80% *vs* 29%; p<0.001) and PR3+ (86% vs 29%; p<0.001) than DP. Non-AAV autoimmune disease diagnosis was more frequent in DP group than MPO+ or PR3+ (43% *vs* 20%; p<0.001 in both cases). DP present more IBD cases than MPO+ (17% *vs* 0%; p=0.025). A higher percentage of renal involvement was observed in both MPO+ and PR3+ patients than in DP (71% vs 40%; p=0.004 in both cases). DP patients show a higher percentage of gastrointestinal involvement than MPO+ (23% vs 6%; p=0.040). DP present a lower incidence of lung (40% vs 69%; p=0.016), cutaneous (0% vs 26%; p=0.002) and ophthalmological (0% vs 34%; p<0.001) involvement than PR3+.

Conclusion: Patients with an MPO+PR3+ result present a lower probability of a vasculitis diagnosis than patients with a simple-positivity, although a diagnosis of non-AVV autoimmune disease is more likely. Thus, double-positivity may suggest an unspecific immune activation state.

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1716 – P1.04.13

Immunology laboratory landscape in the repeated determination of extractable nuclear antigens.

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Purpose: Specificities of the extractable nuclear antigen (ENA) are frequently required for the diagnosis of systemic autoimmune disorders. Nonetheless, immunology laboratories receive requests for ENA from patients who have already been diagnosed, even though many of these specificities are not recommended for autoimmune disease follow-up or who have had negative results in the past. The aim of this study is to examine the results of patients with repeatedly detected ENAs.

Methods: A retrospective study was carried out on patients from our centre, where the presence of ENAs was determined from 2008 to 2023 by immunodot. Both the frequency of seroconversion and the frequency of repeat immunodot of ENAs were determined.

Results: Our lab received 34498 requests for ENA detection between 2008 and 2023; of these, 25300 (73.3%) were negative and 9198 (26.6%) were positive (26.6%). 5719 patients had more than one ENA determination, and a median of 2 determinations (interquartile range: 2-4) and a median of 272 days (interquartile range: 142-512) between determinations. 4738 individuals (82.8%) had no variance in their results; Of these, 3451 (60.3%) had consistently negative results and 1287 (22.5%) had consistently positive results. Seroconversion was observed in 981 patients, representing 17.2% of the total. On the other hand, only patients with more than four determinations (n=1557) were taken into consideration in order to exclude results that were not validated in subsequent analyses. Of these patients, 500 showed some variation in the ENA result. However, only 245 showed a change that could be verified in subsequent analyses.

Among them, there were 110 patiens that had changes to positive, 55 changed to negative, and 80 that were temporary.

Conclusion: Repeat ENA gives the same result in 82.8% of cases. In addition, most of the changes in test results are not confirmed in subsequent determinations.

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2184 – P1.04.14

Plasmin-clipped beta-2-glycoprotein I: novel structures lead to increased antibody binding

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Purpose: Beta-2-Glycoprotein-I (β2GPI) is the main autoantigenic target of antiphospholipid syndrome (APS), these autoantibodies drive clinical manifestations including strokes and recurrent miscarrriage. There are two known structural isomers of β2GPI, an extended and compact form. The functions of β2GPI are contradictory, being capable of both activating and dampening the complement and coagulation cascades, how these contrasting functions are regulated is unknown but stuctural shift is implicated. β2GPI is also a substrate of the protease plasmin, which cleaves within the fifth domain of β2GPI leading to significantly altered protein activity. Very little is currently known regarding the structure or function of plasmin-clipped β2GPI. We present the first comprehensive structural characterisation of plasmin-clipped β2GPI and the associated implications for pathogenic antibody binding for this protein.

Methods: β2GPI was purified from healthy control plasma and cleaved with plasmin. Cleavage was confirmed by electrophoretic mobility by SDS-PAGE. Structural characterisation was undertaken using dynamic light scattering (DLS), small angle X-ray scattering (SAXS), ion mobility mass spectrometry (IMMS) and molecular dynamics simulation (MD). Activity was tested using inhibition of β2GPI ELISAs with APS patient samples and cleaved or non cleaved β2GPI in the fluid phase. Cellular binding was assessed using flow cytometry using HUVEC cells.

Results: Structural characterisation showed significant change for plasmin-clipped β2GPI in comparison to non-clipped β2GPI. DLS revealed a significantly smaller hydrodynamic radius for plasmin-clipped β2GPI ($p=0.0043$) while SAXS and MD analysis revealed a novel S-like structure of β2GPI only present in the plasmin-clipped sample. Ion-mobility-mass-spectrometry (IMMS) showed a different structural distribution in plasmin-clipped compared to non-clipped B2GPI, FACS demonstrated cellular binding was lost post cleavage with significantly less binding of plasmin-clipped β2GPI binding to HUVECs (34% vs 4%). There was also an increased binding of autoantibodies for the plasmin-clipped β2GPI ($p=0.056$), implying a greater exposure of pathogenic epitopes following cleavage.

Conclusion: Cleavage of β2GPI by plasmin results in the production of a unique S-shaped structural conformation with higher patient antibody binding. This may cause increased β2GPI/antibody soluble immune complexes and potentially drive autoantibody production. The altered structure of plasmin-clipped B2GPI may explain its changed functionality in vivo.

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2208 – P1.04.15

Rationale and design of a study to investigate a possible association between group A streptococcal M-peptide antibodies and rheumatic heart disease

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Introduction: Rheumatic heart disease (RHD), a sequelae of a group A streptococcal (GAS) infection, is thought to have an auto-immune basis, given underlying inflammatory responses. Current diagnostic assessment for RHD, following clinical suspicion, needs echocardiographical confirmation, which is expensive and not readily available in high burden populations. Confirmation of findings from early studies reporting long-term persistence of GAS antibodies in sera of RHD patients, may present an opportunity for a an affordable point-of-care test (POCT) to identify at-risk individuals in low-resourced settings, especially In the absence of a vaccine for GAS.

Hypothesis: An underlying immune response drives the development of GAS-related sequela. GAS antibodies persist for an extended period, driving ongoing inflammatory processes including valvulitis, resulting in RHD. Here, we present the rationale and design of a study in RHD African patients to evaluate our hypothesis.

Methods: Previously-collected serum samples within the RHDGen repository, a large continental endeavour performing GWAS to predict RHD disease-susceptible genetic loci in African individuals, will be used for this study. RHD phenotypes in our collection comprise mild to chronic RHD disease states. Representative case (n=100) and control (n=100) samples from the South African cohort will be subjected to ELISA to assess immune responses to a panel of twenty-five Strep A antigens.

Implications and conclusions: This project is expected to provide insight into the role, if any, of GAS antibodies involved in RHD development. In addition to the potential of a POCT possibly identifying at-risk individuals, this work may also contribute to GAS vaccine efforts. Identification of potential biomarkers would assist the development of a diagnostic tool, capable of referring potential cases of ARF and RHD would alleviate the burden of healthcare systems in the developing world.

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2298 – P1.04.16

Role of microbial infections in instigated autoimmune responses: a large scale study

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Virus infections act as predisposing factors for autoimmune responses. Using computational approaches, we have shown that alphaviruses share peptide signatures that are conserved amongst them and share homology with human peptides. Interestingly, these human proteins have been shown to be harbouring autoantibodies against them in arthritic conditions. During the pandemic, with this prior expertise and knowledge, we could address some of the important questions in COVID-19 disease pathology. The origins of extra pulmonary effects in COVID-19 remain enigmatic as the reason for multi-organ damage is **not** always multi-organ infection**.** We speculated autoimmune responses as a plausible cause of exacerbated inflammation and extra pulmonary effects in COVID-19. Using computational methods, we identified human proteins distributed in different organs harbouring regions homologous to SARS-CoV2 peptides that could possibly be acting as autoantigens in COVID-19 patients displaying extra pulmonary tissue damage. Interestingly, these conserved regions are amongst the experimentally validated B cell epitopes of SARS-CoV2 proteins. Thus, our findings formed the basis for explaining the origins of auto antibodies via molecular mimicry of the SARS-CoV2 antigens and provided important insights into a hitherto unprecedented involvement of autoimmune process that could be contributing to the COVID-19 immunopathology. This work resulted in 2 journal publications (https://doi.org/10.1093/infdis/jiaa703; https://doi.org/10.1016/j.molimm.2021. 06.021) and have received more than 60 citations in highly reputed medical journals. We have further analysed unique properties of all experimentally shown microbial peptides (bacterial and viral) that cause autoimmune conditions. Results of this work will be discussed.

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P1.05 ARTIFICIAL INTELLIGENCE AND IMMUNITY

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178 – P1.05.01

Prediction of specific TCR-peptide binding from large dictionaries of TCR-peptide pairs for personalized cellular therapy.

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Purpose: A central avenue of personalized medicine is cellular therapy. Such therapies are often based on the induction of an immune response, through natural or chimeric receptors. For such receptors to induce a response, binding between T cell receptors (TCR) and their cognate target must occur. To optimize such TCRs for a specific peptide-MHC combination (pMHC), good predictors for TCR-pMHC are required.

Methods: To address that, given any TCR and peptide, we employ new Natural Language Processing (NLP) based methods to predict whether they bind. We combined large-scale TCR-peptide dictionaries with deep learning methods to produce ERGO (pEptide tcR matchinG predictiOn), a highly specific and generic TCR-peptide binding predictor. Target peptides and TCRs have different generation mechanisms. To capture these differences, ERGO uses different parallel encoders. At the broad level, we encode the CDR3 of each TCR and each peptide into numerical vectors. The encoded CDR3 and peptide are concatenated and used as an input to a neural network to predict binding.

Results: A set of standard tests are defined for the performance of peptide-TCR binding (upper row plots):

- Single Peptide Binding –Testing whether an unknown TCR binds a predefined target, using TCRs known to bind to this target.
- Multi-Peptide Selection –Given a set of predefined peptides, predict which of those will be bound by a new TCR.
- TCR-Peptide Pairing - TPP-I -test whether a randomly chosen TCR binds a randomly chosen peptide. This was further extended to TPP-II and TPP-III where either the TCR or the peptide are new.

ERGO significantly outperforms current methods in these tests even when not trained specifically for each test. In SPB and MPS, ERGO was tested on previously tested TCR and peptides with better results on most peptides.

Conclusion: We proposed a generic NLP based classifier for TCR-peptide binding using all components of the TCR and the peptide-MHC. We have shown that it is better than current state-of-the-art methods.

The software implementation and data sets are available at https://github.com/louzounlab/ERGO, and http://tcr.cs.biu.ac.il/

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179 – P1.05.02

Counting is almost all you need

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Purpose: The immune memory repertoire encodes the history of present and past infections and immunological attributes of the individual. As such, multiple methods were proposed to use T-cell receptor (TCR) repertoires to detect disease history.

Methods: We propose detecting TCRs associated with a condition and simply counting them or combining them with an attention mechanism.

Results: We here show that the counting method outperforms two leading algorithms. We then show that the counting can be further improved using a novel attention model to weigh the different TCRs. The attention model is based on the projection of TCRs using a Variational AutoEncoder (VAE). Both counting and attention algorithms predict better than current leading algorithms whether the host had CMV and its HLA alleles. As an intermediate solution between the complex attention model and the very simple counting model, we propose a new Graph Convolutional Network approach that obtains the accuracy of the attention model and the simplicity of the counting model.

Conclusion: We present three models for predicting the condition of a host and its HLA from their TCR repertoire, and show they lead to much better predictions than all current algorithms. The code for the models used in the paper is provided at: https://github.com/louzounlab/CountingIsAlmostAllYouNeed.

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181 – P1.05.03

Naive and memory T cells TCR–HLA-binding prediction

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Purpose: T cells recognize antigens through the interaction of their T cell receptor (TCR) with a peptide-major histocompatibility complex (pMHC) molecule. Following thymic-positive selection, TCRs in peripheral naive T cells are expected to bind MHC alleles of the host. Peripheral clonal selection is expected to further increase the frequency of antigen-specific TCRs that bind to the host MHC alleles.

Methods: To check for a systematic preference for MHC-binding T cells in TCR repertoires, we developed Natural Language Processing-based methods to predict TCR–MHC binding independently of the peptide presented for Class I MHC alleles. We trained a classifier on published TCR–pMHC binding pairs.

Results: The classifier obtained a high area under curve (AUC) of over 0.90 on the test set. However, when applied to TCR repertoires, the accuracy of the classifier dropped. We thus developed a two-stage prediction model, based on large-scale naive and memory TCR repertoires, denoted TCR HLA-binding predictor (CLAIRE). Since each host carries multiple human leukocyte antigen (HLA) alleles, we first computed whether a TCR on a CD8 T cell binds an MHC from any of the host Class-I HLA alleles. We then performed an iteration, where we predict the binding with the most probable allele from the first round. We show that this classifier is more precise for memory than for naïve cells. Moreover, it can be transferred between datasets. Finally, we developed a CD4–CD8 T cell classifier to apply CLAIRE to unsorted bulk sequencing datasets and showed a high AUC of 0.96 and 0.90 on large datasets.

Conclusion: A novel classifier can predict the type of the cell (CD4/CD8) and the MHC it binds to.

CLAIRE is available through a GitHub at: https://github.com/louzounlab/CLAIRE, and as a server at: https://claire.math.biu.ac.il/Home.

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366 – P1.05.04

Development of a novel machine-learning prognostic scoring system for patients presenting with spinal metastases utilizing analysis of existing datasets to predict prognosis, morbidity and mortality

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Introduction: Patients with symptomatic spinal metastases comprise a large subset of all cancer patients. The goal of these patients' care is palliation and optimization of quality of life, focusing on pain relief, stabilisation of their spinal column, reversing or preventing neurological deficits. Decision-making in their care is driven by the need to initiate emergent treatment to prevent or reverse neurological injury. An accurate estimate of their predicted prognosis is central to this process. Although life expectancy is critical to clinical decision-making, current available prognostic tools are too inaccurate for reliable clinical use.

Methods: The aim of this project is to establish a comprehensive study database, combining data including inflammatory status, inflammatory-driven conditions such as sarcopenia, as well as novel immune treatments such as immune checkpoint inhibitors from patients who were previously treated for spine metastatic cancer, which will be used to develop and then test a machine learning model, which can more accurately calculate a patient's predicted prognosis. 80% of the data will be used to train a machine learning algorithm to create a model for predicting prognosis. The remaining 20% of the data will be used to validate the model and further validations will be performed using international data.

Results and Disussion: A key factor in determining suitability for surgical management is patient prognosis. Whilst a number of scoring tools have been developed to assess prognosis, they have been superseded by advances in immuno-oncology, radiotherapy and surgical care, rendering them less useful for clinical decision-making. The development of a model to predict prognosis will facilitate accurate predictions of patient prognosis to aid in clinical and specifically surgical decision-making. In addition, the use of a trained and validated machine learning algorithmic model will allow for ongoing future modifications of the model to reflect the impact of immuno-oncology treatments advances and others in the management on cancer patients' prognoses.

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950 – P1.05.05

Leveraging multi-modal data and AI to dissect pathogenic mechanisms and identify novel immunometabolic targets in IBD

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Inflammatory bowel disease (IBD) is a chronic immune disease with increasing global burden. Although several drugs are approved, only a fraction of patients achieves remission. The etiology of IBD entails genetic and environmental factors. Immunometabolism and the underlying adaptation of immune and stromal cells to their microenvironment, have recently emerged as key drivers in the onset and progression of the disease.

We present the development and application of an analytical framework - *MetID* - which leverages the integrated analysis of multiple layers of orthogonal disease data and artificial intelligence/machine learning (AI/ML) to identify key nodal immunometabolic pathways playing a causal role in ulcerative colitis (UC) and Crohn's disease (CD). Our results highlight the role of lipid metabolism and point to *SPHK1*, a sphingosine kinase, as a disease driver for both, UC and CD. *SPHK1* is part of the clinically validated sphingolipid pathway. Our multi-omics data support additional targeting of fibroblast and macrophage metabolism via *SPHK1* and strengthen the relevance of these cell types in IBD.

Furthermore, we demonstrate a distinct role for *SPHK1* in disease relevant assays containing the above-mentioned cell types.

This work highlights the value of combining high-dimensional multi-modal features and AI/ML to identify immunometabolic disease drivers and paves the way to the discovery of novel therapeutic opportunities in IBD. We suggest lipid metabolism and *SPHK1* could be causal nodes and a common pathomechanism for both UC and CD conditions.

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960 – P1.05.06

Identification of distinct NK cell and monocyte subpopulations in recurrent pregnancy loss and recurrent implantation failure through flow cytometry unsupervised analysis

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Purpose: Recurrent pregnancy loss (RPL) and recurrent implantation failure (RIF) pose significant challenges in reproductive medicine, often attributed to underlying immunological dysregulation when considered unexplained. This study aimed to investigate immune subpopulation alterations in RPL and RIF patients compared to controls using unsupervised flow cytometry analysis.

Methods: Peripheral blood samples were collected from RPL and RIF patients, and healthy controls (HC). Immune subpopulations were analyzed using flow cytometry with a panel of markers. Due to the limitations of manual gating, unsupervised analysis was employed to identify novel immune cell clusters based on distinct biomarker expression patterns.

Results: Unsupervised analysis revealed the presence of 14 distinct clusters in natural killer (NK) cells and 24 clusters in monocytes. Four clusters exhibited significant discrepancies between RPL/RIF patients and controls, characterized by distinct phenotypic marker expression profiles. Specifically, Cluster 2 ($NKp30^{-}TIGIT^{+}CD69^{+}$ cytotoxic NK cells) and Cluster 7 ($NKp30^{-}TIGIT^{+}CD69^{-}$ cytotoxic NK cells) were reduced in RIF group and Cluster 11 ($CD3^{+}CD56^{+}Perf^{+}TIGIT^{+}$) and 14 ($CD3^{+}CD56^{+}Perf^{+}TIGIT^{-}$) were reduced in RPL group compared to HC. Additionally, in the monocyte compartment, an increase in Cluster 5 ($CD14^{low}CD16^{+}$HLA-$DR^{+}CX3CR1^{+}CD69^{+}$) and 22 ($CD14^{high}CD16^{-}$HLA-$DR^{-}CX3CR1^{-}CD69^{+}CCR2^{+}$) was observed in RIF group while a reduction of Cluster 17 ($CD14^{low}CD16^{+}$HLA-$DR^{-}CX3CR1^{-}CD69^{+}$) in RIF group and Cluster 18 ($CD14^{high}CD16^{-}$HLA-$DR^{+}CX3CR1^{+}CD69^{+}CCR2^{+}$CD11b Activated^{+}) in RPL and RIF were noted. These alterations in the frequency and activation status of NK cell and monocyte subpopulations were observed in RPL/RIF patients, suggesting potential involvement in the pathogenesis of reproductive failure.

Discussion: The identification of immune subpopulation discrepancies in RPL and RIF patients provides valuable insights into the immunological mechanisms underlying these conditions. The utilization of unsupervised analysis enabled the detection of novel immune cell clusters that may have been overlooked using traditional manual gating approaches. These findings highlight the complexity of immune dysregulation in reproductive failure and underscore the importance of comprehensive immune profiling in understanding disease pathogenesis. Further research is warranted to elucidate the functional significance of the identified immune subpopulations and their potential as diagnostic or therapeutic targets in RPL and RIF.

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P1.06 B LYMPHOCYTE REGULATION AND FUNCTION

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292 – P1.06.01

Dynamicmicropatterns as a novel tool to study immune synapse formation and activation of B cells

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B lymphocytes form a critical part of the adaptive immune system. Their activation and differentiation into antibody-producing cells is dependent on the B cell receptor (BCR), which recognizes a vast repertoire of foreign antigens. While B cells can be activated by soluble antigens, it has emerged that probably the most typical form of antigen encounter is on the surface of antigen presenting cells (APC), which leads to the formation of the immune synapse (IS).

To study the formation of the IS and early B cell activation we use a novel dynamic biotinylation-based micropatterning technique. This method allows us to observe and image individual B cells before and after BCR engagement with high spatio-temporal control of the process. We can analyse both activatory (surrogate antigen) and non-activatory areas in the cell as well as different time points of the activation process, we can also introduce other factors like mechanical stress in the activation process by using different micropattern shapes. This method allows for an array of microscopy modalities to be used both in fixed and live samples.

We have observed several BCR signaling proteins (Btk, PLCg2, Akt and CD79A) in this system and found the different distribution of them along the activation process and the different substrates and the effect of two pattern shapes (5 µm dots and 15 µm triangles) in the protein distribution in the IS. We have also been able to observe the actin cytoskeleton upon activation and the formation of filipodia and lamelipodia during the spreading of B cells and the introduction of actin cytoskeleton inhibitors to observe their effects on IS formation.

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405 – P1.06.02

Exploring B cell-derived autophagosome-like vesicle: mechanism and function

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Purpose: We discovered a novel type of extracellular vesicle (EV) secretion by B cells, termed autophagosome-like extracellular vesicle (ALV), and investigated its impact on B cell differentiation.

Methods: EVs were isolated from stimulated-B cell culture media using different centrifugal forces. Western blotting was performed to compare the ALV marker LC3-II among various combinations of stimuli. To investigate the molecular mechanism of ALV secretion, RNA was extracted from stimulated-B cells and subjected to RT-qPCR. Candidate protein expression was confirmed by western blotting. The role of the candidate gene was assessed using the genetic disruption CRISPR-Cas9 system and reconstitution experiments in B-lymphoma A20 cells. To explore the effects of ALV on B cell differentiation, primary B cells were treated with ALV with or without cytokines. B cells were collected, and flow cytometry was used to analyze exosome endocytosis and cell differentiation.

Results: Interleukin 4 combined with CD40 antibodies (IL-4:CD40) significantly enhanced LC3-II$^+$ALV secretion by B cells compared to other stimuli combinations. This phenomenon occurred by directing autophagosomes towards the endosomal pathway rather than the lysosomal pathway. Moreover, IL-4:CD40 preferentially enhanced the expression of RAB27a, which regulates exosome secretion, at both RNA and protein levels. The RAB27a mutant cells exhibited impaired LC3-II$^+$ALV secretion, which was restored by reconstitution with wild-type RAB27A. ALV combined with IL-4 induced germinal center reaction and IgG1 class switching in vitro, whereas the classical exosome (from IL-4 and lipopolysacchrides co-stimulated B cells) tended to promote plasma cell differentiation. Importantly, neither ALV nor the classical exosome alone affected B cell differentiation, highlighting the indispensable role of cytokines during exosome-mediated effects.

Conclusion: The external IL-4:CD40 co-stimulation enhances LC3-II$^+$ALV secretion by B cells through a RAB27a-dependent pathway. To our knowledge, ALV represents the first instance of secretory autophagy occurring after external stimulation without disrupting autophagosome-lysosome fusion. The secretion of ALV by activated B cells may serve as a positive feedback loop to enhance B cell differentiation, revealing a novel role of secretory autophagy in intercellular communication during B cell activation.

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481 – P1.06.03

Human B-cell intersection between systemic and the upper respiratory tract

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The upper airways are the main port of entry for respiratory pathogens. To prevent respiratory infection, immune protection is mostly needed at mucosal sites, particularly in the upper respiratory tract. B cells contribute to mucosal immunity by producing IgA and IgG antibodies. Antibodies of IgA isotype are secreted by plasma cells residing in the lamina propria and those of IgG isotype derive from systemic immunity and reach mucosal sites by transudation from the serum. Parenteral vaccination does not induce tissue-resident memory T cells (TRM) in the respiratory tract and tissue-resident memory B cells (BRM) have not been demonstrated. Local priming is necessary to generate TRM and BRM, able to rapidly respond to reinfection. In humans, TRMs provide immune surveillance at mucosal sites and can induce rapid antigen-specific recall responses but very little is known about BRMs and most of the data has been generated in mouse models.

Integrin adhesion molecules orchestrate lymphocyte migration and homing to normal and inflamed tissues. The expression of integrins is a prerequisite for the adhesion to the endothelium, but inside-out signaling through the B cell receptor causes the conformational changes that render integrins able to bind their ligands with higher avidity and mediate extravasation.

We have investigated integrin expression on memory B cells (MBCs) of vaccinated adults and children with or without previous COVID-19. We found that recently activated MBCs (actMBCs) and plasmablasts (PBs) in the peripheral blood express the highest levels of integrin molecules and that infection expands both populations. We compared sorted B cell populations from the peripheral blood to those found in nasal and oral swabs. By flow-cytometry, total single cell RNAseq, and VDJ analysis we show that B cells in the swabs are similar to actMBCs and secrete antibodies of IgA and IgG isotypes in vitro.

The frequency of B cells in the nasal and oral swabs is dependent on the local inflammatory conditions suggesting that immunity at mucosal surfaces is induced and does not persist without inflammation.

Thus, although vaccine-induced MBCs are perfectly functional, they cannot prevent infection in the upper airways, because their recruitment follows instead of preceding infection.

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519 – P1.06.04

Early-Onset Common Variable Immunodeficiency in a Patient with Heterozygous Variants in Interferon Response-Associated Genes TRAF3 and IRF4

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Background: Common variable immunodeficiency (CVID) is the most common clinically relevant inborn error of immunity. Causes of CVID are poorly understood and the genetic cause remains unidentified in around 85% of the cases. Disturbed interferon response is known to occur in CVID. In this study, we describe a patient with early-onset CVID that displayed abnormal B cell differentiation, hypogammaglobulinemia, recurrent infections, autoimmune/lymphatic colitis, and lymphoid hyperplasia.

Objectives: This study aims to characterize a novel phenotype associated with heterozygous variants in two interferon response-associated genes, *TRAF3* and *IRF4*.

Methods: Genetic causes of the patient's symptoms were examined with WES. NanoString gene expression analysis was used to study the expression of 50 immune signaling-associated genes. The variants and the characteristics and function of the patient's immune cells were studied with RT-PCR, western blot, flow cytometry, and ELISA.

Results: WES analysis identified one rare missense and one novel protein-truncating variant in cis in *TRAF3:* NC_000014.8:g.[103336572G>A;103369748C>T], NM_145725:r.[34G>A;1116_1135del], p.[Ala12Thr;Gln373Profs*10]. Additionally, an ultra-rare missense variant was found in *IRF4*:NC_000006.11:g.398863C>A, NM_002460:r.(673C>A), p.(Pro225Thr). NanoString analysis demonstrated overexpression in type I interferon-stimulated genes in PBMCs. In concordance, stimulating fibroblasts with poly(I:C) revealed pronounced IFN-β excretion in the patient's cells. The immunophenotyping of the patient's lymphocytes revealed abnormal frequencies of B and T cell populations. The patient had normal total amounts of peripheral B cells with an increased proportion of naïve cells, indicating defective B cell function and differentiation. Although *in vitro* class-switch recombination assay showed that the patient's naïve B cells switch to IgG-producing plasma blasts to a greater extent than controls', IgG ELISA on the culture supernatants indicated nearly absent IgG secretion from the patient's B cells. Further analysis is underway to confirm the role of these variants in disease**.**

Conclusion: Our results suggest that the possible combined effect of *TRAF3* and *IRF4* variants disrupts interferon signaling and B cell differentiation. Further studies are required to elucidate how the variants in interferon response-associated genes contribute to CVID.

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727 – P1.06.06

Enhanced Antibody Responses in CD19-Cre mice

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CD19-Cre is an important and widely used Cre-lox model for B cell-specific genetic manipulation in murine systems. Mice carrying one allele of CD19-Cre are, at the same time, rendered heterozygote for CD19, a crucial coreceptor of the B cell antigen receptor (BCR). As a result, CD19-Cre mice exhibit diminished expression levels of CD19, with potential, yet insufficiently examined, consequences in B cell activation. Here, we report significantly heightened antibody responses upon both T-dependent (NP-KLH) and T-independent (NP-Ficoll) immunizations as well as elevated levels of basal IgM immunoglobulin levels in CD19-Cre mice. *In vitro*, we observed enhanced class-switch recombination and a moderate reduction in B cell proliferation upon LPS and IFNg stimulation, yet no drastic differences in BCR signalling. Our findings warrant careful concideration in the use of CD19-Cre mouse model in B cell research.

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738 – P1.06.07

Antigen-dependent regulation of the early stages of unwanted human B cell differentiation and antibody formation

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Blood transfusion may be required in patients undergoing surgery or having specific diseases. Some patients form antibodies against the transfused red blood cells (RBCs) or platelets, a process called alloimmunization. These antibodies form a problem upon additional transfusion. B cells are ultimately responsible for antibody production. Once formed, antibody-producing plasma cells (PCs) may survive for decades and are challenging to eradicate. Preventing formation of long-lived PCs is thus highly desired. The underlying mechanism of alloimmunization and, more generally, the differentiation of B cells into long-lived PCs remains largely unknown. Limited data show that high antigen density on RBCs promotes alloimmunization, while low antigen density on RBCs induces tolerance[1]. Here, we investigate how antigenic context regulates the early steps of human T cell dependent B cell differentiation into antibody-producing PCs. To investigate this, we compare the effect of antigen in different contexts on B cell differentiation in human B/ CD4 T cell co-cultures. We compare soluble antigen, antigen in complexes (e.g. multimerization into tetramers and dextramers), and antigen incorporated into eukaryotic membranes (e.g. RBC with different amounts of antigen and antigen expressed on HEK293T cells). Our preliminary data show that we are able to detect an antigen specific response in our human B/T cell co-cultures when using soluble antigen. With this study, we intend to find key regulators of the pathways leading to wanted and unwanted antibody formation and thereby identify potential targets for intervention.

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751 – P1.06.08

Regulation of B cell function and expression of CD11c, T-bet, and FcRL5 in response to different activation signals

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The markers CD11c, FcRL5, and T-bet are commonly used to define a subset of B cells that expand during inflammatory conditions, such as infections and autoimmune disorders, where they can make up >30% of mature B cells. However, the association between the proteins and differentiation and function in the host response remain largely unclear. Using an *in vitro* system, we have assessed the co-expression of CD11c, T-bet and FcRL5 among naïve, memory and total B cells in response to stimulation via the B cell receptor (BCR), toll-like receptor 9 (TLR9), and different cytokines. Using flow cytometry, we observed different expression dynamics for all markers, with largely overlapping regulation of CD11c and FcRL5 in response to BCR and TLR9 activation, while T-bet was strongly dependent on IFN-g signalling. Naïve and memory B cells showed similar kinetics as total B cells, although CD11c was more rapidly upregulated on memory cells compared with naïve cells. To further investigate the phenotype of the *in vitro* generated B cells, we ran a qPCR array with 30 selected target genes on sorted naïve and memory B cells after stimulation. Whilst the results showed up- and downregulation of several genes in a manner that was consistent with the expected phenotypical expression of $CD11c^+$ B cells, not all did, indicating that the *in vitro* generated cells may only partially acquire the phenotype seen *in vivo*. Additionally, we observed an increase in gene expression assosciated with plasma cell formation. To further explore possible functions of the *in vitro* generated B cells we analysed the production of antibodies and cytokines, and antigen-presenting cell (APC) functions. There was no association between CD11c, FcRL5, or T-bet expression and antibody secretion or T cell help, rather the functions were associated with TLR9-signalling and B cell-derived IL-6 production, respectively. These results suggest that the expression of CD11c, FcRL5, and T-bet and plasma cell differentiation and improved APC functions occur in parallel and are regulated by similar activation signals, but they are not interdependent. This work helps to further understand the regulation and dynamics of CD11c, FcRL5 and T-bet expression in B cells.

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784 – P1.06.09

FAHD1 as a novel regulator of B cell metabolism

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Purpose: Fumarylacetoacetate hydrolase domain containing protein 1 (FAHD1) is mitochondrial protein that regulates the activity of the TCA cycle through the decarboxylation of oxaloacetate (OAA) into pyruvate and CO_2. Loss of FAHD1 activity results in accumulation of OAA and inhibition of Complex II activity. A pivotal point in the development of humoral responses is the germinal centre (GC) reaction, during which B cells undergo several cycles of intense proliferation and somatic hypermutation. Despite their high proliferation rates, GC B cells rely on fatty acid oxidation and oxidative phosphorylation instead of glycolysis for energy. We hypothesized that FAHD1 plays an important role in the regulation of TCA cycle activity in GC B cells.

Methods: To delineate the role of FAHD1 in B cell responses, we used our previously generated Fahd1-KO mouse strain. To confirm the expression of FAHD1 in B cells, we performed qPCR in sorted splenic cell populations. Furthermore, we performed flow cytometry analysis in spleens and mesenteric lymph nodes of Fahd1-KO and WT mice to detect any developmental or differentiation defects in the absence of FAHD1. Finally, we assessed mitochondrial activity by performing seahorse analysis in freshly isolated and in vitro activated Fahd1-WT or KO B cells.

Results: We were able to confirm the expression of FAHD1 in splenic B cell populations. Furthermore, our preliminary data show altered GC B cell percentages in the mesenteric lymph nodes of Fahd1-KO mice. Specifically, the proportion of dark zone GC B cells seems to be reduced in the absence of FAHD1. Preliminary seahorse analyses supports the hypothesis that loss of FAHD1 reduces mitochondrial robustness and TCA cycle activity in B cells.

Conclusion: In conclusion, FAHD1 is an emerging novel regulator of TCA cycle activity and metabolism. Here we provide the first insight into the importance of FAHD1 for B cell activity.

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808 – P1.06.10

A TNFR2-specific TNF fusion protein with improved vaccine effectiveness

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The germinal center (GC) response plays a crucial role in generating high-affinity antibody producing plasma cells and memory B cells, which is essential for long-term immunity and effective vaccination. However, enhancing the antibody affinity poses a significant challenge. In this study, we discovered that 'NewSTAR2', i.e. targeting TNFRII by a TNFRII-specific TNF fusion protein, could increase the efficacy of keyhole limpet hemocyanin (NP-KLH) immunization. NewSTAR2 significantly enhanced the frequencies of follicular helper T cells and GC-B cells during NP-KLH immunization *in vivo*. Moreover, it augmented the total amount of immunoglobulins in the serum and improved the affinity of anti-NP-specific antibodies. Additionally, NewSTAR2 dramatically increased the expression of CD80 and CD86 on B cells, along with promoting the proliferation and the frequency of GC-B cells *in vitro*. Taking together, these findings suggest that NewSTAR2 could be employed as an adjuvant to promote vaccine effectiveness.

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823 – P1.06.11

Unravelling the B cell immune synapse: septin cytoskeleton as a new master regulator of B cell activation

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The immune synapse (IS) is a cell-cell interaction platform critical in lymphocyte activation by specific antigens. Despite of B cells being able to also respond to soluble antigens, the in vivo importance of the IS and surface-tethered antigen recognition has strongly emerged in the recent years. The IS serves as a dynamic hub for multiple cellular actions but the molecular details of these functions, especially beyond the B cell antigen receptor (BCR) signalling, remain poorly understood. Here, to address the lack in the systems level understanding of the IS, we setup methodology for comprehensive investigation of the composition of primary mouse B cells' IS at proteome level. Utilizing functionalized magnetic beads to mimic antigen presenting cells and trigger IS formation on them, we developed a method to specifically and robustly extract the cell adhesions on the beads, namely the IS or transferrin receptor mediated adhesion as a control.

Our mass spectrometry analysis from the isolated synapses has already unveiled multiple potential new candidate proteins regulating the IS. From these, we are currently focusing on the role of septin7, a cytoskeletal GTPase, linked to several diseases such as Alzheimer's disease, schizophrenia and, notably, autoimmune disorders.

Our preliminary data, using a pharmacological septin inhibitor forchlorfenuron (FCF), support a key role of septins specifically in BCR signaling, immune synapse formation as well as antigen processing and lysosomal integrity.

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1144 – P1.06.12

Belimumab after rituximab combination therapy preferentially targets IgA2+ B cells in systemic lupus erythematosus

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Purpose: Systemic lupus erythematosus (SLE) is a heterogenous autoimmune disease affecting multiple organs. The BEAT lupus clinical trial showed that combination B cell depletion therapy with rituximab, followed by belimumab, an inhibitor of B cell activating factor (BAFF), resulted in fewer severe flares in SLE patients compared to rituximab alone. Although a minor component of circulating antibodies, serum IgA2 anti-DNA antibodies and IgA2+ plasmablasts were specifically reduced after combination therapy. We sought to validate these findings and explore underlying mechanisms.

Methods: 27-parameter spectral flow cytometry followed by clustering analysis was performed on PBMCs from the CALIBRATE trial; a phase-two randomized trial involving 43 patients with lupus nephritis, treated with rituximab alone or in combination with belimumab for 48 weeks. Measurement of autoantibodies and BAFF were performed by ELISA.

Results: IgA2+ (but not IgA1+) plasmablasts and serum IgA2 (but not IgA1) anti-dsDNA antibody levels were reduced after combination therapy but not rituximab alone ($p = 0.04$ and $p = 0.002$ respectively). The expression of BAFFR and BCMA were higher in IgA2+ (vs IgA1+) B cells at baseline, suggesting an increased sensitivity to belimumab. Baseline serum BAFF positively correlated with serum IgA2 levels ($p = 0.0057$) and IgA2+ plasmablasts (0.034). BAFFR expression was decreased and BCMA expression increased in IgA2+ plasmablasts after treatment in the belimumab arm compared to rituximab alone, whereas IgA1+ plasmablasts were unaffected. IgA2+ B cells also possessed a distinct chemokine receptor profile at baseline, with increased expression of gut homing markers CCR9 and ITGB7, whereas mucocutaneous (ITGB1) and follicular (CXCR5) markers were reduced compared with IgA1+ B cells. Belimumab reduced expression of CCR9 ($p < 0.001$) and ITGB7 ($p = 0.017$) only in IgA2+ plasmablasts.

Conclusion: Belimumab after B cell depletion therapy in SLE targets IgA2 + B cells, possibly due to enhanced sensitivity to BAFF, with reciprocal regulation of BAFFR and BCMA expression, and modulation of their tissue homing capacity. These results not only reveal a potential mechanism of action of rituximab/belimumab combination therapy but also suggest that SLE patients with high IgA2 anti-dsDNA antibody production could be stratified for this therapeutic strategy.

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1178 – P1.06.13

Clinical and biological impact of CD6 expression in chronic lymphocytic leukemia

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CD6 is one of the first lymphocyte surface receptors reported in T cells, though soon after it was also found expressed in the normal B1 cell subset as well as in poor and well differentiated B cell neoplasms. Extensive research focused on its role in T cell physiology revealed that CD6 physically associates with the TCR complex and modulates either positively or negatively the activation and differentiation signals delivered upon specific antigen recognition. Moreover, it plays an important role in T-APC synapsis stabilization through its interaction with CD166/ALCAM, a broadly expressed adhesion molecule (in epithelial, endothelial, activated T and B cells, macrophages and dendritic cells). By contrast, little is known about its role in B1 cell physiology. Early studies showed that CD6 ligation protects chronic lymphocytic leukemia (CLL) cells from soluble IgM crosslinking-induced apoptosis. More recently, data from *Cd6*$^{-/-}$ mice revealed an important contribution of *CD6* expression in natural antibody production and in anti-bacterial defense. Within this framework, we have evaluated the clinical and biological impact of *CD6* expression in a CLL patient cohort. Clinico-pathological correlation studies revealed that mRNA *CD6* expression levels were differentially associated with pre-defined CLL prognostic markers, such as the *IGHV* gene mutational status. Moreover, mRNA *CD6* expression levels could predict time-to-first treatment in both multivariate and univariate analyses. Further elucidation of this effect was pursued via transcriptomic data bioinformatic analysis (Gene Set Enrichment Analysis, GSEA), which showed, among other findings, altered expression of genes regulated by the proto-oncogene *MYC*. These results reinforce the notion that CD6 also has a relevant role in B cell physiology, which impacts the clinical behavior of B cell neoplasms.

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1204 – P1.06.14

Retrospective observational study of persistent polyclonal B cell lymphocytosis in our centre

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Purpose: Persistent Polyclonal B-cell Lymphocytosis (PPBL) is a rare and generally benign lymphoproliferative haematological disease of unknown aetiology occurring almost exclusively in women, characterised by chronic, stable and persistent polyclonal lymphocytosis, presence of binucleated lymphocytes, polyclonal increase in serum immunoglobulin M (IgM) and association with the HLA-DR7 haplotype. Those affected are frequently asymptomatic or may present slight splenomegaly. Our objective was to identify all patients that received a PPBL diagnosis at our hospital between 2020-2023 and review their characteristics.

Methods: We compiled clinical and laboratory findings of our sample population regarding: smoking status, presence of splenomegaly, leukocytes & B-cells count, serum immunoglobulin-M (IgM) levels, blood smear morphology, HLA-DRB1 haplotype and BCL-2/IgH genes rearrangement status.

Results: A total of 6 patients were diagnosed with PPBL, all of whom were women with a mean age of 50.8 years (range: 27-70 years). 5 patients self-reported to be smokers or ex-smokers, and 2 of them presented splenomegaly during examination. All 6 patients had shown a mild to moderately-severe lymphocytosis (mean: 13,640/dL, range: 10,550-18,690/dL) with increased absolute count (mean: 3,169/dL, range: 2,378.75-5,291/dL) and percentage (mean: 46%, range: 27.5-74%) of polyclonal B-cells. Serum IgM levels (mean: 501.8mg/dL, range: 381-705mg/dL) were above the upper reference limit in all patients (normal values: 33-293mg/dL). Blood smears were performed in 3 patients; findings included binucleated lymphocytes, smudge cells (Gumprecht shadows) and activated lymphocytes. 5 patients were carriers of the HLA-DRB1*07 haplotype and none of them presented with rearrangements in BCL2/IgH.

Conclusion: Our findings report that PPBL is associated with elevated IgM levels and the HLA-DRB1*07 haplotype, which is in line with the literature. Further studies are needed regarding patient follow-up to determine whether this seemingly benign condition poses any threat of predisposition to other haematological diseases or becoming premalignant and slow-evolving (like monoclonal gammopathies of undetermined significance). Furthermore, it is important for physicians to be aware of PPBL as certain characteristics associated with this disorder such as blood smear anomalies, finding of isochromosome 3q, premature chromosome condensation or multiple BCL-2/IgH rearrangements could lead to a misdiagnosis as a non-Hodgkin lymphoma.

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1267 – P1.06.15

Regulatory B cells expressing granzyme B in renal graft tolerant transplant patients: highly differentiated B cells with a unique pathway, a specific regulatory profile and strong interactions with immune system cells

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Spontaneous operational tolerance, described by the absence of clinical signs of rejection without immunosuppression, is the holy grail of transplantation, with patients maintaining perfect graft function without drug-induced complications. Granzyme B-expressing regulatory B cells (GZMB$^+$ B cells) have been found enriched in the blood of such spontaneous operational tolerant patients after kidney transplantation and we reported that these GZMB$^+$ B cells may be induced in vitro, while keeping their regulatory properties. In order to better characterize them, we analysed two single-cell RNA sequencing (scRNAseq) datasets of: 1) peripheral blood mononuclear cells (PBMC) including natural GZMB+ B cells from 10 kidney transplanted patients including patients with stable graft function on conventional immunosuppression (IS) (STA, n=3), drug-free tolerant patients (TOL, n=3) and patients with ABMR (n=3) and 2) *ex vivo* induced GZMB$^+$ B cells from 4 transplanted patients with same characteristics (TOL, STA, ABMR). We first showed that natural GZMB$^+$ B cells found in PBMCs exhibited a specific regulatory transcriptomic profile associated with reduced expression of HLA molecules-, apoptosis- and inflammatory response-related genes and were enriched in NK cells-specific genes such *NKG7* and *KLRD1*. A pseudo-temporal trajectory analysis showed that these natural GZMB$^+$ B cells were highly differentiated B cells with a *KLF13*-dependent trajectory distinct from that of memory B cells. E*x vivo* induced GZMB$^+$ cells shared with natural GZMB$^+$ B cells a 'regulatory' signature in all patients, exacerbated and highly conserved in tolerant patients, with genes encoding B cell regulatory molecules or associated with migration processes such as *CCR7*, *CCL3* or *CCL4*. In accordance with their migratory-related profile, we found that these GZMB$^+$ B cells were able to infiltrate the graft under inflammatory conditions, suggesting that they may act where immune events take place. In conclusion, these data suggest that GZMB$^+$ B cells acquire a specific phenotype that may be due to the progressive establishment of a protolerogenic environment in these patients. The conserved signature of these highly differentiated GZMB$^+$ B cells that keep their regulatory profile after induction *ex vivo* suggests that they could pave the way for future cell therapy protocols.

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1345 – P1.06.16

The actin regulator WASp associates with chromatin and regulates RNA polymerase II transcription.

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Purpose: The role of Wiskott-Aldrich syndrome protein (WASp) in cytoplasmic actin regulation has been extensively studied, however, its role in the nucleus remains largely unexplored. We aimed to reveal the funtion of WASp in the nucleus of B cells during differentiation from naïve to activated B cells. Our hypothesis is that WASp-mediated actin polymerization in the nucleus may assist dynamic nuclear processes like chromatin remodeling and regulation of gene expression.

Methods: The B cells ability to undergo differentiation relies on processes regulated by the actin cytoskeleton, such as migration, cell-to-cell communication, proliferation, and genomic rearrangement. We examined B cells isolated from wildtype mice and mice lacking WASp or expressing overactive WASp (L272P- and I296T- WASp mutations). B cells were stimulated *in vitro* with LPS and IL-4 to induce their proliferation and differentiation. We performed nuclear cytosol fractionation of naïve and activated B cells, immunoprecipitation, flow cytometry, ChIP-seq and RNA-seq, to obtain a comprehensive view of the function of WASp in the nucleus of B cells.

Results: Nuclear-cytosol fractionation and WASp immunoprecipitation detected WASp in both the cytosol and nucleus of naïve and activated B cells. Using flow cytometry analysis of purified nuclei, we detected increased chromatin association of Histone 3 and RNA polymerase II when comparing wildtype activated to naïve B cells with the mutants. When compared to activated wildtype B cells, WASp KO and overactive WASp B cells had decreased Histone 3 and RNA Polymerase II, suggesting a role for WASp in chromatin dynamics and transcription. Using RNA pol II ChIP-seq and metagene analysis, WASp KO B cells had reduced RNA Pol II transcript coverage.

Conclusion: Our data provide evidence that WASp is directly associated with RNA polymerase II at transcriptional start sites. Moreover, WASp may have a role in chromatin remodeling. B cells represent a unique cell type to investigate WASp and actin in the nucleus and may give insights into the molecular mechanism underlying Wiskott-Aldrich Syndrome and related immune disorders.

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1369 – P1.06.17

Aberrant B Cell Receptor Signaling Responses in Double-negative B Cells of Ankylosing Spondylitis Patients

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Purpose: B cells that have undergone immunoglobulin (Ig) heavy chain class switch recombination and lack expression of IgD and the CD27 memory marker are referred to as double-negative (DN) B cells. They make up a heterogeneous group of B cells that accumulate with advanced age, in chronic infections and autoimmune disease, in which contexts they have been associated with anergy, exhaustion or hyper-reactivity. Because signaling properties of DN B cells are not well characterized, we explored their B cell receptor (BCR) responsiveness.

Methods: We evaluated BCR responsiveness in DN subpopulations that on the basis of their CD21 and CD11c expression were divided into: DN1 ($CD21^{+}CD11c^{-}$), DN2 ($CD21^{lo}CD11c^{+}$) and DN3 ($CD21^{lo}CD11c^{-}$) B cells. To investigate downstream BCR signaling responses, peripheral blood mononuclear fractions were stimulated with anti-Ig or were left unstimulated, and the phosphorylation status of spleen tyrosine kinase (SYK), phosphoinositide-3-kinase (PI3K) p85, and extracellular signal regulated kinase (ERK)1/2 was quantified by phosphoflow cytometry.

Results: In healthy individuals, DN2 and DN3 B cell fractions displayed an anergic phenotype, because BCR-induced phosphorylation of SYK, PI3K p85, or ERK1/2 was absent or very low, compared to DN1 B cells, naïve B cells or $CD27^{+}$ memory B cells. Next, we studied patients with ankylosing spondylitis (AS), a chronic rheumatic autoinflammatory disease of unknown etiology that mainly affects spine joints. Although innate immune cells and T cells are thought to play a major role, autoantibodies have been shown to be present in the serum of patients with AS. In contrast to our findings in healthy controls (n=15), in patients with AS (N=28) the DN2 B cells – but not DN3 B cells - showed significant signaling responses upon BCR engagement.

Conclusion: Our findings indicate that DN2 and DN3 B cells are anergic in homeostatic conditions and that in patients with AS DN2 B cells, but not DN3 B cells, become sensitive to BCR stimulation. As a result, in AS and perhaps also in systemic autoimmune diseases, specifically DN2 B cells may differentiate more easily into pathogenic antibody-secreting cells.

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1372 – P1.06.18

TLR-mediated innate signaling drives early B cell differentiation after influenza virus infection

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Infection-induced B cell activation occurs in discrete steps and in the context of complex innate immune responses, including the elaboration of cytokines, PAMPS and DAMPS and the remodeling of secondary lymph tissues draining lymph nodes. Our long-term goal is to understand how innate signals regulate the adaptive humoral response. Using a mouse model of influenza virus infection, we and others demonstrated immediate early, Type I IFN-induced B cell activation in the respiratory tract draining lymph nodes (dLN) 2-3 days post infection (dpi), prior to the formation of antibody-secreting cells (ASCs) in extrafollicular foci (EF). Among other changes, Type I IFN caused upregulated of TLR3 and TLR7 expression but not of TLR4 by B cells after infection. Using single and double gene-targeted mice, we also showed that protective EF responses required B cell intrinsic signaling via both TLR adaptors, MyD88 and TRIF, which drove upregulation of the transcriptional master regulator of differentiation, IRF4. While MyD88-mediated B cell activation is known to support clonal expansion, the role of TRIF in B cell stimulation is little understood. Here we report that TLR3 is upstream of B cell intrinsic TRIF-signaling during influenza infection and directs B cells towards enhanced B cell differentiation and EF formation. In the context of MyD88-deficiency, the B cell intrinsic lack of TLR3 strongly abrogated EF development. In vitro, stimulation of TLR3 alone did not affect B cell proliferation but acted synergistically with TLR7 to enhance B cell clonal expansion. Moreover, stimulation via TLR3 enhanced upregulation of Blimp-1 and J-chain, markers of ASCs. Mechanistically, TLR3 stimulation in vitro caused upregulation of costimulatory molecules CD69 and CD86 and of the high affinity IL-2 receptor CD25. Addition of IL2 further supported B cell differentiation, while in vivo blockade of CD25 inhibited EF formation. Thus, the EF B cell fate is regulated through TLR-mediated signals that synergize during early influenza virus infection to support differentiation to ASCs. The effects of this early drive towards EF on germinal center formation will be discussed.

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1459 – P1.06.19

Tyrosine kinase 2 modulates splenic B cells through type I IFN and TLR7 signaling

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Tyrosine kinase 2 (TYK2) is involved in type I interferon (IFN-I) signaling, which is crucial in early antiviral responses. In humans, TYK2 deficiency increases susceptibility to infections, and TYK2 loss-of-function variants protects against autoimmunity. However, the role of TYK2 in splenic B cells is not fully understood yet.

Splenic B cell subpopulations [marginal zone (MZ), follicular (FO) and aged (ABC) B cells] from C57BL/6 (WT) and *Tyk2*$^{-/-}$ mice were analyzed *ex vivo* by flow cytometry. Purified B cell subpopulations obtained by fluorescent activated cell sorting were used to study differential gene expression by RNA-seq. CD19^{+} B cells were stimulated *in vitro* with TLR ligands (CL097, Imiquimod, LPS), IFN-α, and anti-CD40 plus IL-4 to evaluate their effect in cell proliferation and differentiation. In addition, cytokine and immunoglobulin secretion were quantified by Cytometric Bead Array and ELISA, respectively.

An altered splenic B cell distribution was observed in *Tyk2*$^{-/-}$ mice: an increase in ABC cells and a decrease in MZ B cells. Transcriptome studies in homeostasis conditions identified genes whose expression was downregulated, such as IFN-I stimulated genes and TLR7. B cell cultures showed that *Tyk2*$^{-/-}$ B cells proliferated and differentiated into plasmablasts (CD138^{+}) to a lesser extent than WT B cells in the presence of TLR7 ligands, whereas TLR4- and T-dependent activation (anti-CD40 plus IL-4) remained unchanged. Analysis of culture supernatants revealed a significant reduction of IL-6 and IL-10 secretion and humoral response in *Tyk2*$^{-/-}$ B cells.

These results highlight the existence of a crosstalk between TYK2 and TLR7 mediated by IFN-I feedback loop, which contributes to the establishment of MZ B cells and B cell proliferation and differentiation.

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1466 – P1.06.20

Extracellular vesicles produced by regulatory B cells: new approach in kidney transplantation

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Purpose: Kidney transplantation remains the best therapeutic option for patients with end-stage renal disease. However, it requires lifelong immunosuppressive therapy, with side-effects and an overall negative impact on long-term graft survival. Some patients display operational tolerance after discontinuation of these treatments. The search for clinical history and biomarkers has enabled us to identify in these patients an increase of regulatory B cells that inhibit the proliferation of effector CD4+/CD8+ T cells. However, the mechanism of action of these cells remains poorly understood. Given the ability of various immunomodulatory cells, such as Tregs, to mediate their mechanism of action through the secretion of extracellular vesicles, we hypothesized that the suppressive effect of Breg cells might be mediated by the secretion of EVs.

Methods: GzmB+ Bregs were expanded for 3 days and subjected or not to mechanical stimulation. EVs produced spontaneously or by stimulation were isolated from the supernatant by differential centrifugation. We characterized their type by cryo-electron microscopy and western blot, and their content by mass spectrometry and in situ hybridization compared with EVs from unstimulated B cells. EVs were then cultured for 72 h with anti-CD3/CD28 bead activated CD4+ T cells to characterize their regulatory potential.

Results: We demonstrated that GzmB+ Bregs release exosomes that are able to dose-dependently inhibit CD4+ T cell proliferation and induce their apoptosis in vitro. We showed that EVs from GzmB+ Bregs are less immunogenic with lower expression of MHCII molecules compared with EVs from non Breg cells. Analysis of their contents revealed a significant reduction in 11 pro-inflammatory miRNA and an enrichment in 11 proteins involved in the regulation of proliferation and programmed cell death of effector T cells. Finally, we showed that shear stress by mechanical stimulation increased EV production by a factor of 3, without altering their suppressive properties.

Conclusion: Due to their inhibitory potential, their lesser immunogenicity, and the possibility to increase their secretion, EVs from GzmB+ Bregs open new approaches in kidney transplantation, as therapeutic targets and biomarkers.

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European Journal of Immunology Clinical-Basic-Translational

1604 – P1.06.21

Regulatory factor X 7 limits B cell activation and lymphomagenesis

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Regulatory Factor X 7 (RFX7) is a transcription factor emerging as an important regulator of immunity. We previously found that it is required for maintaining natural killer (NK) cells' homeostasis by limiting their activation and metabolic rate. Interestingly, elevated metabolism is a hallmark of cancer and *RFX7* mutations have been reported in Burkitt's lymphoma as well as in diffuse large B cell lymphoma (DLBCL), two B cell malignancies originating from antigen-experienced B cells. We thus set out to study the role of RFX7 in B cell lymphomagenesis and activation.

We observed that *RFX7* mutations found in Burkitt's lymphoma and DLBCL cause loss of transcription factor function. Notably, *in vivo* deletion of Rfx7 in B cells accelerated pathogenesis in a Bcl6-driven murine lymphoma model, suggesting relevance for RFX7 in B cell activation processes of the germinal center (GC). Accordingly, Rfx7-deficient B cells exhibited stronger activation in response to B cell receptor triggering *in vitro* and, upon immunization, mice with Rfx7-deleted B cells produced larger germinal centers (GCs). Comparison of activated Rfx7-deficient and control follicular B cells transcriptome revealed alterations hinting at underlying mechanisms that we are currently testing through genetic rescue approaches.

Collectively, these data demonstrate that RFX7 acts as a tumor suppressor in lymphoid malignancy by regulating GC B cell activation. These findings broaden our understanding of the physiological relevance of RFX7, but also our knowledge of the mechanisms controlling B cell-dependent immunity and immune disorders.

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1790 – P1.06.22

Regulation of humoral immune responses differs from COVID-19 in MIS-C cases

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Purpose: MIS-C (multisystem inflammatory syndrome in children) is a clinical condition that may be manifested by symptoms such as fever, abdominal pain, headache, vomiting, and diarrhea that may occur in children with SARS-CoV-2 infection. B regulatory cells (Bregs) are immunosuppressive cells that support immune tolerance and suppress pathological immune responses. B cell exhaustion is associated with weaker antibody responses to pathogens. This study aimed to investigate the role of B cell subsets in the pathogenesis of MIS-C.

Methods: 17 MIS-C cases, 17 pediatric COVID-19 and 17 healthy controls were included in the study. Flow cytometric evaluation was performed using a 10-color mAb panel from peripheral blood samples.

Results: $CD19^+$ B cells were significantly increased in MIS-C compared with COVID-19 and the healthy control group. Consistent with this increase, IgM and IgD expression were found to be increased in MIS-C patients compared to COVID -19 patients and healthy controls. Naive B cells ($CD27^-IgD^+$) were found to be increased in MIS -C patients compared with COVID-19. Switched memory and IgM memory cells ($CD27^+IgD^-$ and $CD27^+$ IgD^+) also showed a similar profile. A significant decrease in MIS-C cases was observed in immature/transient B cells ($CD24^{high}CD38^{high}$) and primary memory B primarily cells ($CD24^{high}CD38^-$) compared to COVID-19 and healthy controls. Regulatory B10 cells ($CD24^{high}CD27^-$) was decreased in COVID-19 and MIS-C compared to the healthy control group. Late/exhausted memory cells ($CD27^-IgD^-IgM^-$) were decreased in MIS-C compared to COVID-19. $CD21^-$exhausted B cells and $PD\text{-}1^+$ B cells followed similar course.

Conclusions: According to the data obtained, the B-cell profile in MIS-C cases differs from COVID-19 in favor of inflammation. The effect of inflammation-limiting regulator B cells seems to be lower in MIS-C than in COVID-19, suggesting the presence of an aggressive and poorly regulated humoral immune response in MIS-C.

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1977 – P1.06.23

Regulatory B cell exhaustion: molecular characteristics and mechanisms of reversion

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Regulatory B cells (Bregs) maintain immunological tolerance in health, and their dysfunction is implicated in various pathological conditions. Chronic lymphocytic leukemia (CLL), characterized by disruptions in B cell homeostasis, offers a unique model to study Breg biology. In this study, we focused on one of the major subpopulations of regulatory B cells in human peripheral blood, memory Bregs (mBregs) that harbor high amounts of cell adhesion molecule CD24 and express co-stimulatory immune checkpoint CD27. CD24hiCD27+ B cells were isolated from peripheral blood of CLL patients and healthy donors using fluorescence-activated cell sorting. Only treatment-naive CLL patients were enrolled in the study to avoid any potential bias introduced by prior treatment. We performed RNA-sequencing on Illumina NextSeq 550 platform and conducted bioinformatic analysis to compare immunosuppressive profiles of mBregs from two cohorts. Our analysis revealed that CLL-mBregs exhibited significantly higher expression of immune exhaustion markers but lower expression of Breg-characteristic functional molecules, indicating compromised immunoregulatory potential. In order to find possible molecular mechanisms regulating the shift from active to exhausted state of Bregs, we employed correlation analysis and discovered genes positively correlating with well-established molecular characteristics of active Bregs and negatively correlating with the exhaustion markers (or vice versa). Expression of these candidate genes (with the majority of them belonging to long noncoding RNAs) was further verified by RT-PCR with subsequent functional validation of their effects using genetic knockdown or overexpression. Our findings provide insights into the molecular mechanisms underlying Breg exhaustion, with potential implications for understanding immune dysregulation across diseases marked by imbalances in pro- and anti-inflammatory responses.

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2100 – P1.06.25

Immunomodulatory effects of epitope-specific CD37 targeting in mouse

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CD37 is glycosylated membrane protein highly expressed in B cells. As other members of tetraspanin family CD37 is involved in regulation of incoming receptor-derived signals, lateral membrane protein interactions and intracellular signal processing. Here we characterize mouse CD37-specific monoclonal antibodies and investigate the effects of CD37 targeting *in vitro* and *in vivo*.

Generated monoclonal antibodies recognized two epitopes in the large extracellular loop of CD37. Clones A23 and A69 both recognize a linear CD37 epitope available on B-lymphocytes, T-lymphocytes and myeloid cells. Clone B1 recognizes a conformational CD37 epitope accessible exclusively on B cells. CD37 antibody clones differ in binding affinity and induction of internalization. Generated monoclonal antibodies were applied to characterize CD37 expression and functional consequences of CD37 targeting *in vitro*. Cells isolated from *Cd37-/-* mice were used as negative control. CD37-specific antibodies were neither activating B cells nor affecting BCR-signaling assessed by Ca^{2+} influx. Added to the purified B cells, CD37 antibodies induced homotypic aggregation and B cell death, strongly potentiated by antibody crosslinking, and abrogated by IL-4 addition. Furthermore, antibody addition to B cells, stimulated with IL-4 and LPS, inhibit B cell proliferation and immunoglobulin class switch to IgG1. Targeting of different CD37-epitopes led to depletion of different B cell subsets *in vivo* studied by flow cytometry and immunohistochemistry. Although B cell depletion was induced in a clone-specific manner, it was not correlating with CD37 expression levels since transitional cells with highest CD37 expression were not depleted by the antibody treatment. Maximal CD37 targeting by single injection of an antibody mix induced a rat IgG_{2a}-specific immune response (IgM and IgG_1) analyzed by bead-based assay accompanied with strong germinal center response. Thus, immunomodulatory effects of CD37 targeting by monoclonal antibodies consist of B cell depletion potentially interesting for treatment of autoimmune diseases and induction/enhancement of immune response important in development of new vaccine technologies.

DFG/RTG1727

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2243 – P1.06.26

The B-cell function in patients with chronic lymphocytic leukemia

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Chronic lymphocytic leukemia (CLL) is characterized by the proliferation of dysfunctional B cells, resulting in significant immune dysregulation. Patients with CLL exhibit varied responses to B-cell receptor (BCR) targeted therapies, emphasizing the need for tailored immunotherapy approaches. This study investigated B cell function in untreated patients with CLL, and we further explored the effects of ex vivo protein kinase C activation on immune checkpoint expression and B cell profiles. Peripheral blood samples were collected from 21 untreated patients with CLL at King Edward Hospital in South Africa, between 2019 and 2022. B cells were stimulated with phorbol myristate acetate (PMA) and ionomycin. Using flow cytometry, the study explored the levels of B cell subsets and immune checkpoint proteins programmed cell death-ligand 2 (PD-L2) and cytotoxic T-lymphocyte associated protein 4 (CTLA-4) expression on various B cell subsets. PMA and ionomycin B cell stimulation upregulated CTLA-4 and PD-L2 expression on B cell subsets ($p < 0.0001$). As expected, monoclonal antibodies targeting PD-1, PD-L1 and CTLA-4 significantly downregulated the CTLA-4 expression of B cell subsets ($p < 0.05$), while PD-L2 exhibited varied responses in different B cell subsets. In addition, these monoclonal antibodies increased the levels of memory B cells ($p < 0.0128$) and activated memory B cells ($p < 0.01$). Protein kinase C activation on B cells stimulates immune checkpoint expression. The use of monoclonal antibodies on B cells play a critical role in the B cell function through the reduction of CD38 expressing activated B cells and upregulation of memory B cells. Moreover, the monoclonal antibody targeting PD-1, PD-L1 and CTLA-4 are effective in reducing the expression of CTLA-4 on B cell subsets.

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2256 – P1.06.27

Mass cytometry immunophenotyping reveals significantly decreased frequencies of circulating B cells in Autoimmune Polyendocrine Syndrome type 1

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Background: AIRE mutation and Aire-dependent regulatory pathway dysfunction result in Autoimmune Polyendocrine Syndrome Type 1 (APS-1) development. Reduced B cell frequencies have previously been observed (ref), but broader proteomic mapping has not been established to fill the research gap in understanding the disease mechanism.

Aim and methods: Aim 1) We aim to create a comprehensive map of the primary immune system subsets and their systemic distribution. **Aim 2)** in-depth understanding of the most associated immune subsets with Autoimmune Polyendocrine Syndrome Type 1 (APS-1). We have adopted a single cell mass cytometry panel with 37 antibody marker panels in Norwegian APS-1 patients (N=18) compared to age and gender-healthy matched controls (N=19).

Results: APS-I patients had significantly decreased numbers of B cells, specifically within the naïve B cell subset, compared to healthy controls. Memory B cells compartment does not show the discrepancies (including switch and non-switch memory cells). We observed similar frequencies of CD38+ plasmablasts among germinal center B cells and transitional B cells. Interestingly, NK and NKT cells were also diminished, although these reductions did not reach statistical significance.

Conclusion: The proteomic mapping has provided additional evidence to support the previous findings that systemic B cells are declining. This new information can help us gain a better understanding and identify the need for further research to explore potential solutions.

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2296 – P1.06.28

Helicobacter infection increases the mitochondrial metabolism of B cells

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Purpose: *Helicobacter felis* (*H. felis*) is a gram-negative, microaerophilic, and spiral-shaped bacterium, which belongs to *Helicobacter spp*. like *H.pylori. H. felis* infection can lead to multiple gastro pathologies such as chronic gastritis, gastric cancer, and peptic ulcer disease. Mitochondria and immune cell functions are closely linked to each other. Studies have shown that high mitochondrial mass and membrane potential indicate active mitochondria. The mitochondrial metabolism of *Helicobacter*-stimulated B cells has yet to be elucidated. Therefore, we investigated the mitochondrial metabolism of B cells upon *H. felis* antigen stimulation.

Methods: B cells were isolated from the spleens of C57BL/6 mice and stimulated with *H. felis* antigen (10 ug/ml), TLR2-ligand PAM3CSK4 (2.5 ug/ml), or TLR4-ligand LPS (10 ug/ml) for various time points. Mitochondrial mass and membrane potential of B cells were assessed using Mitoview Green and Mitoview 633, or TMRE dyes, respectively, via flow cytometry. Additionally, the mtDNA(Cox1 gene)/nDNA(Rps18 gene) ratio and expression of Tfam were measured using Q-PCR to evaluate mitochondrial biogenesis. The mitochondrial ATP5a protein level was also determined using the MetFlow method.

Results: Stimulated B cells exhibited a 20-30% increase in mitochondrial mass and membrane potential at 24 hours, and a 50-60% increase at 48 hours. However, they reduced the mtDNA/nDNA ratio at 24 and 48 hours and increased Tfam expression at 6- and 24-hours post-stimulation. Furthermore, *H.felis*-activated B cells showed elevated expression of ATP5a, a key oxidative phosphorylation protein, compared to the non-stimulated controls.

Conclusions: The findings suggest that activated B cells enhanced their mitochondrial activity and oxidative phosphorylation during *Helicobacter* infection. Despite a decrease in mtDNA content at specific time points, increased Tfam expression indicates ongoing mitochondrial biogenesis. Further investigations are warranted to elucidate the metabolic changes in immune cells within the stomach during *Helicobacter* infection.

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P1.07 BACTERIAL, VIRAL, FUNGAL, AND PARASITIC IMMUNOLOGY

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26 – P1.07.01

Novel GPR84 agonists prime macrophages to enhance phagocytosis and killing of Gram-negative bacteria

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Purpose: Immune adjuvant therapy provides a novel approach for treating bacterial infections. GPR84 is a pro-inflammatory G-protein coupled receptor expressed by neutrophils, monocytes and macrophages. Previous work has shown that activation of GPR84 increases phagocytosis of killed *Escherichia coli* and latex beads by murine macrophages. We investigated the impact of GPR84 agonists on the interaction of macrophages with Gram negative bacteria.

Methods: We investigated the effect of a range of established and in house GPR84 agonists on phagocytosis and killing of viable *E. coli* by THP-1 monocyte derived macrophages (MDM) at different time points. A gentamicin protection assay was used to quantify the number of live intracellular bacteria, with modifications used to quantify bacterial association and attachment. The half maximal effective concentration (EC_{50}) was determined using a four-parameter dose-response curve fit. We examined the uptake of live bacteria using confocal immunofluorescence microscopy and differential staining pre- and post-cell permeabilization to detect internalised and surface attached bacteria. Bone marrow derived macrophages (BMDM) from GPR84 WT and KO C57BL/6 mice were used to examine whether effects on bacterial recovery were GPR84-specific.

Results: We observed a >70% decrease in recovery of *E. coli* from THP-1 MDM stimulated upon addition of GPR84 agonists compared to vehicle treated cells despite increased phagocytosis observed by microscopy described previously. The increased killing was dependent on the dose of agonist, with two synthetic agonists showing picomolar EC_{50}s. Examination of infected cells using confocal microscopy showed a decrease in the number of intracellular but not surface attached bacteria. The effect is rapid, first observed at 30 minutes post agonist stimulation. GPR84 agonist effects were sustained for at least 1 hour after removal of the compounds. This effect was also observed in murine BMDMs. Experiments performed using GPR84 KO BMDM confirmed GPR84-specificity of 6-OAU; two novel agonists were found to act in part *via* GPR84.

Conclusion: Our findings show that GPR84 low molecular weight agonists can reduce bacterial survival in human and murine macrophages in a GPR84-dependent manner. Future experiments will test the effect of GPR84 agonists on Gram-negative bacterial survival using *in vivo* models.

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29 – P1.07.02

Diuretics alone or combined with captopril modulate the phagocytosis of Staphylococcus aureus by mouse macrophages

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Purpose: In experimental and clinical conditions, diuretics have been shown to exert an immunomodulatory effect, promoting the anti-inflammatory activation of immune cells. However, these effects may vary depending on the drug and the underlying mechanisms remain unclear. Therefore, understanding these mechanisms is of great importance for improving the treatment of patients with comorbid inflammatory diseases, including hypertension or chronic heart failure. In such cases, diuretics are administered concomitantly with other antihypertensive drugs, such as angiotensin-converting enzyme inhibitors, which likely modifies the effects of diuretics on the immune system. Thus, our current studies aimed at investigating the impact of selected diuretics administered alone or in combination with captopril on macrophage ability to phagocytose *Staphylococcus aureus*.

Methods: CBA mice were administered intraperitoenally with physiological saline solution of captopril (5mg/kg), furosemide (5mg/kg) or hydrochlorothiazide (10mg/kg), administered alone or in combination (diuretic plus captopril) for 8 consecutive days. On the 3rd day of drug treatment mice were injected with mineral oil, and macrophage-enriched peritoneal exudates were collected 5 days later. To determine phagocytic activity, macrophages were incubated for 20 minutes at 37°C with live *Staphylococcus aureus* (MOI 10), and the growth of surviving bacteria was assessed through microbiological cultures. In parallel, macrophages incubated with heat-killed bacteria were analyzed cytometrically.

Results: Diuretics alone or combined with captopril increased macrophage phagocytic activity, with captopril having the greatest effect. On the other hand, hydrochlorothiazide administration changed the proportion of peritoneal macrophages exposed to bacteria, favoring the infiltrating population and the shift towards pro-inflammatory phenotype with highest expression of surface markers of antigen presentation.

Conclusion: In geriatric population, it is important to find the proper anti- and pro-inflammatory balance to avoid unwanted suppression of antimicrobial immunity for reducing the risk of serious infections which can occur due to age-related immune system dysfunction. Our current studies suggest that furosemide and hydrochlorothiazide do not impair the antimicrobial activity of macrophages, and captopril enhances these beneficial effects, thus contributing to the discussion on the safety of drug use in the elderly.

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96 – P1.07.03

Helminth Co-infection diminishes Antibody Responses to Natural SARS-CoV-2 Infection but not to COVID-19 vaccination: Insights from a Ghanaian Study

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Purpose: The mild COVID-19 outcomes in Sub-Saharan Africa, against expectations, prompted investigation into local factors like helminthic infections, such as Lymphatic Filariasis (LF), which can modify immune responses. The present study examines LF's influence on SARS-CoV-2 immune reactions and COVID-19 vaccination in a Ghanaian cohort to understand its effect on the pandemic's regional dynamics and vaccine efficacy.

Methods: Blood samples were collected from 222 individuals diagnosed with LF in Ghana's Upper-East-Region. The presence of SARS-CoV-2 and filarial-specific antibodies (IgG, IgA) was measured using ELISA and SARS-CoV-2-variant-specific neutralizing capacity was analyzed using Luminex. Analyses were performed using the Kruskal-Wallis followed by Dunn's post-hoc test or Mann-Whitney U tests, along with Spearman correlation analyses.

Results: A notable fraction of unvaccinated participants (n=141) showed seropositivity for SARS-CoV-2 IgA (56%) and IgG (39%) antibodies, despite lacking any confirmed COVID-19 infection history. Vaccinated individuals with Lymphatic Filariasis (LF), post-receiving the AstraZeneca vaccine, demonstrated significantly ($p<0.0001$) enhanced IgA (72X) and IgG (60X) antibody responses specific to the SARS-CoV-2 Spike protein. This enhancement was consistent across IgA and IgG antibodies, improving neutralization efficacy against five critical variants: Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2), and Omicron (B.1.1.529), regardless of the severity of LF pathology or filarial infection status. Conversely, individuals seropositive for *Ascaris lumbricoides* who had recovered from COVID-19 (n=28) exhibited a diminished ($p=0.0264$) IgA response to the infection, though this did not extend to their IgG response or the ability to neutralize the virus. Furthermore, a moderate negative correlation ($r=-0.32$) was identified between *Ascaris* seropositivity and the intensity of COVID-19 symptoms among those who exhibited symptoms (n=36). Notably, individuals with a higher symptom score (n=12) showed a significantly lower ($p=0.0455$) response to *Ascaris*-specific antibodies compared to those with moderate symptoms (n=24).

Conclusion: Our data suggest that COVID-19 vaccination elicits strong immune responses in LF patients, despite the potential influence of filarial infection on natural antibody production against SARS-CoV-2. These findings highlight the intricate ways in which helminthic infections may affect the immunogenicity and clinical outcomes of COVID-19, emphasizing the need for further research into their role in vaccine efficacy and disease progression in endemic regions.

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105 – P1.07.04

Deep immunophenotyping to decipher immunological changes in post-infectious fatigue after SARS-COV-2 infection

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Background: The development of therapeutic strategies against long-lasting symptoms after COVID-19, caused by SARS-CoV-2 virus and referred to as "Long COVID", relies on better understanding of the disease pathophysiology. The immunological correlate of Long COVID, which presents with fatigue and shares features with chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME), has not been demonstrated yet. Therefore, we undertook a prospective cohort study of SARS-CoV-2 infected and uninfected individuals for identifying immune cellular signatures of Long COVID and CFS/ME.

Methods: This Norwegian cohort study included nonhospitalized individuals (aged 12-25 years) who tested positive (n= 404) and negative (n= 105) for SARS-CoV-2 by RT-PCR. All participants underwent a clinical examination, completion of a questionnaire and biomarker analyses at the early convalescent stage and at 6-month follow-up. Participants were classified according to the World Health Organization case definition of Long COVID at follow-up. Mass cytometry was used to profile peripheral blood mononuclear cells (PBMC) collected from selected participants (n=80) divided into four groups (n =20/group) based on SARS-CoV-2 status and chronic fatigue symptoms at follow-up. Pair-wise comparison of immune cell frequencies between Long COVID, recovered convalescents, fatigued controls and healthy controls was performed by negative binomial regression model. Cell types significantly associated with patient categories were put into a machine learning classification model to identify unique signatures of Long COVID.

Results: We demonstrate that higher baseline frequencies of CD56lo-CD16hi Terminal NK cells are associated with chronic fatigue and Long COVID. Moreover, these cells also showed increased PMA-induced inflammatory cytokine production in fatigued and Long COVID patients. Fatigued patients also had decreased frequencies of Transitional and Marginal Zone B cells with a concurrent reduction of Double Negative B cells as compared to healthy controls. Increased frequencies of Tregs were also found to be associated with chronic fatigue and Long COVID. Multivariate analysis and machine learning revealed increased frequencies of Terminal NK cells as the most important feature of chronic fatigue and Long COVID. Moreover, in our analysis, we did not find any difference in immune cellular patterns between chronic fatigue and Long COVID.

Conclusion: Our results indicate a common immune signature in Long COVID and CFS/ME.

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151 – P1.07.06

The role of microbiota and co-localization in the dissemination of vector transmitted pathogens

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Insect vectors are important agents in the transmission of infectious diseases. Through a process called dissemination, many pathogens can enter a host through the bite of an insect vector and then exit the skin to cause a systemic infection. The details about how and when dissemination occurs are still unclear and elucidating this is important for understanding disease pathogenesis and the development of new therapies and vaccines. Leishmaniasis is a vector-borne disease, and in its visceral form, *Leishmania* spp. parasites can disseminate from the skin to internal organs. Various factors from the host, vector, and parasite have been implicated in the dissemination process, including the microbiota derived from the insect vector gut. However, while vector microbiota is required for dissemination, it is not sufficient. The goal of this study is to determine whether the micro-colocalization of parasites, vector- and host-derived factors, including the microbiota that occurs following sand fly transmission is a requirement to facilitate dissemination. The results obtained will contribute to a better understanding of the pathophysiology of leishmaniasis. We hypothesize that bacteria, co-localized with other host and vector factors, influence the physiology of the skin and facilitate the dissemination of *Leishmania* spp. To evaluate this, we are studying dissemination in wild type mice and mice deficient in IL-1b employing a novel model of *Leishmania* spp. infection, the skin prick, that co-localizes these factors in the skin. Later, we will determine the requirement of bacteria and co-localization, using skin-prick model of infection in Germ-Free (GF) mice allied with techniques of flow cytometry. Our initial results suggest that after *L. infantum* and sandfly gut-derived bacteria challenge via skin prick there is an enhanced influx of neutrophils to the inoculation site in the skin. Future experiments will determine whether sandfly gut-derived bacteria impacts on the dissemination from the skin to the internal organs.

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168 – P1.07.07

Colony morphotype governs innate and adaptive pulmonary immune responses to Mycobacterium abscessus infection in C3HeB/FeJ mice

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Purpose: *Mycobacterium abscessus* is a mycobacterial pathogen responsible for pulmonary infection in immune compromised individuals. *M. abscessus* grows with two distinct colony morphotypes, smooth (S) and rough (R), the latter of which is associated with worse patient outcome. While *M. abscessus* colony morphotypes differ in their pathogenicity, It is unknown how immune responses to S and R *M. abscessus* differ in an acute pulmonary infection setting. The purpose of this study was to characterise immunological outcomes of *M. abscessus* infection with S and R morphotypes in an immune competent murine model.

Methods: Female C3HeB/FeJ mice were infected by intranasal (i.n.) route with S or R *M. abscessus*, and at 1, 7 and 21 days post infection (dpi) lungs, spleen and draining lymph node (dLN) were collected for analysis of bacterial burden. Extensive characterisation of innate and adaptive immune responses in the lung was performed by flow cytometry, cytokine/chemokine bead array and histopathological analysis at differing time points following infection. Local immune responses in dLN were also examined by antigenic restimulation and flow cytometry at 7 and 21dpi.

Results: R *M. abscessus* infection was associated with the rapid production of inflammatory chemokines in the lung, combined with the recruitment of activated, MHC-II$^+$ Ly6C$^+$ macrophages both lung and lung draining LN (dLN) at 7dpi. The induction of T helper type 1 responses occurred following both R and S *M. abscessus* infection, but this response was markedly delayed in mice infected with S colony morphotypes. However, despite differences in the development of innate and adaptive immune responses, *M. abscessus* colony morphotype did not affect the rate of bacterial clearance or the development of pulmonary histopathology.

Conclusion: *M. abscessus* colony morphotype is instrumental in shaping the development of pulmonary immune responses following infection, with S *M. abscessus* displaying an 'immunologically silent' phenotype compared to the inflammatory R *M. abscessus* morphotype. This study further informs our understanding of *M. abscessus* host-pathogen interactions.

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177 – P1.07.08

Repeated Plasmodium falciparum infection in humans drives the clonal expansion of an adaptive γδ T cell repertoire

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Repeated *Plasmodium falciparum* infections drive the development of clinical immunity to malaria in humans; however, the immunological mechanisms that underpin this response are only partially understood. We investigated the impact of repeated *P. falciparum* infections on human γδ T cells in the context of natural infection in Malian children and adults, as well as serial controlled human malaria infection (CHMI) of U.S. adults, some of whom became clinically immune to malaria. In contrast to the predominant Vδ2$^+$ T cell population in malaria-naïve Australian individuals, clonally expanded cytotoxic Vδ1$_{effector}$ T cells were enriched in the γδ T cell compartment of Malian subjects. Malaria-naïve U.S. adults exposed to four sequential CHMIs defined the precise impact of *P. falciparum* on the γδ T cell repertoire. Specifically, innate-like Vδ2$^+$ T cells exhibited an initial robust polyclonal response to *P. falciparum* infection that was not sustained with repeated infections, whereas Vδ1$^+$ T cells increased in frequency with repeated infections. Moreover, repeated *P. falciparum* infection drove waves of clonal selection in the Vδ1$^+$ T cell receptor repertoire that coincided with the differentiation of Vδ1$_{naïve}$ T cells into cytotoxic Vδ1$_{effector}$ T cells. Vδ1$^+$ T cells of malaria-exposed Malian and U.S. individuals were licensed for reactivity to *P. falciparum* parasites in vitro.Together, our study indicates that repeated *P. falciparum* infection drives the clonal expansion of an adaptive γδ T cell repertoire and establishes a role for Vδ1$^+$ T cells in the human immune response to malaria.

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185 – P1.07.09

Dynamics of vaginal immunity during murine filarial and Herpes-Simplex-Virus-2 co-infection

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Purpose: More than 1 million sexually-transmitted infections are acquired every day. Sub-Saharan Africa bears approximately 40% of the global burden of STIs, which overlaps with high rates of helminth infections. Host immunity to helminths entails the induction of type 2 immune responses aids in controlling the infection, while increased immune regulation induced by the helminths supports parasite survival. This helminth-driven immune modulation dampens type 1 immune responses, which can lead to impaired control of bacterial and viral infections. Although helminths do not directly colonize or pass through the female reproductive tract, they are a risk factor for HIV and HPV infection. However, the mechanisms remain uncertain. Thus, we developed a chronic filaria HSV-2 co-infection mouse model to analyze immune cell composition and the parasite-caused influence on viral pathology.

Methods: We infected BALB/c wildtype and eosinophil-deficient (dblGATA) mice with the filarial nematode *Litomosoides sigmodontis* (L.s.) (25, 35 or 72 days) followed by an HSV-2 infection for 7 days. To assess pathological, immunological and parasitological data sets, we analyzed samples of the perigonadal adipose tissue (PAT), vagina tissue and lavage (site of HSV-2 infection), and lung tissue and pleural lavage (site of parasite infection) using flow cytometry, Luminex technology and immunofluorescence-based immunohistochemistry.

Results: In HSV-2 and *L. sigmodontis* (L.s.) co-infected mice, we observed increased numbers of eosinophils in the vaginal tissue and lumen and PAT compared to mono-infected mice. Furthermore, we observed increased pro- and anti-inflammatory cyto- and chemokines like Eotaxin-1 in the vaginal lumen. We also showed increased pathology in eosinophil-deficient mice compared to wildtype mice highlighting that L.s.-induced eosinophils play a crucial role in vaginal immunity and pathology during HSV-2 infection. Moreover, we revealed delayed HSV-2 progression towards neurons and reduced epithelial cell lysis within the vaginal tissue of co-infected mice.

Conclusion: Our data demonstrates that the immune-modulation of filarial nematodes affects uncolonized tissues and subsequent STIs. Moreover, the data indicate a crucial role of eosinophils for the vaginal pathology and immunity against viral infections. In detail, we hypothesize that the significantly increased eosinophil numbers might delay HSV-2 progression through the tissue and that L.s. infection exacerbated HSV-2-driven vaginal inflammation.

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196 – P1.07.10

Imbalance of CCR6+ CD4 T cells and IFN-γ CD8 T cells in patients with Long-COVID

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Purpose: Post-acute COVID-19 or Long-COVID (LC) can affect around 20% of convalescent individuals. It is characterised by persistent symptoms (mainly dyspnoea, fatigue and chest pain) for at least 3 months after infection. Among different possible causes driving LC, several authors have pointed towards a dysregulation of the immune system, triggering a persistent hyperinflammatory state. LC patients present differences in activation and exhaustion states of innate and adaptive compartments, as well as in T regulatory cells. However, the role of cells co-expressing different chemokine receptors (CCR), such as CCR6, remains unexplored. The objective of this study is to assess differences in circulating T cell subpopulations co-expressing different CCRs in LC patients.

Methods: Peripheral blood mononuclear cells (PBMCs) from healthy, non-long COVID (NLC) and LC patients were treated with SARS-CoV-2 peptides, including spike (S) and nucleoprotein (NP). T cell subpopulations were determined by flow cytometry and changes in T cell frequencies were assessed by means of unsupervised clustering analysis.

Results: Higher levels of CCR6+CCR9+ and CCR6+ CCR4+ CD4 T cells were found in COVID-19 convalescent individuals upon activation with SARS-CoV-2 S peptides. Further analyses showed a decrease in SARS-CoV-2 specific CD4 T cells co-expressing CCR6 in LC patients compared to NLC, whereas CXCR3+ CCR6- (Th1) and CCR4+ CCR6- (Th2) CD4 T cells were increased in this group. In addition, LC patients showed lower IFN-γ-secreting CD8 T cells after stimulation with SARS-CoV-2 S protein.

Conclusion: These data reveal a possible role of CCR6+ cells in LC pathophysiology. We describe an imbalance of Th1/Th2/Th17 effector cells and of CD8 IFN-γ cells in LC patients, which highlights the role of adaptive immunity during the course of LC.

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207 – P1.07.11

Metabolic profiling of lymphocytes in diseases caused by intracellular pathogens

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Background: Tuberculosis (TB) and Leishmaniasis are caused by intramacrophagic pathogens, namely *Mycobacterium tuberculosis* and *Leishmania donovani* respectively. Till now, studies pertaining to TB and Post Kala-Azar Dermal Leishmaniasis (PKDL) are restricted to their immunopathogenesis, but their immunometabolic profile remains unexplored and was the focus of this study.

Aim: Characterizing the metabolic status of immune cells in patients with TB and PKDL, and delineating their association with the functional status of lymphocytes.

Methods: In peripheral blood mononuclear cells of PKDL (n=16) and TB (n=20) patients along with healthy controls (n=10), the T-cell frequency (CD4/CD8), their activation (CD69) and exhaustion (CD279) status was determined by flow cytometry. The metabolic bioenergetics of lymphocytes was studied in terms of oxidative phosphorylation (OXPHOS) and glycolysis as measured by Extracellular flux analyzer along with quantitative PCR to study expression of OXPHOS (*sdha, Atp synthase, pdk1*) and glycolytic (*hk2 and ldha*) genes, as also *PI3KCA, AKT, mTOR, HIF1A, GLUT1, Smad3 and Cpt1A*.

Results: In comparison to healthy controls, TB cases showed an altered CD4/CD8 ratio, significantly raised T cell activation ($CD69^+$), a metabolic switch towards glycolysis and enhanced expression of glycolytic genes as also raised expression of *PI3KCA*, *AKT, mTOR*, *HIF1A* and *GLUT1*. In contrast, PKDL cases showed T-cell exhaustion ($CD279^+$), OXPHOS was upregulated along with enhanced *Smad3* and *Cpt1A*.

Conclusion: Although TB and PKDL are caused by intramacrophagic pathogens, TB cases demonstrated T-cell activation, upregulation of the PI3K-AKT pathway that resulted in increased *GLUT1*, and a shift towards glycolysis. Conversely, PKDL cases demonstrated T-cell exhaustion, activated *Smad3* and *CPT1* that translated into enhanced OXPHOS. Taken together, drugs targeting immunometabolism can be promising therapeutic options for TB and PKDL.

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255 – P1.07.12

Innate – Adaptive Synergy in Malaria

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Purpose: Natural killer (NK) cells play an important role in the interplay between innate and adaptive immunity in the host response against malaria. Different NK cell subsests have been indicated to play specific roles during the infecion, and memory has been implicated upon reinfection. However, the cellular and molecular regulation of NK cell sudsets after infection remain poorly understood. This study takes advantage of longitudinal prospective blood samples from donors infected with malaria parasites to understand the impact of malaria infection on NK cell diversity and function over time.

Methods: The cohort consists of individuals experiencing their first malaria infection and those with prior exposure. Blood samples were collected at the time of diagnosis, 10 days, and 1, 3, 6 and 12 months post-diagnosis. PBMCs were isolated from these samples for NK phenotypic analysis by flow cytometry and transcriptomic profiling using targeted single-cell RNA sequencing. NK cell cytotoxicity was assessed for different subsets over time through confocal live cell imaging and via flow cytometry.

Results: Analysis revealed a significant reduction in total $CD56^+$ NK cell counts during the acute phase of malaria infection across both primary and recurrent infection cohorts, compared to convalescent stages. However, there were no significant differences between primary and recurrent infections at any time point. Notably, NK cells isolated during acute infection demonstrated better killing capability in live cell imaging assays than the ones at convalescence. Consistent with this, expressions of two key molecular effectors of NK cell cytoxicity, PRF1 (perforin) and GZMB (granzyme B), were significantly up-regulated in NK cells from the acute stage. Proteomic analysis also showed highly elevated levels of soluble granzyme B in plasma, supporting enhanced NK cell cytotoxicity in response to malaria infections.

Conclusion: Our findings show that acute malaria infection significantly but transiently enhances NK cell cytotoxicity for certain cell subsets. This indicates an important role during the acute phase of the immune response against malaria parasites. Further studies to identify the specific signals leading to more efficient NK cell function may improve our understanding and management of malaria parasitemia.

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375 – P1.07.14

Myeloid-Derived Suppressor-like Cells as a Prognostic Marker in Critically Ill Patients: Insights from Experimental Endotoxemia and Intensive Care Patients

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Objective: Polymorphonuclear and monocytic myeloid-derived suppressor cells (PMN-MDSCs and M-MDSCs) are immunosuppressive immature myeloid cells expressed at low levels at homeostasis. Patients admitted to the intensive care unit (ICU) often experience endotoxemia, nosocomial infections and sepsis. MDSCs can have an important impact on the development of infectious diseases, but little is known about their potential predictive value in critically ill patients. Our objective was to characterize the dynamics of MDSCs in healthy subjects challenged with endotoxin and the clinical predictive value of MDSCs for patients admitted to intensive care unit (ICU).
Methods: Blood was sampled from eight healthy volunteers 0-168 hours after endotoxin challenge, and from critically ill patients at risk of developing infections sampled at ICU admission (n=32) and ICU discharge (n=17). Blood was analyzed by multivariate flow cytometry and unsupervised clustering, and by multiplex bead assay to quantify leukocyte subpopulations and cytokines.
Results: PMN-MDSCs and M-MDSCs increased 4-8 hours after endotoxin challenge and returned to baseline levels after 24 hours. PMN-MDSCs and M-MDSCs were elevated in patients at ICU admission and normalized at ICU discharge. A subpopulation of M-MDSC cells expressing intermediate levels of CD15 (CD15int M-MDSCs) negatively correlated with IL-31 concentrations (r=-0.48, P=0.049) and was associated with overall mortality (P=0.02). High abundance of PMN-MDSCs and CD15int M-MDSCs was a good predictor of mortality (P=0.0046 and 0.014), with area under the ROC curve for mortality of 0.70 (95% CI = 0.4-1.0) and 0.86 (0.62-1.0), respectively.
Conclusions: Elevated levels of PMN-MDSCs and CD15int M-MDSCs were independent predictors of mortality in ICU patients. Our observations support the idea that MDSCs represent biomarkers for sepsis, and that flow cytometry monitoring of MDSCs may be used to risk stratify ICU patients for targeted therapy.

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436 – P1.07.15

Characterization of the MAIT cell response to Staphylococcus aureus and the influence of bacterial immune evasion

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Mucosa-associated invariant T (MAIT) cells are a subset of unconventional T cells displaying innate-like characteristics, with rapid secretion of pro-inflammatory cytokines and killing of bacterially infected cells. MAIT cells are abundant in the blood of humans and act as guardians of both mucosal and non-mucosal barriers. Major questions remain regarding the contribution of human MAIT cells in the immune response to invading pathogens such as *Staphylococcus aureus* and the role of potential bacterial immune evasion mechanisms. Here, we studied the MAIT cell-pathogen interplay using peripheral blood from healthy individuals, palatine tonsils and human organ donor matched blood, mucosal barrier, and lymphoid tissues. We also characterized the interplay between the MAIT cell response to *S. aureus* and the immune-evasive function of virulence factors, including the HlgAB leukocidin toxin. Peripheral blood MAIT cells respond to *S. aureus* leading to robust production of cytokines and granzyme B. MAIT cell activation by *S. aureus* is dependent both on IL-12 and IL-18, and activation via TCR triggering in response to MR1-presented antigen. MAIT cells are partially resistant to HlgAB leukocidin toxicity as compared to CD14+ monocytes. This coincides with higher expression level of CCR2, CXCR1 and CXCR2 on monocytes. Interestingly, activation of MAIT cells via TCR triggering or IL-12 and IL-18 reduces MAIT cells susceptibility to HlgAB toxicity. Additionally, MAIT cells display phenotypic and functional diversity according to their tissue localization and stimulation environment, leading to a differential susceptibility to *S. aureus* virulence factors.

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539 – P1.07.16

T cell responses in patients infected with Neoehrlichias mikurensis

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Purpose: *Neoehrlichia mikurensis (N. mikurensis)* is a bacterial species that is transmitted to humans via tick bites. These intracellular bacteria are believed to infect vascular endothelial cells and may cause asymptomatic infections or the infectious disease neoehrlichiosis. Typical symptoms are fever, localized pain, and vascular events. Patients with compromised B-cell immunity suffer from more severe neoehrlichiosis. The aim of this study was to investigate T-cell responses in patients infected with *N. mikurensis*.

Methods: T-cell responses were investigated by stimulating peripheral blood mononuclear cells from infected lymphoma patients (n=8) with recombinant bacterial peptides followed by measurement of T-cell proliferation using flow cytometry. The cells were also incubated with metal tagged monoclonal antibodies for analysis of T-cell populations by multiparameter phenotyping by using mass cytometry. Non-infected lymphoma patients were also analyzed for comparison (n=8). Infection with *N. mikurensis* was determined by PCR analysis of blood samples.

Results: It was only the T cells from the infected patients that responded with proliferation upon stimulation with bacterial peptides, not the T cells from the non-infected control patients. The T-cell responses evoked by this intracellular pathogen in infected patients featured perforin-expressing CD4+ and CD8+ populations that upregulated CXCL10 and interferon-g in response to bacterial peptides. In addition, individuals infected with *N. mikurensis* had higher proportion of perforin-expressing γδ T cells.

Conclusion: Patients infected with *N. mikurensis* develop T cell responses featuring Th1 cells, cytotoxic T cells and γδ T cells, which most likely are important in the defense against this intracellular pathogen.

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584 – P1.07.17

Using weight loss to predict outcome and define a humane endpoint in preclinical sepsis studies

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Objective. Preclinical models are critical for understanding the pathophysiological response to infections and sepsis. In keeping with ethic values, researcher follow guidelines to monitor the health status and minimize suffering of mice. Weight loss is a criteria used as a humane end point, a cut-off value at which animals are euthanized. However, there is no official recommendation for a maximum weight loss leading to euthanasia. Our objective was to analyze the robustness of weight loss cutoff values used in preclinical models of sepsis.

Methods. Data were obtained from 2400 mice infected with *Listeria monocytogenes*, *Streptococcus pneumoniae*, *Candida albicans* and H1N1 influenza virus. Experiments were run over 10 years of research in the laboratory, without weight loss used as a humane endpoint or using a cutoff value of 30%. We performed statistical analyses applying different weight loss thresholds, and in-house data-based power calculation and simulation-based power calculation (more than 100’000 runs).

Results. The proportion of mice that did not survive infection was higher for *L. monocytogenes* (56.1%) and *S. pneumoniae* (57.3%) than for *C. albicans* (38.1%) and H1N1 (45.2%). In all models, independently of the conditions applied to the mice, weight loss segregated mice that survived from mice that did not survive. Statistical analyses indicated that fixing maximum weight loss thresholds at 25%, 20% and 10% of initial weight increased mortality rates (by 5%-13, $p < 0.01$-0.001) of infection by *C. albicans* and H1N1 IV, *L. monocytogenes* and *S. pneumoniae* infection, respectively. In-house data-based and simulation-based power calculations revealed great variability and/or reduction of power as weight loss thresholds approached 20% for *S. pneumoniae* and *L. monocytogenes* models.

Conclusion. To our knowledge, this represents the most extensive study exploring the relationship between weight loss threshold and outcome of sepsis. Our data indicate that weight loss is a valuable predictor of mortality. Yet, weight loss thresholds need to be adapted to each model of infection used in the laboratory to minimize mouse suffering without compromising statistical power and scientific objectives.

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633 – P1.07.18

Unveiling Trypanosoma cruzi extracellular vesicles as masters of host immune regulation

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Purpose: Deepening our understanding on the intricate communication and interaction between parasites and the host immune system may prove to be crucial in addressing the challenges posed by several emerging diseases, such as Chagas disease (CD). Caused by a protozoan parasite, *Trypanosoma cruzi*, CD constitutes a world health problem requiring an integrated and multidisciplinary approach to human and animal health. *T. cruzi* has evolved strategies to persist and disperse inside the mammal host, by evading and subverting host immune defenses. The present work aims to better understand CD related cardiomyopathies, by addressing non-classical *T. cruzi* hosts, allied with innovative *in vitro* cell models and exploiting natural produced parasitic extracellular vesicles (*Tc*EVs).

Methods: Cardiac co-cultures were established combining cardiac explants from *Ovis aries* and *Capra hircus* with autologous peripheral blood mononuclear cells (PBMCs), mimicking the cardiac microenvironment. Co-cultures were exposed to *T. cruzi* parasites or *Tc*EVs, previously isolated, and multiparametric flow cytometry was employed to assess the immunocompetency of the cells, analyzing the expression of major histocompatibility complex class I (MHCI) and class II (MHCII) molecules, along with CD3, CD4, and CD8 markers. Innate immune receptors and cytokines generation were analyzed by real-time-qPCR.

Results: *Tc*EVs rapidly interact with cells, generating a specific immune response, increasing MHCII and CD8 expression in PBMCs. Likewise, *Tc*EVs favors TLR2 and TLR4 gene expression, indicating that *Tc*EVs may activate these receptors. The presence of *Tc*EVs significantly enhances the generation of crucial pro-inflammatory cytokines, including interleukin (IL)-1β and IL-12.

Conclusion: Co-cultures were able to reconstitute the inflammation of the cardiac tissue associated with the establishment of severe cardiomyopathies *in vivo* for different hosts. TcEVs exposition promotes a cytotoxic response which might contribute to control parasite dissemination in cardiac tissue. However, this process may also further increase lymphocytic infiltration and tissue damage. Altogether, these results also highlight that parasitic EVs constitute an intrinsic part of *T. cruzi* biology and may play a role in cardiomyopathies establishment.

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732 – P1.07.19

Gut helminth infection promotes lung anti-viral immunity in a monocyte dependent manner

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Purpose: Infant respiratory viral infections are a major cause of infant hospitalisation and a risk factor in the development of persistent wheeze, airway allergic responses and ultimately asthma. We have previously shown that ongoing infection in mice with the gut helminth Heligmosomoides polygyrus (H. polygyrus) protects against respiratory syncytial virus (RSV) infection, reducing viral load, subsequent immunopathology and airway impairment. This protective effect was independent of adaptive immune responses or helminth secretory/excretory products, and dependent upon the induction of type-I interferons (IFN-I) in the gut and/or lung.

Methods: Mice were infected with H. polygyrus. Flow cytometry and single cell RNA sequencing characterised immune populations in bone marrow, blood and lung. Antibody mediate depletion and adoptive transfers of monocytes prior to RSV infections.

Results: Ongoing H. polygyrus infection induces bone marrow monocytopoiesis, in turn driving an increase in both circulatory monocyte populations and recruited macrophages in the lung. IFN-I signalling is required for the elevation of blood monocytes and lung macrophages. Treatment of infected animals with an anti-CCR2 antibody to deplete the expanded monocyte populations ablates the H. polygyrus induced anti-viral state, whereas elevating monocyte numbers by adoptive transfer replicates the anti-viral effect.

Single cell RNA sequencing of the lung macrophages further characterises the cells responsible for the anti-viral activity. A Ly6C-hi macrophage cluster is significantly expanded in H. polygyrus infection and shows enrichment for antiviral gene expression. RSV infection also induces elevated recruited macrophage counts as early as 8 hours post infection and this is enhanced by ongoing H. polygyrus infection. These RSV induced macrophages show similar anti-viral gene induction to the H. polygyrus induced cells, with co-infection further enhancing this cell signature.

Conclusion: During H. polygyrus infection systemic monocytosis leads to increased numbers of recruited macrophages within the lung that possess an anti-viral gene expression phenotype and are sufficient and essential for mounting an effective immune response to RSV infection.

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737 – P1.07.20

Impact of tissue micromilieu factors on the secretion of extracellular vesicles by Leishmania major and on its survival in phagocytic cells

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Leishmania (L.) major is responsible for chronic but ultimately self-healing forms of cutaneous leishmaniasis (CL). The control of CL requires interferon (IFN)-γ and tumor necrosis factor (TNF) that activate phagocytic host cells for the killing of *L. major* by reactive oxygen (ROS) and reactive nitrogen species (RNS). Besides cytokines, the immune response is shaped by tissue micromilieu factors such as oxygen concentration, temperature or pH, all of which will influence both host cells and parasites. *L. major* is likely to respond to changes in the microenvironment, e.g., by secretion of extracellular vesicles (EVs). EVs manipulate macrophages, the classical antimicrobial effector cells, but presumably also stromal cells (e.g., reticular fibroblasts) in draining lymph nodes, which can function as safe target cells for *Leishmania* and thereby contribute to parasite persistence.

Here, we studied the impact of several micromilieu factors on the secretion of EVs by *L. major* and on the intracellular fate of parasites in macrophages and reticular fibroblasts. Using innovative interferometric nanoparticle tracking analysis (iNTA), we found that a temperature shift from 28°C to 37°C or exposure to acidic pH (pH 6.7) caused an increase or decrease of the number of detected EVs per min, respectively. Next, we pre-treated macrophages and fibroblasts for 2hrs with EVs to study their effect on the infection with *L. major* promastigotes and observed a minor increase in the infection rate as compared to untreated cells. We also explored the influence of hypoxia (3% O_2) or pH 6.7 on the infection of fibroblasts with *L. major* amastigotes or promastigotes for 24-48 hrs. Under these conditions, we saw no change in the infection rate or the number of parasites per infected cell as compared to control cultures, whereas normoxic stimulation with IFNγ and/or TNF led to a decrease of the infection rate.

These findings indicate that the secretion of EVs is regulated by micromilieu factors and that *L. major* is capable to withstand hypoxia and acidity in fibroblasts. In ongoing single cell RNAseq studies, we aim to characterize the host cell niches for *Leishmania* and the impact of micromilieu factors *in vivo* in a more holistic fashion.

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750 – P1.07.21

Pertussis vaccine-induced regulatory T cells suppress protective nasal tissue-resident memory T cells, which can be overcome using novel adjuvants and nasal vaccination.

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Purpose: Respiratory tissue-resident memory T (T_{RM}) cells are essential for sustained protective immunity against *Bordetella pertussis* infection of the nasal mucosa. Current alum-adjuvanted acellular pertussis (aP) vaccines protect against severe disease but fail to prevent nasal infection with *B. pertussis* and suppress induction of T_{RM} cells. With most deaths from pertussis occurring in infants under 1 year old, this failure leaves the youngest infants at risk. This study examined the mechanism of suppression of aP vaccines and explored strategies to induce T_{RM} cells and enhance bacterial clearance from the nose using novel adjuvants and immunization approaches.

Methods: Mice were immunized with the aP vaccine on days 0 and 28 and were challenged with live *B. pertussis* 14 days later. Respiratory T_{RM} cells were assessed by flow cytometry and protection was assessed by CFU counts in respiratory tissues. Draining lymph node (dLN) and spleen cells from immunized mice were used to assess antigen-specific T cell responses and to establish *B. pertussis*-specific T cell lines.

Results: The aP vaccine induced antigen-specific IL-10-producing CD4 and CD8 T cells in dLN and spleen. *B. pertussis*-specific CD4 and CD8 T cell lines established from aP-immunized mice secreted IL-10, expressed the Treg markers, LAG-3 and CD49b and suppressed antigen-specific IL-17 production by CD4 T cells from convalescent mice. Depletion of CD8 T cells in mice at time of immunization with an aP vaccine ameliorated suppression of nasal T_{RM} cells. Blockade of IL-10 signalling during aP immunization or *B. pertussis* challenge enhanced bacterial clearance in the nose and promoted the induction of respiratory IL-17-secreting T_{RM} cells. A novel intranasally delivered adjuvant, LP-GMP, comprising TLR2 and STING agonists, when used instead of or in addition to alum in an aP vaccine, reversed the suppression of antigen-specific IL-17^+ T_{RM} responses and promoted bacterial clearance from the nasal tract.

Conclusion: Our study demonstrates that alum-adjuvanted aP vaccines suppress induction of protective T_{RM} responses by inducing IL-10-seceting Treg cells, but this is reversed by nasal delivery of the aP vaccine with a novel adjuvant.

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773 – P1.07.23

Chlamydia caviae infection in human epithelial lung cells

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Purpose: *Chlamydia caviae*, belonging to the Chlamydia genus, primarily affects guinea pigs but has recently been reported to infect humans, occasionally leading to severe pneumonia. The extent and clinical impact of *C. caviae* infections in humans remain unclear, underscoring the need for additional research to elucidate its health implications.

Methods: We investigated whether *C. caviae* could infect human alveolar basal epithelial cells (A549 cell line). To monitor the progression of the infection, immunofluorescent staining was performed after 6h, 24h, 32h and 48h. We then examined alterations in the expression of E-cadherin, a guardian of epithelial phenotype, α-smooth muscle (α-SMA), a mesenchymal marker, as well as in TGFß1 and TGFß2, involved in epithelial-mesenchymal transition-inducing signaling pathways utilizing qRT-PCR and Western blot. We quantified cytokine secretion in the A549 cells supernatants post-*C. caviae* infection using ELISA.

Results: A549 cells were successfully infected with *C. caviae*, as evidenced by visible inclusions within the cells. qRT-PCR analysis identified a trend towards increased expression of TGFβ1, TGFβ2, E-cadherin, and α-SMA in infected cells. Western blot analysis highlighted a statistically significant decrease in E-cadherin levels in *C. caviae*-infected cells when compared to uninfected controls. In contrast, α-SMA levels remained unchanged between the two groups. Notably, among the various cytokines examined, only the expression levels of IL-6 and IL-8 were statistically significant, with both showing increased levels in A549 cells infected with *C. caviae* compared to the non-infected control cells.

Conclusion: The capability of *C. caviae* to infect human alveolar basal epithelial cells reveals its potential impact on human health beyond its known effects in guinea pigs. The significant changes in IL-6 and IL-8 levels, alongside the decrease in E-cadherin expression in infected A549 cells, imply a specific inflammatory response upon infection. Our work opens new avenues for understanding how *C. caviae* may contribute to respiratory conditions in humans, emphasizing the importance of further research in this area.

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826 – P1.07.24

Candida-triggered oral tumorous epithelial cell responses at the posttranscriptional regulatory level

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Oral candidiasis is frequently observed among the immunocompromised, especially in oral cancer patients. Our prior findings suggest that certain *Candida* species, e.g. *Candida albicans,* enhance oral squamous cell carcinoma progression via several mechanisms, such as altering metabolism, matrix metalloprotease activity, migration and pro-tumour signaling in oral epithelial cells. Here, we aimed to reveal what are the underlying processes, particularly, the posttranscriptional regulatory mechanisms responsible for this phenomenon. As different *Candida* species trigger distinguishable host responses, we further aimed to examine if a 'species-specific' effect is present. To identify tumour-specific effects, we compared the tumorous oral epithelial cell responses to non-tumorous epithelial cell responses.

Therefore, during the study we examined tumour-associated as well as potentially contributing inflammatory responses in oral epithelial cell lines to *C. albicans* and *C. parapsilosis* by wet-lab methods and *in silico* analyses.

Under non-tumorous conditions, *C. albicans* induced strong inflammatory processes, while *C. parapsilosis* activated responses independent of inflammation, but related to hypoxia, carbohydrate metabolism and vascularization. The species-specific effect also manifested in the identified miRNA profiles with no overlap. Under the tumorous condition, *C. albicans* altered the expression of a higher number- and different species- of miRNAs, compared to *C. parapsilosis*, and besides inflammation, general signalling pathways related to transcription factor and receptor regulator activity were also changed. *C. parapsilosis* elicited similar host responses as in non-tumorous epithelial cells. Comparison of the non-tumorous and tumorous conditions showed that the number of differently expressed genes was highest after *C. albicans* presence and cancer-associated genes were most effected in the tumorous condition. According to miRNA-target mRNA analyses, we identified 6 *C. albicans*-induced oral tumor-associated genes under miRNA regulation, that will be in the focus of our subsequent investigations.

To summarize, tumorous oral epithelial cells vigorously discriminate between *C. parapsilosis* and *C. albicans*, as both species evoke a distinguishable host response. Furthermore, the pro-tumor effect of *C. albicans* also manifests at the miRNA level.

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860 – P1.07.26

Dermaseptin S4 improves the innate immunity of gingival cells against oral pathogens (C. albicans and E. faecalis).

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Purpose: Multiple oral pathogens, including C. albicans and E. faecalis cause significant oral infections such as oral candidiasis and root canal infection, respectively. The use of antibiotic/antifungal molecules is limited by possible drug resistance. These limitations could be elevated using antimicrobial peptides (AMPs). Frog skin AMPs (such as dermaseptins) have shown antimicrobial activity against several pathogens and thus could be a medical alternative to prevent/treat these oral infections.

Objectives: To evaluate the effect of dermaseptin S4 (DS4) on oral pathogens (C. albicans and *E. faecalis)*, and gingival cell innate immunity.

Methods: Microorganisms (C. albicans and *E. faecalis*) were exposed to different concentrations (0 to 20 ug/ml) of DS4, then the bacterial growth was assessed after different time points (3 to 24 h). The effects of the DS4 inhibiting biofilm formation were evaluated qualitatively and quantitatively after 3 days. We also evaluated gingival (fibroblast and epithelial cells) and dental pulp stem cells (DPSC) adhesion proliferation after exposure to DS4. Finally, we studied the effect of DS4 reducing proinflammatory responses of the gingival cells when exposed to 5 ug/ml of lipopolysaccharide (LPS).

Results: DS4 showed significant effects, decreasing the growth of C. albicans and E. faecalis. Such growth reduction was confirmed by a significant inhibition of biofilm formation after 72 h exposure to DS4 as shown by scanning electron microscopy and crystal violet staining. The peptide reduced the yeast-to-hyphae transition of C. albicans. Interestingly, gingival fibroblasts, epithelial cells, and DPSC cultured in the presence of DS4 adhered and grew similarly to non-treated cells. Cells stimulated with LPS showed high levels of proinflammatory cytokines (IL-1β, IL-6, IL-8, and TNFa). DS4 significantly reduced the levels of these cytokines.

Conclusion: While it was not toxic to gingival cells, DS4 showed good control of the growth and biofilm formation by C. albicans and *E. faecalis.* Overall, this study suggests using dermaseptin as a medication to control oral infections, including oral candidiasis and root canal infections.

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Abstracts

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899 – P1.07.27

Innate immunity in tuberculosis among type 2 diabetes patients: characterization and clinical correlations

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Background: The increased susceptibility to bacterial infections like tuberculosis is one of the hallmarks of type 2 diabetes, however the underlying mechanisms remains poorly characterized. Alteration in host innate immune responses at early-stage infection might be a reason for inefficient clearance of bacteria, thus leading to dissemination of infection and increased immune pathology. In this study, we have tried to characterize the immune responses of major professional phagocytes, namely polymorphonuclear cells and macrophages which are one of the first cells to encounter Mtb during early infection.

Methods: 20 individuals were recruited under four group namely uncontrolled DM patients with or without pulmonary TB (PTB+DM and DM respectively), only PTB and healthy controls. PMNs and PBMCs were isolated from peripheral blood using density gradient centrifugation. Monocyte derived macrophages were cultured using GM-CSF. Cellular counts and surface marker expression were studied by flow cytometry. Phagocytosis activity was studied by fluorescent labelled bacteria. Bacterial clearance was studied by Colony Forming Unit assays.

Results: Our results revealed reduced phagocytic ability and bacterial clearance capacity of both PMNs and macrophages in PTB+DM patients as compared to PTB patients. Characterization of these innate immune cells have shown altered phenotypes in PTB+DM patients. Reduced number of activated PMNs ($CD62L^{low}$ $CD66b^{high}$) were found in PTB+DM compared to PTB and DM. Also, increased levels of CD47 and CXCR4 were found which positively correlated with increased neutrophilia in PTB+DM patients. Macrophages in PTB+DM patients were found to have an altered phenotype of $CD11b^{low}$ $MARCO^{low}$ $CD206^{high}$.which is positively correlated with the clinical sputum grades(+1,+2,+3).

Conclusion: This study suggests that diabetic milieu induce altered phenotypes of innate immune cells resulting in dysregulated effector immune responses against Mycobacterium tuberculosis. These alterations can potentially be a contributing factor in inability of the host to clear out infection at an early stage and increased immune pathology.

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947 – P1.07.28

Distinct profiles of cytokines and chemokines in cerebrospinal fluid can differentiate bacterial meningitis from viral meningitis.

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Purpose: Distinguishing between bacterial meningitis (BM) and viral meningitis (VM) presents a significant clinical challenge, as timely and precise identification of the causative agent is pivotal for selecting the correct treatment approach and enhancing patient prognosis significantly. Given the inflammatory cascade characteristic of BM, analyzing the patterns of pro- and anti-inflammatory cytokines/chemokines (CTs/CKs) in both cerebrospinal fluid (CSF) and serum could serve as valuable, sensitive markers for distinguishing BM from VM.

Methods: The study aimed to investigate the capacity of cytokines and chemokines (CTs/CKs) to distinguish between BM and VM. To achieve this, biochemical markers and CT/CK profiles were examined in 145 CSF samples and 153 serum samples, split into three groups: BM, VM, and a control group C, consisting of patients with meningism.

Results: In the BM group, CSF levels of TNF-α, MCP-1, MIP-1α, IL-1β, IL-6, ENA-78, IL-10, IL-8, proteins, and white blood cells were significantly elevated, while CSF glucose concentrations were decreased compared to both the VM and C groups ($P<0.01$). Correlation analysis revealed strong positive correlations in CSF for MIP-1α/IL-1β ($r=0.64$), IL-1β/ TNF-α ($r=0.64$), MIP-1α/TNF-α ($r=0.69$) and for IL-6/TNF-α ($r=0.75$). Fewer correlations were observed in the serum (IL-8/MCP-1, IL-1β/MCP-1, and IL-1β/IL-8 ($r=0.83$; $r=0.72$; $r=0.78$, respectively). To determine the optimal cytokine/chemokine (CT/CK) patterns for predicting and classifying BM and VM, a dataset comprising 119 BM and VM CSF samples was split into training (n=90) and testing (n=29) subsets for input into a Random Forest (RF) machine learning algorithm. The RF algorithm correctly classified with 92% sensitivity and 93% specificity 28 out of 29 test samples (15 BM and 14 VM). For serum samples, although the CT/CK levels in the BM serum samples were higher than in VM, the classification of the two groups using the RF algorithm was weak.

Conclusion: These findings suggest that the CSF patterns of CT/CK levels could be used as a valuable tool for differentiating BM and VM

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977 – P1.07.29

Immunological changes during the progression of Clostridioides difficile infection in mouse models

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Background: Current evidence establishes a causal relation between *Clostridioides difficile* infection (CDI), one the most common and severe nosocomial infections, and colon inflammation. Its prognosis and recurrence prediction are still an unsatisfied need, just based on clinical criteria which are not sufficiently accurate. Therefore, the determination of immune profile could throw relevant information about the progression and gravity of CDI.

Objectives: To describe the evolution of the immunological profile in peripheral blood (PB) on a mouse model of CDI over time.

Methods: CDI was induced in 18 C57BL/6 female mice following Cussó et al. (DOI: 10.1007/s11307-019-01408-4) protocol. Mice were divided into mild (ribotype 001, low toxin production) and severe (ribotype 027, high toxin production) groups. Clinical signs score (CSS) was obtained for each animal over time. PB samples of 100 µl were collected at basal, pre-infection (0d), post-infection (+2d) and recovery (+15d); and incubated with 2 multiparametric antibodies panels to analyse lymphoid and myeloid populations. Then erythrocytes were lysate and samples were acquired by flow cytometry using a 16-channel flow cytometer.

Results: Both groups showed a decrease of monocytes (Mo) and dendritic cells (DC) post-infection, compared to basal levels. Within lymphoid populations, activated CD8+ and CD4+ T cells, especially Th1, and NKT cells were increased at post-infection, values recovered after 15 days.; while B lymphocytes raised gradually according to CDI progression. However, at recovery, severe group showed an increase of Th17 cells, pro-inflammatory Mo and mature DC compared to mild group, indicative of more intense inflammatory response. Besides, CSS at recovery positively correlated with these phenotypes, therefore the immune analysis enabled distinguishing between CDI different severity degrees.

Conclusion: The designed antibodies panels are enough sensitive to detect immunological changes and discriminate between CDI stages and severity degrees employing minimal PB samples.

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1018 – P1.07.30

Advancing towards systems immunology: understanding immunological dynamics in human experimental enterotoxigenic Escherichia coli infection through cytometry, proteomics, and computational approaches

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Purpose: Systems immunology provides a comprehensive framework for understanding complex interplay of immune responses. Enterotoxigenic *Escherichia coli* (ETEC) is a major contributor to childhood diarrhea in low- and middle-income countries. Thorough characterization of systemic immune responses may guide vaccine development. In this study, we combined cytometry, proteomics, and computational analyses to investigate immunological dynamics following human experimental ETEC infection.

Methods: Buffy coats and plasma samples were collected from nine volunteers infected with ETEC before, three, and seven days after dose ingestion. Mass cytometry was employed to analyze lymphocytes with 32 markers, yielding 33 cell populations. Plasma samples underwent analysis using 27 markers for inflammation, mucosal injury, kynurenine pathway metabolites, and vitamin B using mass spectrometry and ELISA. Computational methodologies, including Spearman tests, factor analysis (FA), and principal component analysis (PCA) were employed to examine datasets comprising fold changes from baseline, in proportions of cell populations, and protein concentrations, separately for day 3 and day 7 after dose ingestion.

Results: Spearman analysis revealed various significant correlations in fold changes on both days. Notably, N1-methyl-nicotinamide correlated positively with dendritic cells but negatively with gammadelta T cells, Th17-like cells, and plasmablasts. Kynurenine correlated negatively with MAIT cells, gammadelta T cells, TEMRA cells, Th17-like cells. Additionally, S100A9, a calprotectin subunit, showed negative correlations with MAIT cells, gammadelta T cells, and T cell subsets. FA and PCA on day 3 identified inflammatory proteins such as serum amyloid A (SAA), and C-reactive protein (CRP), alongside innate immune cells like gammadelta T, mucosal-associated invariant T, and natural killer T cells, as key variables that explained a significant portion of the total variance. On day 7, key variables encompassed SAA, CRP, tryptophan metabolites, vitamin B1, and calprotectin, with calprotectin negatively correlating with the other key variables. Adaptive immune cell populations, including plasmablasts and Th17-like effector and central memory cells demonstrated notable contributions to systemic immune responses on day 7, with $CD4^+$ TEMRA cells positively correlating with them.

Conclusion: Multivariate analysis characterized immune responses against ETEC, validating univariate findings, and revealing novel biological associations involving gammadelta T cells, TEMRA cells, and calprotectin in a dataset with small sample size.

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1192 – P1.07.32

Elucidating the role of IL4/IL13 signaling in tuberculous granuloma formation

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Tuberculosis (TB), an infectious disease that has coexisted with humans for several millennia, remains a significant global health challenge, causing approximately 1.5 million fatalities annually. The persistence of TB is partly due to the complex interactions between the human immune system and *Mycobacterium tuberculosis* (Mtb), the causative agent of TB. A critical aspect of this interaction is the formation of tuberculous granulomas. These structured aggregates of immune cells arise following infection with Mtb and related mycobacteria. Granuloma act as a double-edged sword: they contain the infection but can also lead to tissue necrosis, promote disease progression and impede antibiotic penetration. Within the granuloma a layer of epithelioid macrophages shields the necrotic core harboring the bulk of the bacterial burden from immune cells and antibiotics in the outer layers. The transcription factor STAT6 has been shown to be vital for the epithelioid transformation of macrophages and necrotic granuloma formation. Since STAT6 is induced by IL4R signaling, we investigate here the influence of IL4 and IL13 signaling on tuberculous granuloma formation and persistence.

To delineate the contribution of IL4/IL13 signaling pathways to the development and architecture of tuberculous granulomas, we employed a zebrafish *(Danio rerio)*-*M. marinum* infection model, which closely mimics human tuberculosis pathology. Genetically modified zebrafish were used to disrupt IL4, IL13 or IL4/IL13 signaling. Development of granuloma was monitored by histological and immunofluorescence staining. Induction and spatial distribution of IL4/IL13 was evaluated by RNAScope over the course of the infection and granuloma forming process (7-21 dpi). Additionally, the bacterial burden was assessed at 14 dpi.

Preliminary findings suggest that IL4/IL13 signaling plays a pivotal role in granuloma formation and maintenance, with expected variations in granuloma structure and bacterial containment between modified and wild-type zebrafish.

By targeting IL4/IL13 pathways, this research aims to unveil the significance of IL-4/IL-13 signaling in the immunopathology of tuberculous granulomas, thereby contributing to the development of potential host-directed adjunctive treatments for tuberculosis.

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1207 – P1.07.33

Exploring distinct T-cell subsets in the upper respiratory tract during Influenza A and Bordetella pertussis infections

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Purpose: The upper respiratory tract (URT) is a site of entry for many public health-concerning pathogens such as SARS-CoV-2, the Influenza virus, or *Bordetella pertussis*. Although the URT represents an important site for initiating and transmitting infection, understanding of the site-specific immunity in the nasal tissue during respiratory infections is limited. The adaptive immune system of URT consists of highly organized nasal-associated lymphoid tissue and dispersed Tissue-resident memory T cells (Trm). Trm cells are a subset of memory T cells, which reside in non-lymphoid tissues. Due to their advantageous location, they can be part of the first line of defense against infections. The development, diversity, and function of T cells in the respiratory tract are not yet completely understood. We hypothesized that different infections such as intracellular viral infection or extracellular bacterial infection give rise to phenotypically and functionally distinct $CD4^+$ and $CD8^+$ T-cell subsets.

Methods: To address this hypothesis we utilized two mice infection models: viral infection (Influenza A) and bacterial infection (*Bordetella pertussis*). Samples from murine URT and lungs were analyzed by flow cytometry and T-cells were sorted for single-cell RNA sequencing.

Results: We observed striking differences between both $CD4^+$ and $CD8^+$ T cells after the infections. Particularly, we identified a unique subset of $CD8^+$ T cells arising after *Bordetella pertussis* infection. These cells were highly clonally expanded and obtained an atypical phenotype characterized by the expression of Granzyme K, transcription factor Eomes, checkpoint-inhibition receptors as well as IL-10 receptor.

Conclusion: We identified a unique subset of $CD8^+$ T cells after *Bordetella pertussis* infection. The function and specificity of these cells will be further studied.

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1226 – P1.07.34

IL-36 receptor expression is required to mount effective dendritic cell responses upon exposure to Staphylococcus aureus

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Purpose: There has been a steady increase in the incidence and severity of antimicrobial resistant infections in healthcare settings worldwide. The need to develop novel treatment approaches to counteract antibiotic resistant pathogens has grown in importance. *Staphylococcus aureus (S. aureus)*, particularly the methicillin resistant strain, is one such pathogen which has emerged as a significant problem in healthcare settings. A deeper understanding of the mechanisms through which *S. aureus* drives inflammation and immunity will be critical towards defining new approaches to counteract infection. The ability of *S. aureus* to drive inflammation in the skin via the IL-36 pathway has recently been documented. Based on these observations, we hypothesised that IL-36 cytokines may also play a role in the ability to mount effective immunity to *S. aureus* and influence the severity of disease outcomes.

Methods: To investigate the mechanisms through which IL-36 contributes to the pathogenesis of *S. aureus* infection, we examined the induction of innate and adaptive immune responses to *S. aureus* exposure in wild type (*wt*) and *il36r-/-* mice using *in vitro* and *in vivo* methods.

Results: Bone marrow derived dendritic cells (BMDCs) from *il36r -/-* mice exhibited a reduced capacity to release inflammatory cytokines (IL-6, IL-12 and IL-23) and chemokines (CXCL1) upon stimulation with heat-killed *S. aureus*, when compared to *wt* cells. In contrast, *S. aureus* mediated induction of surface markers of activation (CD80, CD86, CD40) were not altered. In association with diminished secretion of T cell instructive cytokines, *il36r-/-* BMDCs also displayed a reduced capacity to induce primary activation of effector CD4+ T cell responses under co-culture conditions, with diminished IL-17a and IFN-γ expression after 72 hours. These effects were mirrored *in vivo*, with *il36r-/-* mice having a reduced capacity to mount effective Th17 and Th1 recall responses after sub-cutaneous immunisation with heat-killed *S. aureus*.

Discussion: This data identifies an important role for the IL-36 pathway in regulating innate immune responses to *S. aureus* which impacts the subsequent generation of effector CD4+ T helper responses. The influence of IL-36 cytokines may be an important consideration in the development of effective therapeutic approaches towards combatting *S. aureus* infection.

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1234 – P1.07.35

SLAMF7 and SLAMF8 receptors shape human plasmacytoid dendritic cell responses to intracellular bacteria

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Intracellular bacteria pose significant challenges to the human immune system. Plasmacytoid dendritic cells (pDC) are immune innate cells renowned for their role in antiviral responses, because of their exceptional capacity to produce huge amounts of type I interferon (IFN). pDC have also been implicated in host responses against bacterial infections, although the operating molecular mechanisms remain unclear. Signaling Lymphocyte Activation Molecule Family (SLAMF) members act as microbial sensors and modulate immune functions in response to infectious agents, but their precise role in human pDC is still to be unraveled. Here, human blood transcriptomics revealed the involvement of SLAMF7 and SLAMF8 in various infectious diseases, with elevated levels associated with type I IFN and inflammatory responses in salmonellosis and brucellosis patients. Spectral flow cytometry findings indicate that pDC display the highest basal levels of SLAMF7^{+} or SLAMF8^{+} cells among cell sub-populations, and such expression is heightened by TLR7/8 agonists. Using an acute inflammation inducing-pathogen, *Salmonella*, and a stealthy chronic one, *Brucella*, we further identified SLAMF7 and SLAMF8 as human pDC function regulators. SLAMF receptors absence hinders the maturation state of infected pDC and abrogates cytokine production. SLAMF7 and SLAMF8 signal through NF-kB, IRF7 and STAT-1, and limit mitochondrial ROS accumulation upon Salmonella infection. This SLAMF7/8-dependent control of mitochondrial ROS levels favors bacterial persistence and NF-kB activation. This work constitutes a mechanistic demonstration of pDC anti-bacterial response positively modulated by SLAMF receptors, bridging a critical gap in understanding their molecular activation mechanisms during infection, and envisioning implications for diagnosis and therapeutic development.

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1254 – P1.07.36

The role of extracellular vesicles in oral squamous cell carcinoma- Candida interaction

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Oral squamous cell carcinoma (OSCC) accounts for 90% of oral cancer cases worldwide. In OSCC, the normal oral microbiota is often altered, which may predispose to local infections such as oral candidiasis. A previous study by our laboratory showed that in the case of OSCC, the number and diversity of colonizing yeasts in the oral cavity increases significantly compared to healthy individuals. In addition, the presence of *Candida albicans*, the causative agent of oral candidiasis, contributes to tumor progression by enhancing tumor cell oncometabolite production, the activity of secreted matrix metalloproteinases (MMPs), and signaling pathways involved in tumor progression *in vitro* and *in vivo*. However, the main component causing the changes has not yet been identified. Therefore, in our work, we investigate the effect of *Candida*-derived extracellular vesicles (EV) on the progression of OSCC. As we hypothesize that EVs play a crucial role in cell-cell communication, they may also play an important role in host-pathogen interaction.

During our experiments, we isolated vesicles from *Candida albicans* and *Candida parapsilosis*. In the case of *C. albicans*, it was isolated from both yeast and hyphal forms. The uptake and mechanism of *Candida*-derived EVs were investigated by flow cytometry and confocal microscopy. We also investigated the effect of *Candida*-derived EV treatment on various processes involved in the epithelial-mesenchymal transformation of the tumor, such as the migration, MMP activity and gene expression profile of the HSC-2 human OSCC cells used.

As a result of the experiments, we found that EV treatment affects the migration and morphology of tumor cells. In addition, *C. albicans*-derived EV treatment significantly increase the MMP activity of the cells. Gene expression changes were also detected after *Candida* EV treatment.

These results suggest that *Candida* EVs play a role in promoting OSCC epithelial-mesenchymal transition and thus in tumor progression.

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1256 – P1.07.37

Can trained immunity decrease Candida albicans induced cancer progression in human oral epithelial cells?

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Trained immunity is part of the innate immune system that is regulated by epigenetic modifications and is only temporary. Antibodies are not produced during short-term memory formation therefore the secondary immune response can be activated by similar pathogens whose antigens are recognised by the same pattern recognition receptors. This phenomenon is present in professional immune cells as well as in epithelial cells.

Oral squamous cell carcinoma (OSCC) is a global health problem often associated with various fungal infections caused particularly by *Candida*. Moreover, large amount of evidence suggests that *Candida* can contribute to carcinogenic events in the oral cavity by upregulating oncogenes in non-malignant cells. Recently, our group reported that the immune response induced by *Candida parapsilosis* and *Candida albicans* in OSCC cells is different. *C. parapsilosis* induces a mild host response while *C. albicans* enhances OSCC progression by stimulating pro-tumor signalling pathways and the production of matrix metalloproteinases.

Based on these differences, our goal was to investigate whether immune training with *C. parapsilosis* effect the ability of *C. albicans* to form and enhance cancer progression events. For our experiments, we used the oral epithelial cell line OKF6/TERT-2 which is an immortalized cell line and HSC-2 which is an OSCC cell line as the host, and two distinctive strains of *C. parapsilosis* (GA1 and CLIB214) and *C. albicans* (SC5314 and WO-1) as pathogens. *C. albicans* SC5314 has a much greater impact on epithelial cells than *C. albicans* WO-1. The host cells were pre-treated for 24 hours with live *C. parapsilosis* cells. After that we eliminated the fungal cells with the antifungal agent nourseothricin. After 5 days of rest, we stimulated host cells with *C. albicans* cells for 24 hours and examined the extent of host cell damage, cytokine production, adhesion capacities and gene expression changes.

The pre-treatment with *C. parapsilosis* species decreased cytotoxicity caused by *C. albicans* species in both cell line and increased IL-6 and IL-8 production in OKF6/TERT-2 cell line and decreased in HSC-2 cell line. Adhesion capacity studies and defining gene expression changes are in progress. The pre-treatment holds potential to decrease *C. albicans* induced cancer progression.

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1292 – P1.07.38

Transcriptomic signature of pediatric sepsis

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Purpose: Immune dysregulation is a hallmark of sepsis pathophysiology. First, pediatric immune system is subjected to developmental changes. Second, adult and pediatric sepsis differ in predisposing diseases and sites of infection. However, current sepsis management is the same for both; primarily supportive, including fluid resuscitation, early antibiotic therapy, and cardiorespiratory support. Leukocytes are major players to fight against invading microorganisms. The contribution of immune dysregulation to morbidity and mortality has been described in adult sepsis. However, there is a limited literature describing leukocyte phenotypic characteristics in pediatric sepsis. While adult and pediatric sepsis are clinically distinct as described above, their immunological delineation remained limited.

Methods: We tackled to delineate immunological profiles of pediatric sepsis at a single-cell level by analyzing blood samples from six septic children, at both acute and recovery phases, and four healthy children. Using Adult sepsis data available from the public domain, we also compared the transcriptomic profiles of pediatric and adult sepsis.

Results: 16 single-cell transcriptomic datasets (96,156 cells) were analyzed and compared to adult sepsis dataset. As expected, neutrophilia, T cell lymphopenia and HLR-DR cells depletion were observed in our pediatric cohort. We showed a unique shift in neutrophil subpopulations and functions between acute and recovery phases, along with examining the regulatory role of resistin. Neutrophil signatures were comparable between adult and pediatric sepsis. In contrast, T cell profiles differed between pediatric and adult sepsis. Innate-like CD4 T cells were predominantly and uniquely observed in the acute phase of pediatric sepsis. T2 CD4 cells were dominant in CD4 T cells in pediatric sepsis.

Conclusion: Our study serves as a rich source of information about the phenotypic diversity and trajectory of circulating immune cells during pediatric sepsis.

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1315 – P1.07.39

Memory γδT cells demonstrate a unique metabolic profile resulting in enhanced protection against Staphylococcus aureus.

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Gamma delta (γδ) Tcells are a lymphocyte subset with characteristics of innate and adaptive immune cells. γδT cells are subdivided based on the T cell receptor γ chain. Subsets have been shown to play differential roles in autoimmunity, cancer and are emerging as key players in the pathogenesis of infectious disease. One important aspect of the adaptive characteristics of γδT cells is the acquisition of a memory phenotype to previously encountered antigens. The memory γδT cell phenotype and its importance to effector function in infection is not yet understood. Our group has previously shown that exposure and convalescence from *Staphylococcus aureus* systemic infection results in an expansion of γδT cell subset Vγ6, with concomitant production of IL-17, which is protective during *S. aureus* infection. Critically, these cells could offer a significant advantage when developing novel therapeutics against intracellular pathogens such as *S. aureus*.

Following intra-peritoneal challenge with *S. aureus*, Vγ6 γδT cells are expanded by day four, dominate γδT cell population and remain resident in the abdominal adipose tissue for up to 35 days. Upon reinfection, these resident Vγ6 cells rapidly produce IL-17 and granzyme B, reducing bacterial load. We have further uncovered that the heightened response of these Vγ6 cells is controlled by metabolic changes. Seahorse analysis demonstrates increased baseline oxidative phosphorylation in convalescent γδT cells which is further increased upon re-challenge, in addition to an increase in glycolysis. Inhibition of glycolysis reduced Vγ6 cell production of IL-17 and increased bacterial burden. SCENITH metabolic profiling has further demonstrated that Vγ6 cells exhibit increased glucose dependence but additionally a novel finding that memory Vγ6 cells have an increased dependence on fatty acid oxidation.

Taken together, these data suggest that targeting γδT cell immmunometabolic pathways can potentiate host response to *S. aureus* infection. Host directed therapies against this significant global threat, that focus on γδT cell metabolism is an unexploited avenue for development.

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1365 – P1.07.40

Pneumococcal surface protein A (PspA) prevents killing of Streptococcus pneumoniae by the antimicrobial peptide indolicidin

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Streptococcus pneumoniae is a major human pathogen, responsible for high mortality rates worldwide. An important virulence factor in the pneumococcus is the pneumococcal surface protein A (PspA), a surface-exposed protein that prevents complement deposition on the bacterium. PspA is a widely studied candidate for serotype-independent pneumococcal vaccines. PspA can also bind to lactoferrin, an antimicrobial protein with bactericidal and bacteriostatic effects against a wide range of pathogens. The expression of PspA protects the bacterium from the bactericidal action of lactoferricins – cationic peptides released upon lactoferrin proteolysis. Since the negative charge of the exposed portion of PspA contributes to its ability to prevent lactoferrin-mediated killing, the present study sought to determine if PspA can prevent killing by another cationic peptide, indolicidin. PspA-negative pneumococci were more sensitive to indolicidin-induced killing than bacteria expressing PspA, suggesting that PspA prevents the bactericidal action of indolicidin. Similarly, removal of choline-binding proteins through choline chloride washes increased sensitivity to indolicidin. The absence of capsule and PspA had an additive effect on pneumococcal killing by the AMP. Furthermore, anti-PspA antibodies enhanced the bactericidal effect of indolicidin on pneumococci, while addition of soluble PspA fragments competitively inhibited indolicidin action. Previous *in silico* analysis suggests a possible interaction between PspA and indolicidin. Thus, we hypothesize that PspA may act by sequestering indolicidin, thus preventing it from reaching the bacterial membrane. Taken together, the results reinforce the vaccine potential of PspA and suggest a possible mechanism of innate immune evasion employed by pneumococci, which involves binding to cationic peptides and hindering their ability to damage the bacterial membranes.

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1367 – P1.07.41

Effect of in vitro indolicidin teatment in polymyxin-susceptible and polymyxin-resistant Acinetobacter baumanni

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Microbial resistance to antibiotics is one of the greatest public health challenges. Among the bacteria with broad antimicrobial resistance is *Acinetobacter baumannii*, an aerobic Gram-negative coccobacillus responsible for a high number of nosocomial infections, mainly affecting ICU patients. *A. baumannii* colonizes different host tissues, including the genitourinary and respiratory tracts and post-surgical scars, from where it can spread to the blood, causing systemic infections with high mortality. The high resistance to antibiotics and the ability to form biofilms favor the dissemination of the bacteria and limit the control strategies. Thus, the development of new therapeutic strategies is considered a global priority. Antimicrobial peptides (AMPs) are a group of small molecules produced by different organisms, which have broad antimicrobial activity against viruses, bacteria, protozoa and fungi. Many AMPs have a cationic and amphipathic structure, which favors their insertion into microbial membranes, causing destabilization and death of the microorganism. Unlike antibiotics, bacterial resistance to AMPs is not easily acquired, strengthening the therapeutic potential of these molecules. The present project aims to evaluate the resistance of *A. baumannii* to the cationic antimicrobial peptide indolicidin and to determine its correlation with resistance to antibiotics. The resistance of different clinical isolates to the action of AMP was determined through an *in vitro* bactericidal assay. To determine the effects of antimicrobial resistance on AMP action, resistance to polymyxin was induced by cultivating susceptible strains in increasing concentrations of the antibiotic. Different isolates of *A. baumannii* exhibited variable susceptibility to indolicidin-mediated killing. Preliminary data comparing strains before and after induction of polymyxin resistance showed a similar sensitivity to the AMP, suggesting that different mechanisms are involved in resistance against polymyxin and indolicidin. Further analysis will confirm if resistance to polymyxin affects the ability of *A. baumannii* to survive treatment with indolicidin. The results of this study should contribute to the development of new therapeutic strategies against bacteria that are multi resistant to antibiotics, in addition to elucidating the mechanisms involved in resistance to antimicrobial peptides.

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1485 – P1.07.43

Antibody levels and predictors for low response to childhood vaccines among 7–14-year-old children in Norway

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Purpose: Children in Norway are offered vaccination with 5 doses against diphtheria, tetanus and pertussis (DTP) at 2, 3 and 5 months of age, and 6 and 15 years. A vaccine against measles, mumps, and rubella (MMR) are given at 15 months of age and at 11 years. In this study we analysed antibody levels and factors predicting low antibody responses to vaccinations.

Methods: The study participants came from The Norwegian Environmental Biobank (NEB) and Human Early Life Exposome (HELIX), both sub-cohorts of the Norwegian Mother, Father and Child Cohort Study (MoBa). Plasma samples were collected between May 2014 and September 2017. IgG antibody levels against DTP and MMR were measured in in-house multiplex immunoassays (MIA). Data on immunization and predictors for vaccine response (maternal and child factors) were collected from the Norwegian Immunisation Registry (SYSVAK) and through questionnaires completed by the children's parents, respectively.

Results: Antibodies against DTP and MMR were measured in 901 children aged 7 to 14 years of which 49.7% were female. Among children who had received 4 doses of DTP vaccine (median age 10 years), 5.1% and 20.6% had antibody levels below 0.1 IU/ml against tetanus and diphtheria, respectively. 14.2% and 1.7% had an anti-rubella concentration of <10 IU/ml after 1 dose (median age 9 years) or 2 doses (median age 12 years) of MMR vaccine, respectively. Preliminary analyses suggest that high BMI at sampling age was associated with having antibody levels below 0.1 IU/ml for tetanus and diphtheria. Delivery by C-section was associated with lower levels of tetanus antibodies. There was a trend among children who had received 1 or 2 doses of MMR vaccine that antibody levels against rubella were lower among those who had been breastfeed for >6 months compared to those breastfeed for a shorter time. Data analysis on additional predictors are ongoing.

Conclusion: Despite a high vaccination coverage in Norway, vaccine antibody levels were low in some of the children. Child BMI at sampling time, C-section and duration of breastfeeding could be potential predictors of lower vaccine responses to childhood vaccinations.

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1488 – P1.07.44

Mice deficient for either interleukin-27 receptor-alpha or Eppstein-Barr virus-induced gene 3 are differentially susceptible to Mycobacterium tuberculosis infection

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Within the IL-12 cytokine family, the beta (β)-subunit Epstein Barr virus induced gene 3 (EBI3) forms, together with the alpha (α)-subunits IL-27p28, IL-12p35 and IL-23p19, the three different heterodimeric cytokines IL-27, IL-35 and IL-39, respectively. Whereas IL-27 is mainly produced by dendritic cells and macrophages and signals through the receptor complex IL-27 receptor (R) composed of IL-27Rα and gp130, the immunosuppressive cytokine IL-35 which signals through IL-12Rβ2/gp130 is preferentially secreted in regulatory T (T_{reg}) and B (B_{reg}) cells. The most recently described cytokine IL-39 induces pro-inflammatory immune responses through IL-23R/gp130.

After infection with *Mycobacterium tuberculosis* (Mtb), mice lacking the IL-27Rα exhibit lower bacterial burden in comparison to wildtype mice that correlate with an enhanced IL-17A production. By comparing the outcome of Mtb infection in C57BL/6, IL-27Rα deficient ($^{-/-}$) and IL-27Rα/IL-17A double deficient mice, we observed that the increased protection upon IL-27Rα deficiency is supported by IL-17A. Whereas IL-17A does neither impact the development of Tr1 cells nor the expression of PD1 and KLRG1 on T cells in IL-27Rα$^{-/-}$ mice during infection, it significantly regulates the presence of multifunctional T cells along with the formation of highly stratified granulomas in the lungs.

In contrast to the enhanced protection upon IL-27Rα deficiency, we observed unchanged bacterial loads in mice lacking EBI3 in comparison to wildtype mice. Moreover, despite EBI3$^{-/-}$ mice exhibit increased levels of IL-17A, the frequency of multifunctional T cells was not altered in those animals. Notably, the relative amount of natural killer (NK) T cells was highly reduced in the absence of EBI3.

Together, distinct mechanisms mediated through IL-27R or EBI3 may account for this opposing effect during experimental tuberculosis. To analyze the contribution of T_{reg}-derived IL-35 to the protective immune response, we are presently comparing the outcome of Mtb infection in T_{reg}-specific EBI3-deficient mice (Foxp3^{cre}-positive EBI3loxP$^{/loxP}$), Foxp3^{cre}-negative EBI3$^{loxP/loxP}$ control animals and global EBI3$^{-/-}$ mice. Conclusively, gaining a deeper understanding of the differential role of these two subunits may help to elucidate the complex interplay between protective and pathologic immune mechanisms during Mtb infection.

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1496 – P1.07.45

Exploring the effect of Leishmania infantum and Trypanosoma cruzi extracellular vesicles on dendritic cell-lymphocyte interactions

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Purpose *Leishmania* spp. and *Trypanosoma cruzi* parasites are the aetiological agent of Leishmaniasis and Chagas disease, respectively, and belong to the Trypanosomatidae family. In both cases, the dog represents the main domestic reservoir and these diseases can be fatal if left untreated. Antigen-presenting cells such as dendritic cells (DCs) are seen as important therapy vehicles since they can establish a link between innate and adaptive immunity, therefore reshaping the outcome of infection due to their immunostimulatory role. Moreover, extracellular vesicles (EVs) shed by parasites appear to play an immune modulatory role in disease pathogenesis. Therefore, the present study aimed to examine the interaction of DCs with EVs shed by *L. infantum* (*Li*EVs) and *T. cruzi* (*Tc*EVs) parasites and the subsequent immune response of lymphocytes induced by EVs-primed DCs.

Methods Monocyte-derived DCs (moDCs) were obtained *in vitro* from healthy canine peripheral blood mononuclear cells and exposed separately to *Li*EVs and *Tc*EVs. Afterwards, co-cultures of EVs-primed moDCs and autologous lymphocytes were established, and the gene expression of surface innate receptors (TLRs) and immune mediators evaluated by RT-PCR.

Results The results indicate that *Li*EVs and *Tc*EVs stimulate moDCs to express TLRs, triggering the upregulation of pro-inflammatory cytokines (tumor necrosis factor (TNF)-α and interleukin (IL)-12p40), followed by the generation of the regulatory cytokine IL-10. Furthermore, *Li*EVs-primed moDCs induced lymphocytes to upregulate TNF-α and IL-4 and *Tc*EVs-primed moDCs led lymphocytes to trigger IFN-g and IL-10 gene expression.

Conclusion EVs from both parasites trigger analogous activation of moDCs, which can be justified by the similarity between these trypanosomatids. Although EVs-primed moDCs direct lymphocytes to exhibit a mix pro and anti-inflammatory response, specific immune mediators are generated depending on the parasite. Taken together, these findings indicate that EVs shed by *T. cruzi* and *L. infantum* parasites can establish communication with DCs and specifically shape the adaptive immune response, highlighting the great immunomodulator potential of parasitic EVs.

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1664 – P1.07.46

Exploring the immunomodulatory properties of zymosan on human peripheral blood mononuclear cells: an in vitro study

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Purpose: Recent evidence suggests that symbiotic fungi play a critical role in shaping host immunity, with fungal cell wall components influencing immune cell activity. Pattern recognition receptors (PRRs) are crucial in recognizing fungal antigens, yet further research is needed to elucidate their precise impact on immune cell biology. This study aimed to evaluate the effects of the significant fungal cell wall component, zymosan, on the expression of C-type lectin receptors (CLRs) representatives (Dectin-1 and Dectin-2) on human peripheral blood mononuclear cells (PBMCs) and its potential to activate these cells for antifungal immune mechanisms, such as reactive oxygen species (ROS) generation and cytokine (IL-17, IL-23, TNF) synthesis.

Methods: PBMCs at a concentration of $1x10^6$ cells per mL, cultured *in vitro* at 37°C in a 5% CO2 humidified atmosphere, with or without zymosan (1 μg/mL and 10 μg/mL), derived from *Saccharomyces cerevisiae* cell wall, and harvested after 72 hours of incubation. Quantitative RT-PCR (qRT-PCR) was used to assess the mRNA gene expression of CLRs and cytokine in human PBMCs. Spectrofluorimetry was used to examine zymosan-induced ROS generation in PBMCs.

Results: Zymosan induced an increase in the mRNA expression level of Dectin-1 (1.7- and 4.5-fold) and Dectin-2 (2.3- and 2.5-fold) in PBMCs stimulated with 1 and 10 μg/mL, respectively when compared to unstimulated control cells. Exposure of PBMCs to zymosan at concentrations of 1 and 10 μg/mL resulted in a slight up-regulation of IL-17 mRNA levels by 1.6- and 2-fold, respectively, while significant up-regulation of IL-23 mRNA expression by 31- and 51-fold, and TNF mRNA expression by 45- and 55-fold, respectively, compared to non-stimulated cells. PBMCs exhibited substantial ROS production upon stimulation with zymosan at concentrations of 1 μg/mL ($p<0.001$) and 10 μg/mL ($p<0.0001$) compared to unstimulated cells.

Conclusion: This study's findings underscore the pivotal role of zymosan in modulating the immune response through the upregulation of CLRs and cytokine expression and the induction of ROS production in human PBMCs. These insights shed light on potential targets for enhancing antifungal immune mechanisms and offer avenues for further exploration in therapeutic interventions against fungal infections.

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1752 – P1.07.48

Development of an ex vivo bronchoalveolar lavage model towards in vivo neonatal non-human primate models of respiratory infections

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Purpose: Respiratory infections, particularly in neonates, result in a large amount of hospitalisation and death. Respiratory syncytial virus (RSV) and *Streptococcus pneumoniae* (SP) are the leading cause of bronchiolitis and pneumonia in those under 5, respectively. It is imperative to understand the pathophysiology underpinning them, but difficult to determine in neonatal cohort for many reasons, thus requiring use of an animal model. Our team is developing neonatal non-human primate (NHP) models to help understand immunopathogenesis of RSV and SP infections, while providing a model for testing novel therapeutics. To help reduce the use of neonatal NHPs, we developed an *ex vivo* bronchoalveolar lavage (BAL) model of infection, primarily alveolar macrophages, to assess and compare cellular immune profiles following exposure to RSV and SP.

Methods: BALs from healthy adult macaques were collected, cultured and exposed to RSV M37 (strain used in human challenge and obtained from C. Chiu, Imperial College London) and/or SP at different MOIs for 6hrs, 24hrs and 48hrs. Cells were collected and stained with optimized antibody-panels for myeloid cell characterization by flow cytometry, and culture supernatants were analysed for cytokines/growth factors expression by multiplex bead-based immunoassay. Immunohistochemistry staining was also performed on infected and non-infected cells.

Results: We successfully cultured and exposed cells from NHP BAL, primarily alveolar macrophages, with M37-RSV. Our data show positive staining with anti-RSV antibody compared to mock uninfected cells. The majority of vacuolated and/or multi-nucleated BAL cells were alveolar macrophages. Supernatants at 24 hours from infected cells showed increased IL-10, TGF-a, IL1Ra, IL-18 and VEGF, with decreased MIP-1a, IL-6 and TNF-a. Flow cytometry showed a significant decrease in HLA-DR expression in exposed alveolar CD163+ macrophages compared to non-infected cells. Altogether, these results suggest that RSV infection induce a more immunosuppressive profile of the cells. Results from SP infections (ongoing experiments) will be compared to RSV.

Conclusion: We were able to establish an *ex vivo* model of NHP alveolar macrophages to study immunopathogenesis of RSV and SP. Results from this model will complement future *in vivo* studies of neonatal NHP models to better understand the pathophysiology of RSV and SP severe infections.

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1793 – P1.07.49

Interleukin-32 producing CD8+ memory T cells define IDO1 / PD-L1 niche in human cutaneous leishmaniasis

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Immune checkpoint (IC) expression has been extensively studied in vitro, revealing potential regulatory pathways, particularly in myeloid cells. However, these in vitro data fail to explain how IC expression is regulated at disease sites, such as leishmaniasis lesions or the tumor microenvironment. To understand IC regulation in the context of the complex cellular and molecular niches present in vivo, it is necessary to study IC expression in situ. To address this, we used spatial transcriptomics to study IC expression in skin lesions from patients with cutaneous leishmaniasis (CL), a disease characterized by chronic skin pathology. Previously, we found that indoleamine 2,3-dioxygenase 1 (IDO1) and programmed death-ligand 1 (PD-L1) are enriched in CL lesions, and reduced PD-L1 early after treatment predicts cure in Sri Lankan patients. Here, we used spatial cell interaction mapping to identify IL-32-expressing CD8+ memory T cells and regulatory T cells as key components of the IDO1/PD-L1 niche in CL lesions across patient cohorts from Sri Lanka, Brazil, and India. Importantly, the abundance of IL-32+ cells and IL-32+CD8+ T cells at treatment onset was prognostic for cure rate in Sri Lankan patients. This study provides a unique spatial perspective on the mechanisms underlying IC expression during CL and identifies novel biomarkers of treatment response, with implications for understanding IC regulation in the complex in vivo environments of other chronic inflammatory and infectious diseases.

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1811 – P1.07.50

Impaired NK cell response contributes to ageing-associated susceptibility to pneumococcal pneumonia

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Streptococcus pneumoniae (the pneumococcus) is the leading cause of bacterial pneumonia, with high morbidity and mortality worldwide, and the elderly are the most significantly affected population. This is thought to be related to ageing-associated immunological changes, which impair the host defence against infection. In the context of pneumococcal infection, defects in immune components and responses that are specifically related to disease progression and severity in aged lungs is poorly understood.

Pneumococcal carriage in the nasopharynx is a prerequisite for the development of pulmonary infection. Considering the potential influence of prior carriage events on host immunity, we established a secondary pneumonia model by challenging mice that had a prior carriage exposure to the same strain of pneumococcus. Using this re-infection model, we explored how ageing affects leukocyte trafficking during pulmonary pneumococcal infection, with a specific focus on the role of NK cells.

We found that most adult mice were protected from the secondary pneumonia challenge whilst aged mice remained susceptible, which closely replicates increased susceptibility to pneumococcal pneumonia with age in human. In adult mice, a more efficient NK cell recruitment was observed in those mice that did not develop bacteraemia, suggesting a protective role of NK cells at preventing systemic dissemination of bacteria. Compared to adults, elderly mice showed a delayed recruitment of neutrophils which accumulated in the vasculature, and more surprisingly, a completely abolished systemic recruitment of NK cells. Furthermore, NK cells from aged adults (both in mice and humans) had markedly lower production of IFNγ and granzyme-B in response to pneumococcal stimulation. The elderly had an expanded effector $CD8^+$ T-cell population, which compensated for the diminished IFNγ production by NK cells but not the cytotoxic response against pneumococcus. Treating aged mice with IL-15/IL-15Ra complex significantly increased NK cell trafficking to the lungs during infection, and this increase was correlated to reduced bacterial loads in blood.

Our results suggest that competent NK cell responses are critical for protective anti-pneumococcal immunity in the lung and that ageing leads to deficient NK responses to pneumococcal infection, which is a major contributing factor to increased susceptibility to disease.

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European Journal of Immunology Clinical-Basic-Translational

1839 – P1.07.51

Towards novel COVID-19 therapy based on natural products with anti-SARS-CoV-2 activity

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In this multidisciplinary project, we aim to discover new natural products that can treat or prevent COVID-19 infections caused by SARS-CoV-2. Thus far, 171 natural products from endemic plants originating from northern Europe and Tanzania have been isolated and elucidated by employing nuclear magnetic resonance (NMR); many of which are novel or rare.

In order to evaluate the potential selective inhibitory effects of natural products against the virus without harming the cells, cell cytotoxicity assays were performed. For this, in vitro MTT and XTT assays were utilized on A549 human pulmonary epithelial cells . The antiviral efficacy was evaluated using a neutralization assay using pseudo typed viruses encapsulating spike proteins of prevalent SARS-CoV-2 variants, namely Omicron (XBB 1) and Delta (B.1.617.2). An ACE2 and TMPRSS2-expressing A549 cell model was utilized in the assessment of viral entry inhibition.

Most of the compounds had little cytotoxic effects on the cells. From 171 compounds tested, seven exhibited significant inhibitory activity regarding viral cell entry, achieving up to 100% inhibition at 10μM concentrations with minimal cytotoxic effects. The next step will be to assess potential effects on viral replication using infectious SARS-CoV-2 isolates and quantitative RT-PCR with TaqMan probes. With that we will elucidate whether the natural compounds hinder viral propagation solely at the entry phase or extend their inhibitory action to subsequent stages of the viral life cycle. In addition, we will continue with computational docking studies with the most promising compounds to predict the interaction between these compounds and viral proteins.

This research signifies a strategic expansion in the search for antiviral agents derived from natural sources. The compounds identified offer promising options for the development of anti-SARS-CoV-2 therapeutics. The outcomes of this study will potentially facilitate the progression of these phytochemicals, underscoring the importance of natural products in addressing contemporary health challenges.

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1862 – P1.07.52

Serum calprotectin in severe and mild COVID-19 patients

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Purpose: The aim of the study is to evaluate the levels of serum calprotectin between healthy controls and COVID-19 patients.

Methods: Serum samples were collected from 16 healthy controls and 50 COVID-19 hospitalized patients, 17 with severe evolution and 33 mild evolution, at two times: hospitalization (T0) and 11 days post hospitalization (T1). Serum samples were stored at -80 Cº and serum calprotectin was subsequently determined with the Chemoluminiscent immunoassay QUANTA Flash® Circulating Calprotectin, according to the manufacturer's specifications. Statistical analyses were performed with Graphpad prism v5.0 software.

Results: COVID-19 patients showed statistically higher levels of serum calprotectin at T0 and T1 compared with healthy controls. Statistically significant differences have been detected in serum calprotectin of patients with COVID-19 between the time of hospitalization and 11 days later.

Conclusion: Elevated levels of serum calprotectin observed in COVID-19 patients could reflect that neutrophils participate in the COVID-19 pathophysiology.

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1879 – P1.07.53

Regulation of NLRP3 inflammasome-related gene expressions in the progression to chronicity in brucellosis with bone joint involvement

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Purpose: Bone joint involvement (BJI) is commonly observed in the zoonotic infection, brucellosis, caused by the intracellular pathogen Brucella. Caspase-1 and caspase-11 initiate joint inflammation and proinflammatory cytokine production and participate in infection control thereafter. Caspase-1 and Caspase-11 involve inflammation by activating the NLR family pyrin domain containing 3 (NLRP3). The role of NLRP3 dysfunctions in bone and joint diseases was shown, however, the function of NLRP3 in the chronicity of BJI+ Brucellosis remains unknown. Our study determined the mRNA levels of genes involved in NLRP3 inflammasome activation during the chronicity process in BJI+ brucellosis.

Method: The study included ten acute and six chronic BJI+ brucellosis cases and eight healthy controls. Cases were classified as acute (0-2 months) and chronic (>12 months) according to the onset time of symptoms. A human mRNA microarray analyzed the expression of over 30,000 genes in mononuclear cells separated from peripheral blood by Ficoll density-gradient centrifugation. An independent sample T-test analyzed the changes of genes in BJI+ brucellosis. A KEGG pathway analysis determined the association of those genes with NLRP3 expression and activation.

Results: NLRP3 mRNA was decreased in BJI+ chronic brucellosis compared to control (p=0.015). Supporting this, TRIM31, which is involved in the degradation of the NLRP3 inflammasome, increased in these cases compared to the control (p=0.009). In contrast; P2RX7, which plays a role in forming the NLRP3 complex by uptake of extracellular ATP into the cell, was increased in BJI+ acute brucellosis compared to the control (p=0.015). Similarly, NEK7, which acts as a component of the NLRP3 inflammasome complex, increased in these cases (p=0.019 and p=0.013).

Conclusion: Our findings suggest that the expression NLRP3-related genes increase in BJI+ acute brucellosis, whereas these pathways are suppressed at the mRNA level in BJI+ chronic brucellosis.

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1912 – P1.07.54

Schistosoma mansoni proteases and their effect on host extracellular vesicle production: A strategy to modulate the immune system

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Background: In schistosomiasis, caused by *Schistosoma mansoni*, the parasite invades the host through the skin. The parasite is using proteases like Cathepsin B1 (SmCB1) and SmCL3 to degrade tissue and blood. Extracellular vesicles (EVs) play a key role in intercellular communication and in the response to parasitic infections. EVs can be characterized by the presence of tetraspanins. These transmembrane proteins play a crucial role in all phases of EV biogenesis**.**

Methods: The recombinant proteins SmCB1 and SmCL3 were produced in yeast *P. pastoris*. Their activity was assessed at different pH values using an enzymatic activity assay. Human adenocarcinoma basal epithelial cells (A549), human monocytes THP-1 and mouse macrophage cells (RAW) were treated with active and E-64-inactivated SmCB1 or SmCL3. EVs were collected by ultracentrifugation and size exclusion chromatography. Size and concentration of EVs were measured using Zetasizer. Western blot analysis was performed to detect the tetraspanins CD63, CD81 and CD9 in the cells. Mice were treated intranasally for three consecutive days with active and E-64-inactivated SmCB1 or with EVs from SmCB1-treated A549 cells. Lung cells were analysed by flow cytometry.

Results: The activity of SmCB1 was optimal at or below pH 7.0, while SmCL3 was active at all pH values tested. Treatment with SmCB1 (active and E-64-inactivated) and SmCL3 resulted in the release of EVs from A549 cells. These EVs were mainly ~100 nm in diameter. Each of the cells expressed CD63, CD81 and CD9. Intranasal treatment with active SmCB1 reduced the number of alveolar macrophages and CD11c+ cells, and inactivation with E-64 abolished this effect. SmCB1-induced EVs reduced the number of alveolar macrophages, CD11c+ cells and NK cells in the lung compared to sham-treated controls.

Conclusion: Our results show that SmCB1 triggers the release of EVs from A549 cells and that these vesicles have immunomodulatory potential. This study sheds light on the complex interplay between *S. mansoni* proteases and host EV dynamics and contributes to our understanding of the immune response to S*chistosoma* parasites**.**

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European Journal of Immunology Clinical-Basic-Translational

1926 – P1.07.55

Toll-like receptor 4 improves replication of *Chlamydia trachomatis* serovar D in the uroepithelial T24/83 cell line

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Chlamydia trachomatis infection remains mostly symptomless but can cause tissue modulating processes like hydrosalpinx, pelvic inflammatory disorder or lead to infertility. Uroepithelium is the point of entry for the chlamydial infection. In order to identify the immune signaling cascades activated by *Chlamydia*, compared to polyI:C or LPS, we infected T24/83 human uroepithelial cell line with *C. trachomatis* serovar D. Here we used microarray analysis and quantitative Real Time PCR, and observed an induction in the mRNA transcription of inflammatory genes such as il-1β, il-6 and tnf-α. However, the ELISA showed, that the corresponding cytokines were not secreted upon infection. T24/83 cells secrete the mentioned cytokines after a stimulation with polyI:C or LPS which are ligands for Toll-like receptor (TLR) 3 and 4, respectively. Thus, the pattern of the gene induction is different compared to infection with *C. trachomatis*. Additionally, we identified a set of genes like matrix metallopeptidase 1 and 3 and histatin 1, which were most induced upon *C. trachomatis* infection. It was reported, that TLR3 and TLR4 play an important role in the host pathogen interaction. Therefore we wanted to identify the role of TLR3 and TLR4 in T24/83 cells using CRISPR/Cas9 knock-outs. Unexpectedly we could show that in TLR4KO cells *C. trachomatis* serovar D replicates less well than in the wildtype. Whereas TLR3KO cells showed no difference in chlamydial replication compared to the wild type host cells. Hence, we assume that chlamydia can use TLR4 as a supporter for its own growth.

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1943 – P1.07.56

Antibody-based approaches for studying potential adhesion proteins of *Gardnerella spp.*

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Bacterial vaginosis (BV) is characterized by a disruption of lactobacillus-dominated vaginal microbiota and the overgrowth of *Gardnerella* spp. and other anaerobic bacterial species. This shift in vaginal microbiota is associated with women's reproductive health issues. The defining characteristic of BV is a polymicrobial bacterial biofilm primarily dominated by *Gardnerella*. The displacement of lactobacilli, colonization, and biofilm formation depends on the adherence capacity of *Gardnerella*. *Gardnerella* surface-associated proteins, such as the collagen-binding protein (CNA) and the M protein repeat protein (MPR), are suggested to be to play a role as bacterial adhesins. This study aimed to explore the interactions between CNA and MPR with extracellular matrix (EM) proteins and assess their presence on the surface of *Gardnerella* spp. using newly developed monoclonal antibodies (MAbs).

Hybridoma technology was utilized to generate mouse MAbs targeting CNA and MPR proteins. MAb-based ELISA revealed an interaction between CNA and human fibrinogen, while MPR demonstrated interaction with fibrinogen and fibronectin. Flow cytometry using both MAbs and polyclonal antibodies against CNA and MPR was then employed to assess potential adhesins expressed on various *Gardnerella* strains. Before analysis, optimization steps for CFDA-SE dye and antibody quantities were conducted. Results indicated a stronger interaction between surface MPR and corresponding antibodies compared to surface CNA.

The utilization of recently developed MAbs revealed the interaction between CNA and MPR with EM proteins, signifying the role of these surface proteins as adhesins. Flow cytometry findings provided valuable information regarding adhesins on *Gardnerella* spp. surfaces, which could inspire future investigations on their interactions with EM proteins on epithelial cells. This study contributes to advancing our understanding of BV molecular mechanisms and lays the groundwork for developing targeted strategies for its diagnosis and treatment.

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1963 – P1.07.57

Promising vaccine antigens against Klebsiella pneumoniae reduce bacterial burden in a sepsis model

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Klebsiella pneumoniae is a Gram–negative bacterium, which causes septicaemia, respiratory tract infections, urinary tract infections and soft tissue infections. It is a major opportunistic pathogen, accounting for 11.9 % of reported bacterial species in 2021 in the EU/EEA, according to EARS-Net surveillance data, and antimicrobial resistant strains are associated with high mortality rates.

The aim of this project was to identify proteins used by *K. pneumoniae* to attach to host epithelial cells and their potential as novel protective vaccine antigens. In total, 31 bacterial adhesins from *K. pneumoniae* were identified by the Cell Blot approach developed by the McClean Lab, 24 of which were novel. Three antigens were selected as potential vaccine candidates. These were confirmed to play a role in *K. pneumoniae* attachment to lung cells *in vitro*, as BL21 cells expressing recombinant proteins showed 14.3–, 7.22–, 6.48–, and 13.3–fold (p=0.012, p=0.0199, p=0.0011, p=0.045) increased levels of attachment to 16HBE14o$^-$ cells respectively. Immunisation of mice with Antigens O, D or A individually showed reduced *K. pneumoniae* burden in peritoneal cavities (2.50 log, 2.69 log, 1.54 log (p=0.0007, p=0.0007, p=0.0080), respectively) in a sepsis challenge model and also reduced dissemination to the spleen (1.01 log, 1.44 log, 0.86 log (p=0.0003, p=0.043, p=0.0263), respectively). Strong anti–specific IgG titers were raised in mice immunised with Antigen L and Antigen O compared with mice treated with adjuvant alone. Serological analysis showed the expression of high antigen–specific antibody responses compared to the control group (titres ~ 3.9×10^6), the ratio of IgG1 / IgG2a (ratio = >5) suggests that Th2 responses were induced by both Antigen L or Antigen O immunisations. T–cell recall response analysis following immunisation of Antigen L or O demonstrated stimulation of IL–17 and IL–22 expression. Overall, these antigens were protective against *K. pneumoniae* sepsis and have potential as vaccine candidates.

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1981 – P1.07.58

Genetic deficiency of long pentraxin 3 dampens *Staphylococcus aureus* infection in a murine model of osteomyelitis

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Purpose: Osteomyelitis (OM) is a severe bone infection originating from hematogenous spreading of microbes or contamination of surgery/fracture. OM is primarily caused by the opportunistic bacterium *Staphylococcus aureus* (SA). This evades the host immune response, acquires antibiotic resistance, and chronically colonizes the musculoskeletal tissue[1], yet the underlying molecular and cellular processes are largely unclear. Here we aimed to characterize the pathogenetic mechanisms of SA-OM by focusing on the long pentraxin 3 (PTX3), a soluble pattern recognition molecule and bone component that is emerging as a new player in osteoimmunology[2] and a diagnostic marker of periprosthetic joint infections (PJI), a common form of OM[3].

Methods: A murine model of OM, based on intra-bone injection of SA, was developed that closely mimicked surgery/trauma-related OM in humans and allowed addressing the role of PTX3 in gene-modified (*Ptx3*$^{-/-}$) animals. Local and systemic infection and inflammation were assessed via microbiology, flow cytometry, histochemistry and microCT techniques.

Results: SA-injected mice developed chronic infection with pronounced inflammation and extensive tissue remodeling. Concentration of inflammatory markers (including IL-6, IL-1b, and TNF-a) and PTX3 in the serum and bone homogenate was higher in SA- than vehicle-injected WT animals. Noticeably, we found more bacteria in WT than *Ptx3*$^{-/-}$ mice at 6 and 14 days from challenge, with viable SA confined to the treated limbs only. Furthermore, administration of a PTX3-targeting antibody dampened infection in the bones of SA-infected WT mice. These also had enhanced systemic inflammation compared to *Ptx3*$^{-/-}$ littermates, with expanded innate immune compartment in the spleen and increased serum levels of inflammatory mediators. However, the local (in the bone) inflammatory reaction was more pronounced in the absence of PTX3 with increased levels of Th1 cytokines (IL-1b) and antimicrobial chemokines (CXCL9).

Conclusion: In a mouse model of SA-OM, genetic deficiency of PTX3 protected from infection and inflammation, pointing to this pentraxin as a crucial player in OM pathogenesis and a novel therapeutic target in bone infections.

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2019 – P1.07.59

Chemical and infectious colitis models show different immune profiles

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Background: Ulcerative colitis is a type of inflammatory bowel diseases (IBD) characterized by a chronic immune response imbalance within the gastrointestinal tract. While the precise origins remain elusive, researchers have turned to animal models to explore the pathogenesis, causes and diagnosis of human IBD. Among these models, one of the most prevalent involves the use of dextran sodium sulfate (DSS) to induce damage to the intestinal epithelium. Its advantages include the rapid onset, reproducibility, and precise control. Another widely used model is a *Clostridioides difficile* infection (CDI) model, which, after treatment with antibiotics, has been shown to develop a colitis similar to that suffered by patients. However, although both models generate similar clinical symptoms to ulcerative colitis, being two different insults (chemical and infectious), they may generate a different immune response.

Objectives: To define the differences in the immunological profile between the DSS and CDI models.

Methods: DSS model was induced by 2,5% (w/v) DSS in drinking water continuously for 5 days in 10 C57BL/6 female mice. CDI model was induced in 9 C57BL/6 female mice following the protocol by Cussó et al. (DOI: 10.1007/s11307-019-01408-4), employing 10^6 CFUs of ribotype 027. 100 µl of peripheral blood samples were collected at basal and 2 days post-treatment; and incubated with 2 multiparametric antibodies panels to analyse lymphoid and myeloid populations. Then erythrocytes were lysed, and the samples were acquired by flow cytometry using a 16-channel flow cytometer.

Results: The results of flow cytometry analysis showed that DSS group presented a marked increase in granulocytes, proinflammatory monocytes and NK cells, and a significant decrease in dendritic cells compare to CDI mice. While, DSS model did not present variations in lymphoid populations, CDI model was characterized by an increase in B cells, especially plasmablasts, T lymphocytes and NKT cells. Within T cells, effector memory and activated phenotypes of both CD4+ and CD8+ T cells were increased in the CDI group compared to DSS model.

Conclusion: Although clinically and histopathologically both models are similar, their immune response is clearly different and must be taken into consideration when selecting one model over another.

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2223 – P1.07.60

Triacetylfusarinine C (TAFC) as a potential diagnostic biomarker of invasive pulmonary aspergillosis

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Purpose: Invasive pulmonary aspergillosis (IPA) is a severe fungal infection caused by *Aspergillus* spp. and usually occurs in immunocompromised patients. The most common causative pathogen is *Aspergillus fumigatus*. This pathogen causes a range of manifestations, from allergic forms to invasive and chronic infections or underlying pulmonary dysfunctions. The mortality rate of IPA ranges between 30-95 %. Diagnostic tools for detection biomarkers (galactomannan, 1,3-β-D-glucan, IgA, and IgG specific to *A. fumigatus*, *Aspergillus* DNA) and medical imaging constitute the basis of the screening approach, although both have some limitations in specificity. *A. fumigatus* synthesizes several siderophores, the major extracellular hydroxymate siderophore being triacetylfusarinine C (TAFC). Siderophores are low molecular weight iron-specific chelators that are essential virulence factors. Detection of TAFC may be a promising biomarker of IPA in urine and BALF samples.

Methods: We developed recombinant lipocalin (Lcn) coupled to agarose resin for affinity purification of selected siderophores, including TAFC. His-tagged Lcn1/Lcn2 were prepared in a prokaryote expression system where the designed sequence was inserted into the pET28B+ plasmid. The transformation was performed into *E. coli* BL21 expressing bacteria and coupled with Ni-NTA agarose resin in a centrifugation minicolumn. Lcn 1 was analyzed by mass spectrometry. Biological samples were loaded onto an affinity column, and TAFC was eluted by molecular excess of rhodotorulic acid. Detection of TAFC was performed by mass spectrometry.

Results: Recombinant Lcn1 was successfully expressed and amino acid sequence analysis by mass spectrometry revealed 13 peptides with 44,9% sequence coverage. The interaction of Lcn1 with TAFC was probed by native mass spectrometry. The non-covalent complex was not detected, contrary to enterobactin-Lcn1 complex, run in parallel in a control experiment. This observation rationalized the negative binding observed with the affinity column we prepared for patients´ sample analysis. In the next step, two other protein-TAFC binding systems will be designed and tested.

Conclusion: An engineered microarray for pre-concentration of siderophore TAFC from urine and BAL samples for subsequent detection using MALDI TOF technology is needed for routine siderophore detection useful in diagnosing IPA**.**

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2234 – P1.07. 61

B-cell responses induced by *Neoehrlichia mikurensis* infection in patients with malignant B-cell lymphomas

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Purpose: *Neoehrlichia mikurensis (N. mikurensis)* is an intracellular tick-borne bacterium that belongs to the family of *Anaplasmataceae*. It is associated with persistent infection of vascular endothelial cells, leading to severe infections in individuals with compromised B-cell immunity, including patients with malignant B-cell lymphomas. As B cells seem to play a crucial role in the defense against *N. mikurensis*, the purpose of this study was to characterize the B-cell subsets induced by *N. mikurensis* infection in immunocompetent individuals and individuals with malignant B-cell lymphomas.

Methods: Peripheral blood mononuclear cells were isolated from the blood of immunocompetent individuals, infected with *N. mikurensis* (n = 6), non-infected immunocompetent individuals (n = 4), individuals with malignant B-cell lymphomas, infected with *N. mikurensis* (n = 3), and non-infected individuals with malignant B-cell lymphomas (n = 3). The cells were incubated with monoclonal antibodies directed against 28 B-cell markers and analyzed by Cytometry by Time-of-Flight (CyTOF). The multiparameter data was processed through cluster analysis to create minimum spanning tree plots, illustrating the sizes and phenotypes of various B-cell subsets, alongside univariate analyses.

Results: The characteristics of B-cell subsets in immunocompetent individuals and immunocompromised individuals with malignant B-cell lymphomas were compared with non-infected, immunocompetent individuals and non-infected individuals with malignant B-cell lymphomas. Regardless of immune status, infected individuals expressed a higher proportion of plasmablasts, memory B cells and resting memory B cells. In contrast, non-infected individuals expressed a higher proportion of naïve B cells and CD5+ B cells.

Conclusion: There were significant differences observed in B-cell subset distributions among immunocompetent and immunocompromised individuals infected with *N. mikurensis* compared with the non-infected individuals, fortifying the notion that B cells play a crucial role in the host defense against *N. mikurensis*.

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2242 – P1.07.62

Immune programs in human dendritic cells upon immunization with *Chlamydia trachomatis*

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Purpose: *Chlamydia trachomatis* (Ct) is responsible for more sexually transmitted infections worldwide than any other bacterium. However, to date the immune response in humans against Ct remains incompletely understood. From studies in mice we know that immunization with live Ct leads to priming of protective immune responses by activation of effector T cells, whereas immunization with UV-inactivated Ct produces responses of tolerance by activation of regulatory T cells. We aim to assess the role of human dendritic cell (DC) subsets in Ct infection to understand the natural immune response against Ct and its manipulation by vaccination strategies.

Methods: We are using monocyte derived DCs (moDCs) to test the direct effect of Ct on DCs. In addition, we successfully established an *ex vivo* Ct infection model of cervix samples from donors undergoing hysterectomy. To investigate immune mechanisms in Ct-immunized DC, we performed flow cytometric analysis, RNA-Seq and functional *in vitro* experiments.

Results: MoDCs stimulated with Ct or UV-Ct show differential transcriptional immune programs regulating cytokine response and apoptosis. One striking difference between moDC infected with live and UV-Ct was inclusion formation in a proportion of moDC infected with live Ct, indicating active proliferation of Ct in immune cells, which might influence their T cell priming properties. Furthermore, we observed decreased levels of CCR7 expression upon infection with UV-Ct. In comparison to LPS-stimulated moDC, migration is in general lower in Ct-primed cells, which could explain the absence of long-lasting immunity upon Ct infection. When assessing the *ex vivo* Ct infection model in cervix-derived immune cells, we observed the main immune cell subset taking up Ct being CD14+CD11c+ and CD14-CD11c+ antigen presenting cells.

Conclusions: Our studies provide a first step towards understanding antigen uptake and presentation during human Ct infection, which, ultimately, can lead to new vaccine strategies against this important bacterial sexually transmitted disease.

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2252 – P1.07.63

Microbial SIMs: role in inflammatory balace

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The two important aspects of an infection include the incoming pathogen and the defending host immune system. *Mycobacterium tuberculosis* (Mtb) is an intra-cellular pathogen that has developed specific mechanisms to invade and survive within its host cells. Immunomodulation in tuberculosis by Mtb has been discussed only as a virtue of the pathogen; however the mechanistic intricacies of the pathogen mediators of immune response alterations have received less attention. Several bacterial proteins have been scrutinized for their immunogenicity since proteins are known to be antigenic in comparison to other macromolecules. However, virulence of Mtb has also been ascribed to a plethora of cell surface lipid moieties for their abundance in the Mtb cell wall. Whilst such large macromolecules and surface associated signatures have often received the deserving attention, the role of small secondary molecules (SIMs) synthesized by the pathogen and their consequence on the host-mycobacteria balance has remained elusive. This talk will discuss preliminary results towards identification of such alternative bacterial mechanisms that may contribute to subversion of host immune responses allowing better survival with least fitness cost to the host.

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2282 – P1.07.64

Immunometabolic profiling of myeloid cells in pediatric patients represents a tool how to depict the dynamics of sepsis progression

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Background: Sepsis is heterogeneous syndrome with dynamic progression, in late phases associated with immunosuppression, which can persist many months after sepsis onset. The exposure of myeloid cells to environmental stimuli such as invading pathogens or pathogen-originated ligands is followed by their activation leading to a metabolic switch, represented by increased glycolysis linked with anabolic processes. Hence, immunometabolism represents the main governing force for immunity and its defensive functions. Changes in immunometabolic profile occur within minutes and represent the major source of intermediate molecules for defensive processes and also for epigenetic changes associated with long-lasting immunosuppression. This immunometabolic switch ensures the gain of required amount of energy and intermediate metabolites for defensive processes, vital for host protection and his subsequent recovery.

Objectives: The metabolic changes in immune cells represent possible approach how to depict the sepsis dynamics and can reflect the functional ability of cells during sepsis progression. This project aimed to characterize immunometabolic pattern of myeloid cells within sepsis progression.

Methods: Blood samples from pediatric septic shock patients were collected in two time points to cover sepsis progression (TP1 – within first day after ICU admission, TP2 – 5-7 days after ICU admission). We performed the flow cytometry profiling of single-cell energetic metabolism by SCENITH completed with detailed immunophenotypic analysis of myeloid cell subpopulations and their associated functional status.

Results: Using SCENITH we investigated specific metabolic pattern of myeloid cells and their glucose dependence or fatty acid oxidation capacity. We also focused on detailed analysis of myeloid cells, their subsets and activation status by CD86, CD163, CD354 or HLA-DR, together with altered cytokine production. All markers were correlated to the severity of sepsis (SOFA score) to obtain comprehensive information associated also with clinical picture of patients.

Conclusion: In summary, we showed a detailed immunometabolic analysis of myeloid cells of pediatric septic patients together with a characterization of sepsis-altered cells' subset frequency and their ability to perform defensive processes.

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P1.08 BIOINFORMATICS AND IMMUNOLOGY

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509 – P1.08.02

A comprehensive immune single-cell atlas of (auto)immune-related diseases: exploring disease and treatment risks in cancer immune-checkpoint therapy

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Immune-checkpoint therapy (ICT) is one of the most successful treatments against cancer; however, some patients develop immune-related adverse events (irAEs) during the treatment, such as colitis or arthritis. Our study aims to identify immunotherapy-related disease and treatment signatures through the integration of a *Human immune single-cell atlas of (auto)immune-related diseases* comprising ICT, irAEs, and matching autoimmune-related single-cell transcriptomics data.

We built the atlas by integrating six publicly available scRNA-seq datasets, comprising cancer patients under ICT (82 patients; mostly melanoma), with and without irAEs (22 colitis and 8 arthritis patients), and autoimmune datasets matching the irAEs (51 patients). Disease and treatment signatures were assessed through pseudobulk differential gene expression analysis by cluster cell type.

Our human immune single-cell atlas of (auto)immune-related diseases comprises 484,446 cells, 54 clusters, and 27 harmonized cell types. The comparison between ICT-induced and spontaneous colitis signatures identified two cell clusters that shared significant genes (FDR<0.05): naive helper T cells and regulatory T cells. In the former, we identified a 4-gene signature that was upregulated in induced but downregulated in spontaneous colitis, whereas in the latter, a 3-gene signature upregulated in both was identified.

Our atlas can aid identification of disease and treatment signatures in ICT at the immune cell level.

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523 – P1.08.03

Towards deciphering the T cell receptor-antigen-HLA code with single cell genomics

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T cells can recognise a plethora of different antigens, presented on HLA molecules, through specific T cell receptors (TCRs) that are unique to individual cells. However, predicting the specificity of a given TCR from its genomic sequence currently presents a major bottleneck for improving our understanding of immune repertoires and developing data-driven approaches for immunotherapies. Recent technological advances in single-cell sequencing have enabled joint profiling of cellular transcriptomes with paired-chain TCR sequences, offering unprecedented insights into disease states and human TCR repertoires. However, interpretation of the data is not straightforward and existing computational tools only focus on individual analysis tasks, such as resolving TCR clones (e.g. Dandelion, MiXCR), sequence similarity-based clustering (e.g. GLIPH, tcrdist3), structural modelling (e.g. AlphaFold), or predicting antigen-HLA binding (e.g. netMHCpan). In order to build a more integrative analysis tool that can facilitate advances in TCR specificity prediction, we have developed Cell2TCR, an open-source package that segments paired-chain TCR sequencing data into functional groups called TCR motifs. Cell2TCR identifies convergent TCR motifs triggered by SARS-CoV-2 infection that are shared across different donors, multiple tissues, and strongly confined to distinct activated T cell states. We experimentally validate the specificity of these TCR motifs and provide compelling statistical evidence for their HLA-restriction. Cell2TCR offers integrated database querying capacities to infer putative antigen specificities for TCR motifs and can inform structural TCR modelling. We showcase the broad applicability of Cell2TCR in various disease contexts, such as cancer and autoimmunity. Overall, Cell2TCR represents a powerful and integrative analysis tool for single cell genomics that opens up new avenues for deciphering the TCR-antigen-HLA code.

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592 – P1.08.05

Identification of transcriptional regulatory networks that control variable human immune responses.

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There is a strong variability among individuals regarding responses to immune challenges. The “Milieu Intérieur” (MI) project was developed to assess the factors controlling immunity in a healthy population. To do this we established a cohort of 1,000 healthy individuals, balanced for age and sex, and from a homogenous genetic background. From this cohort we previously identified features such as age, sex, genetics, smoking behavior, cytomegalovirus serostatus (CMV) and socioeconomic status (SES) as important factors driving inter-individual differences in human immune response. To have a better understanding of the underlying molecular processes in this variation, transcriptomic data were generated for 200 individuals, aged 20 to 29 years and 60 to 69 years. This data set consists of bulk RNA-Seq data derived from whole blood samples, which were stimulated for 22h with diverse immune agonists including bacterial (LPS), viral (poly(I:C), gardiquimod, ODN and R848) and T-cell activator stimuli (SEB).

Differential analysis of the RNA-Seq data followed by dimension reduction with Regularized Generalized Canonical Correlation Analysis (RGCCA) identified groups of individuals with differential immune responses. Testing associations between these groups of individuals and the characteristics of donors revealed associations of gene expression profiles with the donors’ sex, age, smoking behavior, and cytomegalovirus (CMV) serostatus. Our work thus highlighted subgroups of individuals with different immunological outcomes based on their transcriptional profiles. These findings suggest a strong stimulus specificity in immune responses and also highlight the influences of socio-demographic and immunological factors on gene expression profiles.

Based on these subgroups of individuals we will next study differential transcriptional regulation induced by immune stimulations, by inferring transcriptional networks using tools such as CRCmapper, GRNboost2, or Genie3. Analysis of the differences in these regulatory networks between subgroups of individuals should provide a better understanding of the underlying immune processes and causal role of immune variability in determining disease risk and differential responses to treatments, for improved prevention and patient management strategies.

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713 – P1.08.06

Exploring systemic immune response and faecal microbiome associations in the Milieu Intérieur healthy donor cohort

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The gut microbiome has been shown to have a significant impact on immune responses in multiple models. However, many of these studies have been performed in disease states, when both the immune response and the microbiome are perturbed. Few studies have examined the impact of the microbiome on healthy immune states. We asked the question of how faecal microbiome and systemic immune responses are associated in a healthy adult human context: the Milieu Intérieur cohort, a genetically homogeneous cohort of 1,000 healthy donors of French ancestry, equally distributed between males and females and between the ages of 20 and 69.

To do this we first defined systemic immune response phenotypes by calculating gene expression scores after whole-blood stimulation with diverse microbes in the donors of the Milieu Intérieur cohort. To characterise the microbiome, we used shotgun sequencing datasets obtained from paired faecal samples. To test for significant associations, we applied either linear regression models or PERMANOVA, on microbiome and blood immune phenotypes, while correcting for potential confounders: age, sex, day of sampling, genetics, BMI, smoking, diet, and frequency of major immune cell components.

While we found no consistent significant correlations between immune scores and alpha diversity measurements, for beta diversity, we saw significant correlations with specific immune responses after Influenza, BCG, and *C. albicans* stimulation. Analysis at the taxa level identified significant associations between the relative abundance of Lactobacillales and Influenza-induced IFNγ, IL-1B, and TNF gene scores. An association was also found for TNF secretion at the protein level. In parallel, we observed significant associations between the relative abundance of Christensenellaceae and Poly:IC-induced IFN-I gene score, which was also found at the protein level for IFNα.

In summary, we identified significant associations between the relative abundance of specific faecal microbiome taxa with systemic immune responses. This included the identification of novel associations as well as confirmation of previously described associations in other contexts. Ongoing and future work will further dissect the immune pathways affected, and test specific hypotheses related to microbiome metabolite immune interactions.

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762 – P1.08.07

A unique human cord blood CD8+CD45RA+CD27+CD161+ T cell subset identified by flow cytometric data analysis using Seurat

Duan Ni[1], Julen Reyes[1], Brigitte Nanan[1], Gabriela Pinget[1], Lucie Kraftova[1], Thomas Ashhurst[1], Felix Marsh-wakefield[1], Claire Wishart[1], Jian Tan[1], Peter Hsu[1], Nicholas King[1], Laurence Macia[1], Ralph Nanan[1]

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Advances in single-cell level analytical techniques, especially cytometric approaches, have led to profound innovation in biomedical research, particularly in the field of clinical immunology. This has resulted in an expansion of high-dimensional data, posing great challenges for comprehensive and unbiased analysis. Conventional manual analysis is thus becoming untenable to handle these challenges. Furthermore, most newly developed computational methods lack flexibility and interoperability, hampering their accessibility and usability. Here, we adapted Seurat, an R package originally developed for single-cell RNA sequencing (scRNA-seq) analysis, for high-dimensional flow cytometric data analysis. Based on a 20-marker antibody panel and analyses of T cell profiles in both adult blood and cord blood, we showcased the robust capacity of Seurat in flow cytometric data analysis, which was further validated by Spectre, another high-dimensional cytometric data analysis package, and conventional manual analysis. Importantly, we identified a unique $CD8^+$ T cell population defined as $CD8^+CD45RA^+CD27^+CD161^+$ T cell, that was predominantly present in cord blood. We characterized its IFN-γ-producing and potential cytotoxic properties using flow cytometry experiments and scRNA-seq analysis from a published dataset. Collectively, we identified a unique human cord blood $CD8^+CD45RA^+CD27^+CD161^+$ T cell subset and demonstrated that Seurat, a widely used package for scRNA-seq analysis, possesses great potential to be repurposed for cytometric data analysis. This facilitates an unbiased and thorough interpretation of complicated high-dimensional data using a single analytical pipeline and opens a novel avenue for data-driven investigation in clinical immunology.

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916 – P1.08.08

Comprehensive post-translational modifications mapping of centrosomes in T cell activation

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Purpose: Studies have unveiled a crucial role for the centrosome in T cell activation and that the transition from quiescent to effector T cells is specifically regulated at the translational level. Post-translational modifications (PTMs) are now seen as an increasingly relevant target of investigation due to their great effect on the functional diversity of proteomes and their association to several pathological processes. Newly developed bioinformatics and statistical tools, such as "open search" engines and weighted spectrum, peptide, and protein (WSPP) models, allow the assessment of the proteome-wide variety of PTMs as well as their quantification. Our project aims to handle the unknown wide diversity of PTMs in the T cell centrosomal proteome from both a qualitative and a quantitative point of view using novel algorithms and software specifically developed for this type of -Omic data.

Methods: iTRAQ 4-plex LC-MS/MS data of resting and activated T lymphoblasts centrosome-enriched fractions obtained from thirty healthy donors were analysed. Results were validated in separate label-free T lymphoblast centrosome samples as well as in a TMT 10-plex T lymphoblast activation kinetics.

Results: This work describes the comprehensive T cell centrosome PTM map and assesses the differential PTM levels analysis, showing coordinated changes of specific modifications during T cell activation.

Conclusion: Altogether our results underscore PTMs as a potential regulatory element of the centrosome role in T cell activation.

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975 P1.08.09

Unraveling the autoimmune cellular and histological transitions of Sjögren's Syndrome

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Purpose: Autoimmune diseases are characterised by an immune response against self-antigens. This dysregulated response evolves as the disease progresses, and is dependent on a plethora of complex yet scarcely explored cellular interactions. Primary Sjögren's Syndrome (pSS) is an autoimmune disease that chiefly affects salivary and lacrimal glands, causing a deterioration of the tissue and its function. Over time, infiltrating immune cells organise into Tertiary Lymphoid Structures (TLS), which will contain active Germinal Centres responsible for the continued maturation and activation of self-reactive B cells. In a subset of patients, this potentiates the appearance of intraglandular B cell lymphoma. However, it is still unknown which alterations in infiltrating immune cells and the tissue milieu correlate with disease progression, and how they promote the appearance of this malignancy.

Methods: In order to reveal the varying cellular heterogeneity in the immune and non-immune populations of salivary glands, we recruited pSS patients at early (<2 years since diagnosis) and late (>10 years since diagnosis) disease stages, and performed high-throughput single-cell RNA-seq (scRNA-seq) on minor salivary gland biopsies, with paired blood samples.

Results: Using data integration, we identified increased infiltrating B and T cell proportions with a heightened immune activation correlating with disease progression. Furthermore, cell-cell communication analysis revealed crucial ligands produced by various stromal cell populations important for maintaining the TLS immune functions.

Conclusion: By identifying these early-to-late patterns in the immune cells infiltrating the salivary gland, we will be able to survey the patient's associated blood sample and assess which genes can be used as biomarkers to track disease progression in the clinic.

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1045 – P1.08.10

Blood immune cells of post-COVID-19 syndrome patients present with distinct alterations in their transcriptional states

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Purpose: Months after acute COVID-19 disease, a substantial proportion of patients suffer from long-term symptoms known as post-COVID-19 syndrome, which comprises a heterogeneous multitude of symptoms involving different organs. As a newly arising disease, the underlying pathomechanisms of post-COVID-19 syndrome are not resolved yet, but ongoing inflammation is discussed as one of the drivers of these long-term sequelae. Focusing on patients suffering from post-COVID-19 syndrome with persistent fatigue and exertion intolerance after mild to moderate acute disease, this study was designed to examine if there are transcriptional changes in circulating blood immune cells of these patients - at steady state or after ex vivo stimulation - that could help clinical diagnosis as well as guide the development of therapeutic strategies.

Methods: Single-cell RNA sequencing of blood immune cells from post-COVID patients and control donors, fully recovered from SARS-CoV-2 infection, was performed at steady state and after *ex vivo* whole blood stimulation.

Results: Peripheral blood mononuclear cells presented with distinct disease-associated transcriptional changes pointing towards an inflammatory state in the circulation of post-COVID-19 patients. Specifically, NK cell subpopulations characterized by cytokine production and a *FCGR3A* (CD16)-expressing cytotoxic T cell population, previously described in acute COVID-19 disease and shown to induce endothelial cell damage, were more abundant in post-COVID-19 patients. Further, monocytes displayed an inflammatory transcriptional profile in patients with post-COVID syndrome. Ex vivo stimulation revealed a comparable transcriptional response of monocytes from post-COVID-19 patients and recovered individuals to TLR4/TCR- and TLR3-stimulation, while the response to SARS-CoV-2 spike protein was heterogeneous among the post-COVID-19 patients especially in the expression of several chemokines.

Conclusion: This study provides insights into the systemic immunological alterations of post-COVID-19 patients during the disease's steady-state as well as under immune challenge, contributing to a better characterization of this disease paving the way for biomarker development and exploration of therapeutic strategies.

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1053 – P1.08.11

The dog as the immunologist's best friend for identifying potential genomic biomarkers for atopic dermatitis: an integrated bioinformatic analysis with translational potential.

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Purpose: Canine atopic dermatitis (AD) is a complex skin disease commonly seen in veterinary practice that resembles human AD, particularly in clinical and immunological aspects. AD may be influenced by multiple environmental and genetic factors in both species, with a strong breed predisposition described in dogs. Considering that the dog is the most suitable spontaneous model for several human genetic diseases, exploring the molecular mechanisms underlying canine AD also may contribute to better understand the pathogenesis of human AD. Thus, we aimed to identify shared genomic variants among dog breeds highly susceptible for AD, that also differ from breeds with the lowest incidence of the disease, as they might influence the pathogenesis of AD.

Methods: We integrated multiple bioinformatic approaches to analyse the whole-genome sequences of 171 purebred dogs from 14 AD-susceptible and 14 AD-protected breeds. Firstly, we applied the XP-EHH method, followed by a GWAS, functional enrichment analyses for GO terms related to biological processes and KEGG pathways and, finally, a SIFT-value estimation for predicting the effects of candidate variants on protein functions. Analyses were performed using PLINKv1.90 and Rv3.2.2. The threshold for statistical significance was set at p-value ≤0.05.

Results: We identified 11 genomic regions that significantly differed between susceptible and protected dog breeds and contained 32 candidate genes (e.g. *SOCS6, IL9, CD4, CD27, MAPK8, C1R, C1S,* among others) that were enriched in GO terms related to immune response. Furthermore, we detected a total of 549 variants predicted to have detrimental effects on protein function (SIFT-value ≤0.05) that were also located in these regions. Functional analyses revealed that candidate genes specifically participate in the following biological processes: complement activation via classical pathway, Th2 cell differentiation, humoral immune response mediated by circulating immunoglobulin, and PI3K-Akt signaling pathway, which is essential during inflammation, as it regulates the activation of neutrophils and mast cells, and promotes phagocytosis and cytokine production.

Conclusion: Genomic variants located within the candidate genes here identified are likely to play a key role in the immunopathogenesis of AD. Further studies are warranted to validate their potential use as biomarkers in human and veterinary clinical practice.

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1076 – P1.08.12

Computational stabilization and functional characterization of single-chain T cell receptors targeting a cancer epitope

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Advances in the development of targeted immunotherapy with T cell-based therapeutics have led to an increased interest in the T cell receptor (TCR) and its natural ligand, the peptide loaded major histocompatibility complex (pMHC). TCRs are heterodimeric proteins, which complicates handling in research and their application as therapeutics. For the structurally similar antibody Fab region, this problem has been solved by generating single-chain variable fragment (scFv). In return, a single-chain TCR (scTv) construct would be highly beneficial, combining the assets and flexible applications of scFv's and the specific TCR binding properties. Here, we apply rational protein design to increase stability of the highly unstable scTvs. Mutants were expressed *in vitro* and evaluated for stability as well as functionality.

To produce scTvs with increased stability compared to wild type, the known 1G4 (targeting NY-ESO1$_{157\text{-}165}$ peptide bound to HLA-A*0201) was chosen as a template. The scTv was rationally designed using ProteinMPNN, a deep learning-based method for the design of protein sequences (Dauparas J *et al.*, 2022), and Rosetta, a software-suite that allows for protein design with a physics- and statistics-based energy function (Kuhlman B, 2019). The resultant mutants were expressed in Expi293 cells. Functionality of the scTvs was evaluated *in vitro* using biolayer interferometry (BLI), determining scTv pMHC binding kinetics compared to wild type and TCR-like antibodies as a positive control.

Computational protein design of the chosen scTv led to improved expression rates for the tested constructs compared to wild type. A BLI assay was established and used to evaluate the kinetics of the pMHC-scTv interaction. The measured affinities of scTvs and control antibodies agreed well with K_D values known from literature, confirming the functionality of the stabilized scTvs. In conclusion, we introduced a pipeline for the computational stabilization and expression of pMHC-binders, which can then be tested for functionality in a robust BLI assay, thus facilitating the streamlined design and optimization of specific T-cell epitope binders for different pMHCs of interest.

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1084 – P1.08.13

A simple and robust bulk TCR sequencing-based method for identification of antigen-specific TCRαβ pairs

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Purpose: Despite progress in the recent years, there is still high need for simple, robust and easily multiplexible methods for identification of TCRs specific to antigens of interest, including identification of native TCRαβ pairs. In this study we aimed to develop a bulk TCR sequencing-based approach for antigen-specific TCR identification that exploits the ability of T cells to respond to antigenic stimuli by proliferation in vitro.

Methods: Peripheral blood mononuclear cells (PBMCs) isolated from healthy donor blood were stimulated with peptide mixes for 7 days in the conditions allowing for antigen-specific proliferation. Proliferation was monitored by the dye-dilution assay and flow cytometry. Following the cultivation stage, cells were lyzed and used for TCR repertoire library generation. Pre-cultivation CD4 and CD8 T cell repertoires were obtained from PBMCs magnetically enriched for the respective subsets. Subsequent bioinformatic analysis consisted of 1. identification of antigen-expanded clonotypes, 2. attribution of the identified clonotypes to the CD4 or CD8 subset, and 3. frequency correlation-based TCRαβ pairing.

Results: The described approach allows to identify expanded CD4 T cell clonotypes with initial frequency as low as 1 in 100000 cells, and CD8 T cell clonotypes with initial frequency of 1 in 10000 cells. For approximately 25% of TCRβ clonotypes, a corresponding TCRα paired clonotype could be identified. Comparison with single cell TCR sequencing data revealed 45% sensitivity and 71% specificity of our pairing approach. CD4/CD8 attribution of paired clonotypes further supported accuracy of this pairing approach, with no attribution conflicts observed for paired clonotypes.

Conclusion: We believe that the approach described hereby will further enable and simplify TCR discovery in various fields, including cancer immunology, autoimmune and infectious disease research.

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1159 – P1.08.14

Development of an User-Friendly Application for Automated Analysis of Skin Immune Landscape based on the Multiplex Annotated Tissue Imaging System technology.

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Purpose: The aim of the project is to develop an user-friendly interface to automate the analysis of 3D multiplexed images generated with Multiplex Annotated Tissue Imaging System (MANTIS) and explore complex immune environments in human tissue. MANTIS allows to quickly generate and explore 10 fluorescent signals acquired simultaneously in 3D using conventional confocal microscopes. This application aims to facilitate a comprehensive analysis of the skin immune landscape with integrated cell attribution, spatial analysis, and statistical comparisons.

Methods: The application was previously designed for automated phenotypic attribution based on selected combinations of biomarkers to identify immune subsets, while considering structural compartments (e.g. vessels, dermis or epidermis). Leveraging multiplexed 3-D imaging data and unsupervised bioinformatics, we developed an interactive data exploration pipeline. This includes graphical representations of cell counts, digital maps for spatial localization, and clustering analysis via t-distributed stochastic neighbor embedding (t-SNE). Additionally, it facilitates spatial visualization of biomarker expression, heatmap generation for biomarker intensity, automatic identification of regions of interest (ROI) using an alpha shape algorithm, calculation of cell-to-distances and statistical comparisons between conditions. Analysis can be performed per sample, per donor, or per cohort. We analyzed and compared acral lesions from patients with systemic lupus erythematosus, Kawasaki syndrome, or COVID-19–associated skin manifestations.

Results: By integrating advanced analytical tools into our user-friendly interface, we observed that severe pathological lesions from systemic lupus erythematosus, Kawasaki syndrome, or COVID-19–associated skin manifestations shared common quantitative immune features while displaying a nonrandom distribution of cells with the formation of disease-specific dermal immune structures. Notably, COVID-19-associated acral lesions were preferentially enriched in CD8 $CD57^{high}$ T cells, a subset previously described to damage tissues during viral infections, that heavily infiltrated epidermal layers.

Conclusion: The MANTIS user-friendly application enabled to combine the power of automated processing and interactive visualization tools, to offer a comprehensive solution for exploring, understanding, and interpreting complex immune environments in the skin.

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1188 – P1.08.15

Glycosyltransferase and glycosidase changes in Natural Killer (NK) cells after cytokine stimulation; a bioinformatic analysis

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Purpose: Protein glycosylation is a ubiquitous post-translational modification occurring in the endoplasmic reticulum (ER) and Golgi apparatus. The types and frequency of protein N-glycosylation is modified in different cellular states and directly influences the functional properties of cellular proteins. Natural killer (NK) cells are innate cytotoxic lymphocytes with important anti-tumour functions, however, the role that glycosylation plays in the function of NK cells is poorly understood. Glycan profiles are studied through the expression and activity of biosynthetic enzymes, namely glycosyltransferases and glycosidases. This study aims to define the changes in expression of glycosylation enzymes before and after cytokine stimulation of *ex vivo* populations of NK cells.

Methods: Next generation RNA sequencing datasets of cytokine stimulated human NK cells were identified and selected for suitability from the genome expression omnibus (GEO) data repository. Utilising the Galaxy EU web platform fastq files were aligned to the Human Reference Genome hg38 by HISAT2. Read counts were determined by *htseq-count* in reference to hg38 annotated features. Differential gene expression was examined using the DESEQ2 tool. From the data, read counts of glycan biosynthetic enzyme transcripts were collated and statistically analysed.

Results: A total of nine datasets compared unstimulated NK cells to NK cells following 12-72 hours of cytokine stimulation. The results showed significant upregulation of enzymes involved in early biosynthesis of glycan core structures and transfer to proteins prior to folding in the ER, suggesting increased demand for glycan synthesis after NK activation. Mannosidases trim mannose moieties from initial high mannose templates, as glycoproteins move from the ER to Golgi apparatus. These enzymes regulate the transition to multi-antennary complex and hybrid glycans. Differential expression hints at increased production of hybrid glycans. The remaining enzyme changes indicate increased poly-N-acetyllactosamine (LAcNAc) chain formation, fostering the formation of ABO and Lewis antigens, and serving as ligands for galectin carbohydrate-binding proteins (CBP). The CBP family has been shown to increase activation of innate immune responses and induce proinflammatory cytokine production from monocytes.

Conclusion: Our results show that upon cytokine stimulation, glycan biosynthesis increases, with a trend towards LacNac chain formation, possibly enhancing galectin binding activity.

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1205 – P1.08.16

Genetic signature for early prediction of anti-TNF response in Rheumatoid Arthritis Patients

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Rheumatoid arthritis (RA) is a chronic inflammatory disease affecting approximately 0.5-1% of the global population, being higher in females. It is characterized by synovial joint inflammation and destruction, progressive disability, and reduced quality of life. In fact, 80% of insufficient treated patients develops misaligned joints, and half of them are unable to work within ten years after the diagnosis.

Anti-TNF agents have revolutionized the treatment of RA, significantly improving clinical outcomes. However, almost 30% of treated patients show an inadequate response and reliable response predictors are not described until now. Therefore, the aim of our study was the determination of biomarkers useful in the prediction of the response to anti-TNF therapy in RA patients. Here, we hypothesized that transcriptomic data could serve as a prognostic and predictive biomarker in RA patients under anti-TNF. Indeed, we reported a transcriptomic signature with high capacity to predict the response to anti-TNF treatment in patients with RA, before its initiation.

We perform a retrospective analysis of whole peripheral blood mononuclear cells (PBMC) transcriptomic profiling of patients with RA, to define the small-scale signature genes involved in the response to anti-TNF treatment. We carry out differential gene expression analysis and ranked the most discriminatory genes to establish a small-scale genetic signature.

Based on the initial analysis of RNA-Seq results, 53 differentially expressed genes were identified between responders (R) and non-responders (NR) and the selection of the 18 most discriminatory genes reached a predictive accuracy of 88.75%When we evaluated the minimal number of genes with relevant discriminatory power using ROC analyses, we stated a predictive model composed of 6 genes that effectively discriminate R and NR, with an area under the curve (AUC) of 0.81.

Our 6-gene genetic signature could be used as a method to distinguish between R and NR to anti-TNF treatments and therefore, to predict the response of RA patients to these therapies.

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1602 – P1.08.17

Employing single-cell RNA sequencing dataset analysis to probe the functional and metabolic state of NK cells infiltrating Glioblastoma tumour sites, and comparing them to the transcriptomic profiles of circulating NK cells derived from healthy donor datasets

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Purpose: Glioblastoma Multiforme (GBM) is the most common and aggressive type of brain cancer in adults, with a poor 5-year survival rate. A prominent contributing factor to this poor prognosis is the hostile tumour microenvironment (TME) of GBM, that induces immune failure and impedes efficacy of immunotherapies. Therefore, it is vital to characterise the epigenetic changes that tumour infiltrating NK Cells (TINKs) undergo to become dysfunctional. As innate responders to cancer, illustrating the differences in gene expression of healthy circulating NK cells and TINKs may be insightful for underlining the fundamental factors of NK cell dysfunction immune failure in GBM. Utilising single cell RNA-seq compiled from multiple datasets can be highly informative for exploring these questions. We hypothesised that GBM TINKs convey have dysregulated gene expressions that are reflected both in function and metabolism.

Methods: Data used in this bioinformatic research was sourced from the pre-published work of Netskar et al., 2023. This preliminary paper integrated data from over 40 single-cell and bulk datasets, sourced from cancer patients and healthy donors, into a ready-to-use, open access data resource. From this, we specifically focused on examining NK cell transcriptome profiles from healthy and GBM patient datasets.

Results: The resultant work highlighted the altered transcriptomic profiles of TINKs compared to Healthy peripheral NKs. This included upregulated gene signature for reactive oxygen species (ROS) and associated mitochondrial complexes - sources of ROS in TINKs. Further alterations of gene signatures implicated in NK Cell dysfunction included TGF-Beta signalling pathway and ER stress etc. Finally, we modelled specific metabolic and cellular signalling pathways that could help explain TINK dysfunction in GBM.

Conclusion: Our data provides an insightful perspective to understanding the TME's impact on NK cell dysfunction. The findings produced from this work can inform practical approaches to examining the immune challenges of GBM and help in the design NK cell immunotherapies.

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1640 – P1.08.19

Integrated analysis of single-cell and bulk RNA sequencing data reveals a memory-like Natural Killer cell subset associated with Mycobacterium tuberculosis latency

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Natural killer (NK) cells are innate-like lymphocytes that belong to the family of type-1 innate lymphoid cells and rapidly respond to virus-infected and tumor cells. In this study, we have combined scRNA-seq data and bulk RNA-seq data to define phenotypic and molecular characteristics of peripheral blood NK cells. While the role of NK cells in immune surveillance against virus infections and tumors has been well established, their contribution to protective responses to other intracellular microorganisms, such as *Mycobacterium tuberculosis* (Mtb) is still poorly understood. In this study we have combined scRNA-seq data and bulk RNA-seq data to illuminate molecular characteristics of circulating NK cells in patients with active tuberculosis (TB) disease and subjects with latent *Mycobacterium tuberculosis* infections (LTBI) and compared these characteristics with those of healthy donors and patients with non-TB other pulmonary infectious diseases and have validated relevant results by flow cytometry on a small cohort of subjects. We show here that LTBI subjects have a significant expansion of NK cells characterized by the prevalence of a memory-like $CD52^{+}NKG2C^{+}$ NK cells. Altogether, our results provide some new information on the role of NK cells in protective immune responses to Mtb.

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1720 – P1.08.20

Identification of relevant tissue-specific factors regarding the formation and maintenance of human tertiary lymphoid structures

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Purpose: Immune cell infiltration can lead to the formation of Tertiary Lymphoid Structures (TLS), composed of cells highly engaged in antigen presentation, T cell activation, and B cell maturation in Germinal Centre-like structures. The presence of such structures in disease-afflicted tissues significantly shapes the prognosis of multiple autoimmune diseases and cancers.

We aim to identify human tissue-specific factors, cell-cell interactions, cell types, and cell states taking part in the formation and maintenance of TLS. Besides gaining a better insight into the poorly explored biology of TLS in a tissue-specific context, we seek to possibly establish a starting point for diagnostic (patient stratification) and therapeutic approaches.

Methods: Single-cell RNA sequencing data of autoimmune diseases from various tissues were collected and analysed to obtain a cell type and state census for each tissue. Cell-cell interaction inference methods were used to predict general and tissue-specific signalling events related to TLS. The relevance of these genes is then assessed by comparison with disease-specific databases.

Results: A collection of annotated cell types and interactions, associated with different tissues and autoimmune diseases.

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1961 – P1.08.22

Human DNA adaptome: the unbiased multiplex PCR-based profiling of the whole set of TCR and BCR chains at the DNA level

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Sequencing of T and B-cell receptor (TCR and BCR) genes is an insightful practice in basic and applied immunological research that indicates normal and pathological conditions. Here, we present the first-ever ultra-multiplex DNA-based PCR system designed to simultaneously analyze all TCR chains (TRA, TRB, TRG, TRD) and all BCR chains (IGH, IGK, IGL) in a single assay.

The study analyzed DNA from the venous blood of 40 healthy donors and bone marrow of 10 patients with B-cell leukemia before and after therapy. The sequencing data was processed using MiXCR software.

The actual DNA adaptome assay includes multiplex primers annealing to V- and J-gene regions, and upstream of D-gene regions. To reduce PCR bias, primer concentration is optimized using a new computational approach based on the ratio of weighted and unweighted frequencies of V, D, and J genes among nonproductive TCR and BCR rearrangements. The results obtained for normal samples show the ability to identify all functional V and J genes in productive (in-frame) and nonproductive (out-of-frame), partial (D-J, V-D) and complete (V-D-J and V-J) rearrangements in TCR and BCR loci. In fact, we detected 47 TRAV, 59 TRAJ, 56 TRBV, 13 TRBJ, 12 TRGV, 5 TRGJ, 8 TRD, 4 TRDJ, 63 IGHV, 6 IGHJ, 46 IGKV, 5 IGKJ, 44 IGLV, 5 IGLJ genes across the analyzed samples. Productive rearrangements have been presented in samples in ratios proximal to physiological. Tested DNA load from 100 pg (15 cells) to 400 ng (60 000 cells) per 25 uL assay shows flexibility and sensitivity suitable for most applications. Applying adaptome assay for leukemic samples, we identified abnormally expanded clonotypes (rearrangements) in onset time points. Using these clonotypes as biomarkers for Measurable Residual Disease we were able to detect the presence of remaining leukemic cells in patient samples after therapy.

The presented adaptome assay is characterized by low time consumption and a high level of simplicity in use, which prevents human errors and allows automatization. It allows the extraction of maximum information from the analyzed sample with high sensitivity and precision, required for research and diagnostics.

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1983 – P1.08.23

VISH-Pred: An ensemble of fine-tuned ESM models for protein toxicity prediction

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Purpose: Peptide and protein based therapeutics are becoming a promising treatment regimen for myriad diseases. Toxicity of proteins is the primary hurdle for protein-based therapies. Thus, there is a need for accurate *in silico* methods for determining toxic proteins to filter the pool of potential candidates.

Methods: To address this challenge, we proposed an ensemble framework, called VISH-Pred, comprising models built by fine-tuning ESM2 transformer models on a experimentally validated and curated large dataset of protein and peptide toxicities. The primary steps in VISH-Pred framework is to efficiently estimate protein toxicities taking just the protein sequence as input, employing an under sampling technique to handle the humongous class-imbalance in the data, and learning representations from fine-tuned ESM2 protein language models which are then fed to machine learning techniques such as Lightgbm and XGBoost.

Results: The VISH-Pred framework is able to correctly identify both peptides/proteins with potential toxicity and non-toxic proteins, achieving an MCC of 0.737, 0.716 and 0.322 and F1-score of 0.759, 0.696, and 0.713 on three non-redundant blind tests respectively, outperforming other methods by over 10% on these quality metrics. Moreover, VISH-Pred achieved the best accuracy and AUC scores on these independent test sets, highlighting the robustness and generalization capability of the framework.

Conclusion: By making VISH-Pred available as an easy-to-use web-server, we expect it will serve as a valuable asset for upcoming endeavors aimed at discerning the toxicity of peptides, enabling efficient protein-based therapeutics.

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2263 – P1.08.25

Multi-omic single cell analysis reveals immune cell perturbations in sepsis

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Purpose: Sepsis is a life-threatening condition that is characterised by an abherrent immune response to trauma or infection. Throughout the course of sepsis, the function and diversity of peripheral blood mononuclear cells (PBMCs) become perturbed. These alterations persist post recovery from sepsis, however many of those who survive experience long term sequelae. Understanding these alterations may identify prognostic and potential therapeutic markers to help reduce the burden of this condition.

Methods: This study used a multi-omic approach involving the BD Rhapsody™ single cell sequencing pipeline, utilising PBMCs from people with sepsis, bacteraemia without sepsis and 'healthy' controls. We leveraged the concurrent measurement of transcriptome and surface protein expression to identify and characterise PBMC populations.

Results: Using this multi-omic approach, we identified 18 distinct cell types, including 14 T cell populations, 1 NK cell and 3 B cell populations using the surface protein expression of conventional and phenotypic markers. Among the T cell populations, we detected a population of *CD69*+ naïve T CD4+ T cells via their expression of naïve markers CD45RA and CD197 surface protein and *CD69* gene expression. Interestingly, this population of T cells was present at higher proportions (1.99 log2 FC) in people with sepsis compared to the control group. Other increased PBMC populations in the sepsis group included Th17 (0.99 log2 FC), Treg (0.99 log2 FC), Th2 (0.75 log2 FC) and Th1 (0.7 log2 FC) cells compared to controls. Conversely, when compared to both the control and bacteraemia groups, sepsis samples presented with significantly decreased proportions of cytotoxic cell subsets, including NK cells (-2.19 & -0.61 log2FC *vs* control and bacteraemia respectively), CD56+ T cells (-2.13 & -2.17 log2 FC), terminally differentiated CD8+ T cells (-1.27 & -3.23 log2 FC), and γδ T cells (-0.74 & -0.83 log2 FC).

Conclusion: Overall, these results highlight the value of using a multi-omic approach to studying immune cell populations and highlight the proportional perturbations to functional immune cell subsets in sepsis.

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P1.09 CANCER IMMUNOTHERAPY

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51 – P1.09.01

Programmed Salmonella blocks cancer metastasis by activating NK cells in an IFN-γ-dependent manner

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Purpose: To investigate the potential of bacterial cancer therapies, specifically attenuated Salmonella typhimurium strain YB1, as a novel approach for evoking anti-tumor immune responses and suppressing metastasis in various cancer types.

Methods: We utilized an attenuated Salmonella typhimurium strain YB1 engineered by our lab to study its effect on cancer metastasis. We analyzed the induced cytokines, identified the indispensable factors, and used CyTOF analysis and antibody-mediated cell depletion to determine the major immune cells involved in Salmonella-provoked metastasis suppression.

Results: Our study revealed that attenuated Salmonella effectively suppressed cancer metastasis, regardless of cancer types and genetic background, by evoking strong anti-metastatic immune responses. We identified IFN-γ as an indispensable factor for inhibiting cancer metastasis, and NK cells as the major immune cell type responsible for Salmonella-induced metastasis suppression. We found that IFN-γ was primarily produced by NK cells during early Salmonella infection, which in turn promoted the accumulation, activation, and cytotoxicity of NK cells.

Conclusion: Attenuated Salmonella typhimurium strain YB1 has a potent suppressive effect on cancer metastasis by evoking strong anti-metastatic immune responses, primarily through IFN-γ-dependent NK cells. This bacterial cancer therapy represents a promising alternative approach for targeting solid tumors and warrants further investigation for potential clinical applications in cancer treatment.

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52 – P1.09.02

Ex vivo modeling of precision immuno-oncology responses in lung cancer

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Immunotherapy has changed the treatment landscape in some cancers and even led to favorable outcomes in previously incurable cancer types. Despite the potential benefits, only a fraction of patients respond to current immuno-oncology (IO) treatments. Lack of predictive biomarkers, unknown mechanisms of immune resistance, complex tumor-immune interactions, and the understudied role of the tumor microenvironment pose significant challenges to the use of IO drugs. Moreover, predicting which patients would benefit from the expensive new treatments remains a significant challenge.

To address these challenges, we have developed a personalized ex vivo tumor model, which can be used to screen for immunotherapy responses and discover immunotherapy biomarkers in lung cancer. We are doing so by first stimulating the patient's immune cells with autologous tumor organoids. This is followed by a tumor-killing assay utilizing the stimulated immune cells with or without different immunotherapy combinations. Lastly, we single-cell sequence different treatment conditions to reveal changes occurring to the immune cells after their interactions with the tumor organoids.

By using this set-up, we were able to model lung cancer anti-PD-1 (Nivolumab) primary resistance ex vivo, and identify individual responders to anti-PD-1 combined with chemotherapy agents. In addition, we have discovered a novel IO-drug combination of anti-PD-1 and another immune checkpoint inhibitor that was able to overcome Nivolumab resistance in some patients. Furthermore, we have identified potential biomarkers for both combination resistance and response, which will be further validated in the future.

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94 – P1.09.03

CD40 agonist affects maintenance of B-cell acute lymphoblastic leukemia in patient-derived xenograft mouse models

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Purpose: We investigated the effect of a CD40 agonist antibody on B-cell acute lymphoblastic leukemia (B-ALL) using patient-derived xenograft (PDX) mouse models. Specifically, the study aimed to assess the impact of CD40 stimulation on B-ALL cell proliferation, signaling pathways, apoptosis induction, and leukemia-initiating cell frequency.

Methods: Patient-derived xenograft (PDX) mouse models were utilized to study the effect of a CD40 agonist antibody on B-ALL. Intravenous injection of the CD40 agonist antibody was administered to the PDX mice. Various assays were employed to evaluate the effects of CD40 stimulation, including RNAseq analysis, western blotting, flow cytometry, and proteomic analysis. Specific parameters assessed included alterations in cell proliferation, activation of signaling pathways, apoptosis induction, cell cycle progression, and changes in protein expression profiles.

Results: The i.v. injection of the CD40 agonist significantly altered the proliferation and growth of B-ALL cells in vivo. CD40 stimulation rapidly activated the ERK1/2 pathway in B-ALL cells. Flow cytometry analysis demonstrated that the CD40 agonist induced apoptosis and affected the cell cycle and proliferation of B-ALL cells in PDX mice. Proteomic analysis revealed alterations in proteins associated with B-cell receptor signaling, the mitotic cell cycle, and homeostatic processes, suggesting significant impacts on crucial signaling pathways involved in B-ALL cell expansion and maintenance. Administration of the CD40 agonist profoundly altered the frequency of leukemia-initiating cells and the development of leukemia in PDX mice.

Conclusion: The study findings indicate that CD40 agonists represent a promising immunotherapeutic strategy for the treatment of pediatric B-ALL. These preclinical results provide a basis for future clinical trials aimed at evaluating the efficacy of CD40 agonists in the treatment of B-ALL, offering potential avenues for improving patient outcomes in this disease.

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135 – P1.09.04

Fractalkine: a chemokine with pro-tumourigenic effects in oesophageal adenocarcinoma that can be attenuated by CX3CR1 modulation.

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Purpose: Oesophageal adenocarcinoma (OAC) is a poor prognosis cancer with a 5-year survival rate of less than 25%. Current response rates to chemo-radiotherapy are only ~30% and new therapeutics are urgently needed. The pro-inflammatory chemokine fractalkine plays a role in the disruption of anti-tumour immunity in OAC via its recruitment of Natural Killer (NK) cells to the visceral adipose tissue (VAT). Furthermore, our group have shown that CX3CR1 modulator E6130 can redirect NK cells away from chemotactic cues of OAC omentum and towards tumour. Here, we propose that fractalkine may also directly promote OAC tumour growth and that E6130 can attenuate this effect.

Methods: OAC cell line OE33, metastatic OAC cell line FLO-1 and FLO-1^{LM}, OAC radiosensitive OE33P and radioresistant OE33R were pre-treated with CX3CR1 modulator E6130 (0.1-1000nM) and/or treated with recombinant fractalkine (0.1-200ng/mL) for 24 hours. Cell viability and proliferation was assessed using CCK8 and BrdU assays. Expression of epithelial-to-mesenchymal transition markers SLUG, Vimentin and E-cadherin was assessed by flow cytometry. Tumour samples from OAC patients were treated with E6130 and LDH release was measured to assess for tumouricidal activity and expression levels of IFN-y and IL-2 were assessed by ELISA.

Results: Fractalkine significantly increased OE33, FLO-1, FLO-1^{LM}, OE33P and OE33R cell viability and proliferation. Pre-treatment with E6130 attenuated these effects in all cell lines. Fractalkine elicited no significant changes in SLUG, Vimentin and E-cadherin expression in OE33, FLO-1 and FLO-1^{LM} cells, suggesting limited effect in tumour cell metastasis. Furthermore, E6130 elicited tumouricidal effects on OAC patient tumour, while decreasing IFN-y and increasing IL-2 release.

Conclusion: Our data show that fractalkine significantly increases OAC tumour cell survival and proliferation and that E6130 can significantly attenuate such increases. E6130 reduces IFN-y in line with fractalkine's induction of this cytokine in previous studies, but also increases IL-2 suggesting that this drug may elicit activation of NK cells in the TME. Future *in vitro* and *in vivo* studies will further elucidate the role of fractalkine in OAC tumour growth, resistance to cell death, inflammation, metastasis and metabolism, and confirm the therapeutic utility of CX3CR1 modulation in this hard-to-treat cancer.

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157 – P1.09.05

ANTI-TUMORAL POTENTIAL OF THE NEW GRANULYSIN-BASED AND Tn/MUC-1-TARGETED 5E5GRNLY INMUNOTOXIN

Ana Pilar Tobajas[1;2], Raquel Ibáñez-Pérez[1;2], Patricia Guerrero-Ochoa[1;2], Ruth Soler-Agesta[1;2], Evelyn Galano-Frutos[1;2], Laura Cambronero[1;2], Blanca Conde[1;2], Eva Barrio[1;2], Ramón Hurtado-Guerrero[1;3], Alberto Anel[1;2]

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Purpouse: 5E5GRNLY is an immunotoxin composed by granulysin (GRNLY), a human protein with cytolytic activity against microbes and tumors present in the granules of human CTLs and NK cells, and a single-chain fragment variable (scFv) derived from the mAb 5E5 directed against the Tn antigen, generated by the aberrant glycosylation of the MUC-1 membrane protein in several types of cancer.

Methods: 1) Recombinant GRNLY and 5E5GRNLY were produced in *Pichia pastoris* and purified by affinity chromatography. 2) Immunotoxin binding on the surface of cells expressing Tn antigen were demonstrated and quantified by flow cytometry. 3) Cell death induced on human tumor cell lines by GRNLY or 5E5GRNLY was assessed by quantifying Annexin-V-FITC binding to phosphatidylserine and/or 7-AAD to DNA by flow cytometry. 4) The *in vivo* anti-tumor capacity of 5E5GRNLY or GRNLY was tested in athymic mice xenotransplanted with the Tn-positive multiple myeloma H929.

Results: The new granulysin-based anti-Tn immunotoxin 5E5GRNLY was produced in *Pichia pastoris* and purified with good yield. 5E5GRNLY specifically bound to cancer cells expressing the Tn-MUC1 antigen, such as the acute lymphoblastic leukemia Jurkat, the multiple myeloma H929 or the pancreatic adenocarcinoma Capan-2. 5E5GRNLY exerted cytotoxicity against these cell lines, reducing the IC50 of GRNLY at least 2.5-fold. Finally, *in vivo* experiments based on the systemic injection of GRNLY or 5E5GRNLY in athymic mice xenotransplanted with H929 cells showed that both GRNLY and the immunotoxin efficiently inhibited the development of this tumor.

Conclusion. 5E5GRNLY augments the cytotoxicity of GRNLY on cells that express the Tn-MUC1 antigen and is also effective *in vivo*. We intend now to test 5E5GRNLY in another *in vivo* model using Capan-2 cells, which are less sensitive to GRNLY than H929 cells, and which shows a higher *in vitro* improvement of the IC50 by the immunotoxin.

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160 – P1.09.06

Combination of the granulysin-based and CEA-targeted MFE23GRNLY immunotoxin with a BCLXL-selective BH3-mimetic in a lung cancer model

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MFE23GRNLY is an immunotoxin composed by the scFv anti-carcinoembryonic antigen (CEA) MFE23 fused to granulysin (GRNLY), a human protein with cytolytic activity against microbes and tumors present in the granules of human CTLs and NK cells. It has shown specific tumor targeting activity and potent anti-tumor effect after systemic administration in preclinical tumor models. Here, we explore MFE23GRNLY combination with a BH3-mimetic selective for the anti-apoptotic protein Bcl-x_L in order to increase its therapeutic potential *in vitro* and *in vivo*. MFE23GRNLY was produced in *Pichia pastoris* and purified by affinity chromatography as previously described. Cell death induced on human tumor cell lines by MFE23GRNLY alone or in combination with the Bcl-x_L-selective BH3 mimetic drug A-1155463 was assessed by flow cytometry, quantifying Annexin-V-FITC binding to phosphatidylserine and/or 7-AAD to DNA. We had previously demonstrated in other tumor cells that MFE23GRNLY induced cell death through activation of the mitochondrial apoptotic pathway, resulting finally in caspase activation and cell dismantling. However, when testing CEA-positive A549 lung adenocarcinoma cells, the general caspase inhibitor Z-VAD-fmk did not inhibit cell death induced by the MFE23GRNLY immunotoxin *in vitro*, in contrast to results obtained in other cell lines. This result correlated with a high level of expression of the anti-apoptotic protein Bcl-x_L in this cell line and we hypothesized that combination of MFE23GRNLY with the Bcl-x_L-selective BH3 mimetic A-1155463 could have a synergic effect, something that was clearly demonstrated in *in vitro* experiments using the methodology outlined above. Since we had previously demonstrated that MFE23GRNLY alone was able to inhibit by 70% the development of this tumor in athymic mice after systemic injection, we have now initiated an *in vivo* experiment in this mice model to test the anti-tumoral potential of its combination with the BH3 mimetic A-1155463.

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260 – P1.09.07

Effect of the HLA-G/ILT2 pathway as an immune system checkpoint in gastric cancer

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Introduction: Approximately 80-85% of patients do not benefit from conventional Immune Checkpoint Inhibitor (ICI) therapies (anti-PD1/PDL1/CTLA4) in the context of gastric cancer. Therefore, identifying new potentially treatable immunosuppressive pathways is crucial for the development of innovative immunotherapy strategies in gastric cancer.

The HLA-G/ILT2 pathway plays a role in immunosuppression in both physiological contexts (maternal-fetal tolerance) and pathological conditions such as cancer. The interaction between HLA-G and the ILT2 receptor promotes the activation of inhibitory phosphatases (SHP1/2) that block the immune response mediated by the T-cell receptor (TCR).

Objectives: The aim of this study is to investigate the presence of HLA-G and its receptors in patients with gastric cancer and its effect on the activation of T lymphocytes, in order to open new therapeutic possibilities for these patients.

Results: Using multiparametric flow cytometry, we observed an increase in the ILT2+ cell population in the peripheral blood of patients with gastric cancer compared to a control cohort, both in total T lymphocytes (27.5% vs. 5.6%, $p<0.0001$) and more significantly in the CD8 T lymphocyte population (49.0% vs. 11.1%, $p<0.0001$). Furthermore, we identified elevated expression of HLA-G and ILT2 in the tumor tissues of patients (immunohistochemistry). Using HLA-G (+) and HLA-G (-) tumor cells, we formed conjugates with CD8 T lymphocytes from patients with gastric cancer. No defects were found in the formation of conjugates with HLA-G (+) cells (40.7±10.1%) compared to HLA-G (-) cells (44.4±6.7%, $p>0.05$). Immune synapse formation assessed by confocal microscopy showed that CD8 T lymphocytes were capable of generating activating synapses with HLA-G (+) cells (60.8±3.8%) and degranulating perforin and granzyme (45.3±12.1% and 57.5±22.5%) similarly to HLA-G (-) cells (66.1±6.2%, 49.4±20.9%, 57.9±18.7%, $p>0.05$). However, co-cultures of ILT2+ T lymphocytes with HLA-G (+) cells reduced its activation capacity upon TCR stimulation, measured by IFNy production (paired T-test $p=0.0006$) and proliferation (10.0±13.4% vs. 24.6±17.0%, $p<0.0001$) compared to co-cultures with HLA-G (-) cells.

Conclusion: Molecules in the HLA-G/ILT2 pathway are overexpressed in patients with gastric cancer. HLA-G does not prevent the formation of tumor-T lymphocyte conjugates but inhibits the activation of ILT2+ CD8 T lymphocytes, becoming a potential new immune checkpoint.

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317 – P1.09.08

UniCAR T-cell potency – A matter of affinity?

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Over the past decades, there has been a significant focus on reprogramming immune cells for targeting tumor cells, with Chimeric Antigen Receptor (CAR) T-cells emerging as a highly promising technology. Despite remarkable efficacy in hematologic malignancies, CAR T-cell therapy can cause severe to life-threatening adverse events. To address these safety issues, we and others have developed adaptor CAR platforms, such as the Universal CAR (UniCAR) system. In the UniCAR system, T-cells are engineered to express a UniCAR directed to the non-tumoral peptide epitope E5B9 derived from the nuclear La protein. UniCAR T-cell activation relies on the presence of a Target Module (TM), a bridging molecule that contains the E5B9 epitope and a binding moiety that specifically recognizes antigens on the tumor cells. The redirection of the UniCAR T-cells to target cells thus involves two interactions with different affinities, between (i) the TM and the CAR T-cell or (ii) the TM and the target cell.

So far, little is known about how the affinity between adaptor molecules and adaptor CAR T-cells impacts their functionality. In the case of the UniCAR platform, the interaction between the UniCAR and the TM can easily be tuned by engineering the amino acid sequence of the E5B9 peptide, without the need to re-modify the CAR.

Here, we investigate whether and how the amino acid sequence of the UniCAR epitope, including the amino acids flanking the E5B9 epitope in the native La protein, may affect the interaction between a UniCAR TM and UniCAR T-cells. By engineering the E5B9 peptide in previously published UniCAR TMs directed to the Fibroblast Activation Protein (FAP), we assess whether the modified E5B9 epitope (named E5B9L) affect the potency of UniCAR T-cells.

Overall, our work indicates that the binding affinity between the TM and the UniCAR T-cells does not play a crucial role. This affinity can fluctuate across a broad spectrum, ranging from low picomolar to nanomolar values, without significantly impacting the functionality and/or potency of the UniCAR T-cells.

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382 – P1.09.09

Development Of Patient-Derived 3D Tumor Organoids Combined With Immune Cells, As Preclinical Testing Platform For Personalized Cancer Immunotherapy

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Currently available models to validate preclinical efficiency of Immunotherapies lack in mirroring or poorly recapitulate the original patient's tumor. Active immunotherapy, which is dependent on HLA-TCR interaction, can only be tested in fully human based *in vitro* systems or humanized mice (complex, expensive, and time-consuming). Therefore, we need to develop more sophisticated systems to address immunotherapies correct mode of actions in humans.

Successful in recalling the tumor heterogeneity and complexity *in vitro*, Patient-Derived Tumor Organoids (PDTOs) can provide a robust *ex vivo* system for modelling human cancer, holding great potential as pre-clinical human platform for testing personalized therapies.

My work is focused on combining patient-derived immune cells and PDTOs to develop complex immuno-organoids that retain both patient's tumor unique features and immune system-mediated response. This allows to test tumor cell killing by the patient's own immune system after a personalized immunotherapy treatment.

Moreover, after in-depth patient´s tumor characterization, we design a personalized immunotherapy approach to specifically target the patient´s tumor.

Oncolytic vaccines have a dual mechanism: direct oncolysis of cancer cells and release of tumor antigens, causing cytotoxic T cell activation against cancer cells. To develop patient-specific oncolytic vaccine-based immunotherapies, our strategy is coating oncolytic vaccines with patient-specific tumor peptides (PeptiCRAd technology).

We established PDTOs from Renal Cell Carcinoma and Bladder Cancer, which we characterized by DNA sequencing, immunofluorescence, drug screens. HLA ligandome analysis of the PDTOs allowed the identification of 52 patient-specific tumor peptides. We validated the immunogenicity of these peptides by stimulating healthy donor Peripheral Blood Mononuclear Cells (PBMCs) and analyzing IFN-gamma secretion or CD107a degranulation. We will also use patient-derived PBMCs to unravel the best candidate peptides able to recall a T cell antitumor response. Finally, we will investigate how an oncolytic vaccine decorated with patient-specific tumor peptides (personalized PeptiCRAd) is able to trigger tumor-specific adaptive immune response, measuring specific T cell killing of PDTOs with impedance-based Xcelligence system, LDH release and live cell microscopy.

Establishing such a pre-clinical human model will pave the way for testing patient-specific responses, developing individualized treatments and accelerate their track to the clinic.

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389 – P1.09.10

Unveiling the potential of oncolytic virotherapy in inducing tertiary lymphoid structures for enhanced anti-tumor responses

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Tertiary lymphoid structures (TLS) are organized clusters of immune cells that develop in non-lymphoid tissues in response to chronic inflammation. Mature TLS, resembling lymph nodes, correlate with favorable prognoses in solid tumor cancers and predict immunotherapy responses in patients. This study investigates oncolytic virotherapy's potential to induce TLS in the tumor microenvironment (TME) for enhanced anti-tumor responses.

To assess the oncolytic vaccinia virus (OVV)'s ability to induce TLS in the TME, we administered it via intranasal instillation in TC-1 lung tumor-bearing mice. Compared to modified vaccinia Ankara virus (MVA), known to induce TLS in the lungs of naïve mice following intranasal instillation, OVV elicited a higher density of TLS, likely due to its oncolytic properties.

To enhance this property, we generated a recombinant OVV expressing three murine chemokines (OVV-3mCK). We validated its oncolytic properties, as well as its capacity to secrete chemokines both in vitro and in vivo across syngeneic murine models. Chemotaxis assays revealed a synergistic effect of the three chemokines secreted in the supernatant of infected cancer cells. Both the unarmed OVV and the OVV-3mCK enhanced immune cell infiltration in subcutaneous tumors, overcame tumor resistance to anti-PD-1 treatment, and restricted tumor growth compared to the control group. Although no difference in therapeutic efficacy was observed between the viruses, a significant increase in T and B cell aggregates was noted in MC38 hot tumors treated with OVV-3mCK. We are currently conducting research in a humanized mouse model using a virus armed with human chemokines (OVV-3hCK). Initial results have shown enhanced infection of human tumors by chemokine-armed OVV in this model and up to a 50-fold increase in chemokine secretion.

Our results confirm our hypothesis regarding the role of these three chemokines in TLS formation, as well as the relevance of using OVV for this purpose. However, no positive correlation between TLS presence and therapeutic outcomes has been observed yet. By focusing now on specific T cell responses, we aim to discern whether this lack of correlation is due to model invasiveness or the inefficiency of ectopic TLS induced by a different inflammatory signal than that provoked by the tumor.

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418 – P1.09.11

First-in-class inhibitors of ERAP1 generate novel cancer antigens as targets for MHC-I-directed therapies

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Purpose: The antigen processing machinery of a cell shapes the repertoire of peptides presented for immunosurveillance on the cell surface. Human leukocyte antigen class I (HLA-I) molecules present peptides that are recognised by both $CD8^+$ T cells and NK cells, and these complexes play a vital role in the recognition and eradication of transformed cancer cells. However, selective pressure by $CD8^+$ T cells in many tumour types often results in immunoediting that can drive tumour evolution and progression, and many tumour-infiltrating lymphocytes also display exhausted phenotypes following chronic antigen stimulation. Endoplasmic reticulum aminopeptidases (ERAPs) process peptides translocated into the ER following intracellular proteasomal degradation, thereby editing the final pool of ligands able to compete for binding to HLA-I molecules. Hence, modulation of ERAP processing presents an opportunity to profoundly alter the immune landscape of cancer, and elicit priming of de novo $CD8^+$ T cell responses against a novel suite of tumour-specific epitopes which additionally comprise novel targets for HLA-I-directed therapeutics (TCR-bispecifics and vaccines) to enhance anti-tumoural efficacy.

Methods: Here, we developed a highly potent and selective allosteric inhibitor of ERAP1. Using diverse immunopeptidomics approaches, we show that pharmacological inhibition of multiple ERAP1 haplotypes across several cancers is able to unmask an alternative suite of tumour-associated peptides that are presented by an array of HLA-I molecules.

Results: Crucially, we demonstrate that (i) ERAP1 inhibitors are able to significantly enhance the potency of TCR-based therapeutics targeting A*02:01-restricted tumour-specific epitopes (ii) several epitopes either significantly upregulated or uniquely presented following ERAP1 inhibition are recognised by pre-existing $CD8^+$ T cell populations in multiple healthy human donors and (iii) $CD8^+$ T cell populations recognising these ERAP1 inhibitor-dependent epitopes demonstrate anti-tumoural efficacy in vitro.

Conclusion: These advancements demonstrate that combination of MHC-I-directed therapies in concert with pharmacological inhibition of ERAP1 are able to both augment anti-tumour immunity with the potential to subvert tumour immune evasion mechanisms, and have culminated in our lead candidate molecule entering clinical trials in 2023. Current work is focused on validating optimal ERAP1 inhibitor-dependent cancer-specific epitopes across multiple cancer types which can be taken forward for TCR-based immunotherapeutic development.

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453 – P1.09.12

CCL20 is upregulated in KRAS-driven non-small cell lung cancer via a FOXL2 - NF-κB axis and displays prognostic ambivalence

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Purpose: Immune Checkpoint Blockade (ICB) led to better outcomes in non-small cell lung cancer (NSCLC) but only a subset of patients benefits from current treatment regimens. Different molecular subtypes show diverse treatment responses. It was reported that patients with KRAS-mutant tumors respond better to ICB therapy, in contrast, EGFR-mutant tumors show higher resistance to this type of therapy. In order to evaluate distinctions between these subtypes, a thorough characterization of the immune environment (IE) was performed in patients with untreated NSCLC.

Methods: A cohort of 54 NSCLC cases underwent targeted RNA sequencing, with results validated using public datasets and further explored through in vitro and in vivo experiments. Potential therapeutic targets were evaluated in flank tumor mouse models.

Results: Elevated CCL20 expression was observed in KRAS-mutated NSCLC, driven by tumor cells through an NF-κB-FOXL2 axis. In an orthotopic mouse model combined with a tumor-specific CCL20 knockout, reduced tumor formation was observed. Additionally, antibody-mediated CCL20 blockade in a mouse tumor flank model led to slower tumor progression and decreased regulatory T cell infiltration compared to isotype controls. Survival analysis revealed conflicting prognostic impacts of high CCL20 expression in KRAS-mutated NSCLC under chemotherapy and ICB, validated across multiple datasets. Specifically, KRAS G12C mutated cell lines with TP53 co-mutation displayed an elevated activity in the NF-κB-FOXL2 axis and other inflammatory pathways. Further analysis of the TCGA-LUAD dataset revealed an elevated CD8+ T cell frequency with these specific mutations indicating a more responsive IE to ICB.

Conclusion: A novel KRAS-CCL20 link was identified and validated, suggesting CCL20 targeting as a potential therapeutic avenue, with further investigation warranted, particularly in the context of ICB. Additionally, CCL20 merits exploration as a biomarker for identifying ICB-responsive patients.

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487 – P1.09.13

Breaking Boundaries: Next-Level γδ-T Cell Isolation with REAlease

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Purpose: γδ-T cells represent a unique subset of immune cells that exhibit characteristics of both the innate and adaptive immune systems. Their non-MHC-bound nature makes them promising candidates for allogeneic CAR T cell therapies. Due to their low frequency, the enrichment of γδ T cells is often a necessary step in many experimental workflows. Therefore, there is a great need for protocols that enable the enrichment of highly purified γδ T cells with intact functionality to simplify research, improve the understanding of their biology and facilitate therapeutic development. The new StraightFrom REAlease technology provides a direct method for the enrichment of highly purified, unlabeled cells from various blood materials, while also offering the possibility of full automation for standardized separations.

Methods: : γδ T cells were isolated from Leukopak, Buffy Coat, and Whole Blood using REAlease γδ T-cell MicroBead Kits. Flow cytometric analysis assessed cell separation performance, γδ T cell subtype frequency, and activation status of enriched or TransAct expanded cells. MACSplex technology was used to analyze cytokine secretion profiles in stimulated cells, while the cytotoxic potential of enriched and expanded γδ T cells against target cells was investigated in co-culture experiments

Results: The newly developed REAlease γδ T-cell MicroBead Kits allowed the isolation of high purity γδ T cells from Leukopak, Buffy Coat and Whole Blood. Isolated cells exhibited no expression of the activation markers CD25 and CD69. Stimulation effectively activated the cells, showcasing their full functionality. Expansion rates of up to 300x for Vd1 cells and 90x for Vd2 cells after 8 days were achieved. Expanded cells exhibited efficient killing of target cells, validating their cytotoxic potential

Conclusion: StraightFrom REAlease technology demonstrates efficacy in isolating functional γδ T cells with high purity from various blood sources. The results highlight the potential of this technology to streamline research efforts and advance therapeutic strategies related to γδ T cell biology and allogeneic CAR T cell therapies.

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511 – P1.09.14

Transforming growth factor β inducer as a novel secreted immune checkpoint counter-inhibiting tumor-associated T cells

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Purpose: We investigated the hypothesis that Transforming Growth Factor Beta Inducer (TGFBI), an extracellular matrix protein secreted within the tumor microenvironment (TME) of various tumors, functions as a secreted immune checkpoint (sIC), and contributes to the restriction of anti-tumor T cell responses.

Methods and Results: Serum TGFBI concentrations were elevated in colorectal cancer (CRC) and hepatocellular carcinoma (HCC) patients compared to healthy individuals, and correlating with high protein TGFBI expression on cancer tissues and poorer survival outcomes. Notably, TGFBI exhibited abundant expression on tumor cells but also on monocytes, $CD4^+$ T cells, $CD8^+$ T cells, B cells, and NK cells from tumor tissues and periphery of cancer patients. Its neutralization significantly enhanced both CD4+ and CD8+ T cell activation *in vitro*. This effect was highlighted by utilizing freshly purified TGFBI-secreting CD4+ and CD8+ T cells, which, upon TGFBI neutralization, exhibited a significant enhancement in their effector functions (IFNg and TNFa production) and capacity to differentiate into cells expressing the requisite phenotype for migration into tissues and tumors. These insights were further supported by *in vivo* evidence, showing increased T cell activation and reduced tumor volume following TGFBI blockade in a colon cancer mouse model.

Conclusion: These findings unveil a counter-regulatory loop via T-cell-intrinsic TGFBI secretion as a novel immunosuppressive mechanism within the TME, which can be restored through TGFBI neutralization.

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630 – P1.09.15

Split identity of IFNγ in remodeling of undifferentiated pleomorphic sarcoma cell line JBT19 towards chemotherapeutic resistance and anti-tumor immunity and its implication for adoptive cellular immunotherapy

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Purpose: IFNγ is a key proinflammatory cytokine produced by macrophages, natural killer T cells, NK cells, cytotoxic $CD8^+$ T cells, and Th1 $CD4^+$ T cells. The cytokine modulates the function of both immune cells and their targets, including tumor cells. However, IFNγ can have either pro- or anti-tumorigenic activities depending on the mechanisms involved. Thus, the impact of IFNγ on the therapeutic efficacy of oncologic treatment is difficult to determine.

Methods: The patient-derived UPS cell line, JBT19, was treated with IFNγ (200 µg/ml) for seven days (γJBT19). The γJBT19 phenotype, proliferation, and sensitivity to docetaxel treatment were determined, respectively, by flow cytometry and MTT assay. The γJBT19 immunomodulatory potential was evaluated by co-culture with monocytes upon their GM-CSF (concentration)- and M-CSF (concentration)-mediated differentiation into M1 or M2 macrophages. The γJBT19 immunogenicity was evaluated through *ex vivo* enrichment and expansion of JBT19-reactive lymphocytes.

Results: The γJBT19 cells had abrogated proliferation and were resistant to docetaxel. The IFNγ treatment increased their surface expression of CD44, CD47, CD95, MHC-I, and PD-L1, and, *de novo*, induced the surface expression of MHC-II. On the other hand, γJBT19 cells could abrogate the differentiation of monocytes into M2 macrophages in the presence of M-CSF and licensed the JBT19 cells to a robust *ex vivo* enrichment and expansion of JBT19-reactive lymphocytes, which were able to *in vitro* efficiently eliminate both JBT19 and γJBT19 cells.

Conclusions: IFNγ engages different mechanisms in JBT19 cells, which, on the one hand, transform the cells into minimally proliferating and highly chemoresistant cells and, on the other hand, into efficient immunomodulatory and immunogenic cells that can induce efficient antitumor cellular immune responses. These findings can have substantial ramifications for therapeutic strategies combining chemotherapy and immunotherapy.

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667 – P1.09.16

Improving CAR-T cell immunotherapy with CRISPR/Cas9 gene editing

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Chimeric antigen receptors (CARs) are synthetic receptors comprised of the extracellular antigen recognition antibody-derived single chain variable fragment (scFv) and the intracellular T cell signaling domains. CARs endow T cells with the designed specificity and function. Since 2017, CAR-T cell immunotherapy is an approved cancer treatment approach for patients with certain types of leukemia and lymphoma. Here, we present the general principles of CAR-T cell therapy, the CAR structure, the production of the cellular product, and some of the major challenges in existing therapies, including the limited persistence of CAR-T cells and the lack of tumor-specific targets. We aim to produce safer and more effective mouse CAR-T cells by improving the individual design steps. One such improvement is developing CAR-T cells with the knock-out (KO) of the endogenous T-cell receptor (TCR). TCR KO prior to the introduction of the CAR molecule to the T cells reduces alloreactivity and contributes to the production of a more homogenous, and therefore safer, cellular product. We tested the knock-out of the TCR with the CRISPR/Cas9 system and designed sgRNAs targeting TCR α constant (TRAC) locus in the Jurkat cell line and primary mouse $CD4^+$ and $CD8^+$ T cells. Flow cytometry analysis showed that the KO efficiency was high in both Jurkat cell line (up to 90 %) and mouse primary T cells (up to 60 %). Therefore, in addition to upgrading CAR-T cell functions, manufacturing of the cellular product represents an opportunity to make CAR-T cells safer, more effective, and broadly available. The advantage and rationale for focusing on mouse CAR-T cells is the ability to comprehensively access mechanisms of action in the presence of an interacting immune system in future preclinical *in vivo* studies.

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719 – P1.09.17

Targeting cellular metabolism for modulation of T lymphocytes function

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Purpose: Recent discoveries in field of Immunometabolism presents a unique opportunity for improvement of existing immunotherapies. T cells differentiation and effector functions are intrinsically interconnected with cellular metabolism, therefore compounds that target metabolism could be used as adjuvant therapy. A glycolysis inhibitor 2-deoxy-D-glucose (2DG) is a promising compound that has also immunomodulatory effects, therefore we set to investigated how it modulates T cells from healthy donors.

Methods: Peripheral blood mononuclear cells (PBMCs) were isolated and activated with anti- CD3/anti-CD28 antibodies. Following activation, PBMCs were treated with 2DG and analyzed. ELISA and flow cytometry were used for immunophenotyping, cellular respiration and rate of glycolysis were measured with Seahorse analyzer. In parallel, samples were prepared for RNAseq.

Results: We show that treatment with 2DG decreased PD1 in a dose dependent manner, at low dose 2DG also increased CD69 expression. The effect of low 2DG was partially caused by inhibition of protein N-glycosylation rather than glycolysis. Interestingly, 2DG treatment of T cells followed by re-stimulation led to an increase in IFN-γ at low 2DG concentration while at higher concentration the levels returned to control levels. Transcriptomics showed differences in transcription of genes involved in metabolism, activation and differentiation, exhaustion as wells as genes related to ER stress and unfolded protein response. Bioenergetics measurements showed shift from glycolytic to oxidative metabolism.

Conclusion: We demonstrate that 2DG at physiologically achievable concentrations decreases PD-1 and increases IFN-γ secretion mostly due to inhibition of N-glycosylation. Together with the effect of 2DG on PD-1/PD-L1 axis, this provides important evidence that 2DG could potentially improve the T cell anti-tumor responses. The transcriptomics analysis showed multiple alterations relevant for T cells anticancer functionality in line with 2DG multimodal action. Our findings revealed that 2DG have important effects on T cells metabolism, function and differentiation. Altogether our results are promising and demonstrate that 2DG could be used to improve the T cell anti-tumor responses, but further research is needed.

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759 – P1.09.18

Semaglutide as a potent activator of anti-tumor immune response in breast cancer

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Breast cancer is the most common malignant tumor in women and is usually associated with early metastasis, representing a significant health problem. The immune response against cancer is based on the effector actions of cells of the immune system, among which NK cells and $CD8^+$ T lymphocytes are the most significant. Semaglutide is an antidiabetic, belonging to the group of incretin mimetics. It exerts its effects through agonistic action on receptors for glucagon-like peptide-1 (GLP-1). The anti-tumor effect of GLP-1 receptor agonists manifests as inhibition of growth, proliferation, migration, and invasiveness of tumor cells. The aim of this study is to investigate the anti-tumor effect of semaglutide in the 4T1 experimental model of murine breast cancer. After induction of breast cancer, BALB/c mice were treated with semaglutide intraperitoneally. Semaglutide application significantly delayed the onset of palpable tumors and slowed tumor growth. At the same time, the antidiabetic did not show a direct anti-tumor effect in *in vitro* experiments. The results of the study indicate that semaglutide affects the functional phenotype of $CD3^-CD49b^+$ NK cells by enhancing the expression of activation receptors CD69 and NKG2D, while reducing the expression of the PD-1 inhibitory receptor and IL-10. Additionally, semaglutide increased the infiltration of $CD3^+CD49b^-$ T lymphocytes into the tumor as well as the expression of activation markers CD69 and NKG2D, and enhanced granzyme production. It also significantly reduced the expression of PD-1 and IL-10 on $CD3^+CD49b^-$ T lymphocytes. Semaglutide did not affect the overall number of tumor-infiltrating dendritic cells but significantly increased the expression of the costimulator CD86. These results indicate that semaglutide has the potential as a therapeutic agent for stimulating the immune system against tumors.

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818 – P1.09.19

2-deoxy-D-glucose and metformin alter effector functions of T cells from peripheral blood mononuclear cells

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Purpose: The most prominent development in cancer treatment in recent years has been T cell targeted immunotherapy. However, poor response to therapy has led to a continuing search for ways to improve anti-tumor T cell responses. Immunometabolism has revealed that T cells functions are intimately tied to changes in cellular metabolic processes. With modulating immune cell metabolism, we could therefore improve cancer immunotherapy. Metformin, a standard first line antidiabetic drug, and glycolysis inhibitor 2-deoxy-D-glucose (2DG) are metabolic drugs with several promising anti-cancer and immunomodulatory effects. We conducted a transcriptomics study to explore their effect on various pathways in PBMCs, especially in relation to exhaustion, effector functions and T cell metabolism.

Methods: Peripheral blood mononuclear cells (PBMCs) were isolated from buffy coats of healthy donors, activated with anti-CD3/anti-CD28 antibodies for 48h, treated with 2DG or metformin for 24h and prepared for transcriptome sequencing. In parallel, bioenergetics in terms of respiration and rate of glycolysis was measured with Seahorse analyser, the expression of differentiation and activation cell surface markers on T cells was determined by flow cytometry.

Results: Transcriptomics showed differences in expression of genes involved in crucial metabolic pathways of PBMCs. We have also observed differences in expression of activation, differentiation, and exhaustion markers, as well as in effector properties (cytokine expression). Both treatments similarly downregulated ROS generating pathway. Specifically, 2DG treatment increased mRNA level of genes related to ER stress and unfolded protein response (UPR), while high dose of metformin decreased effector functions of T cells.

Conclusion: The transcriptomics analysis showed multiple alterations relevant for T cells anticancer functionality in line with their multimodal action, with the specific differences between the two compounds. Our findings revealed that metformin and 2DG have important effects on T cells metabolism, function, differentiation, and persistence. This can be explored in further development of adjuvant therapies that could improve cancer immunotherapies.

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895 – P1.09.20

Targeting the sialic acid-Siglec glyco-immune checkpoint in melanoma

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Immune checkpoint blockade (ICB) therapies have revolutionized the treatment of melanoma in the past decade. These therapies interfere with immune checkpoint molecules present on T cells, which lead to immune suppression upon engagement with their ligands. However, many patients still do not show a beneficial response from ICB, so the search for novel targets and combinatorial therapies is ongoing. Particularly, a recent interest in identifying immune checkpoint molecules on myeloid cells paves the way for achieving higher response rates to ICB. The increased expression of sialic acid glycans found in melanoma plays a key role in anti-tumor immune responses through engagement of Siglec receptors expressed on both lymphoid and myeloid cells. Hence, the sialic acid-Siglec axis has emerged as a novel myeloid and lymphoid glyco-immune checkpoint to target in cancer therapies.

In order to manipulate the sialic acid-Siglec axis in the tumor microenvironment (TME) of melanoma, we used the B16OVA melanoma cell line and knocked out the gene CMAS that encodes an essential enzyme for sialic acid expression, which was found to be altered in melanoma patients. After subcutaneous injection *in vivo*, the sialic acid negative CMAS KO tumors grew significantly slower than the sialic acid positive MOCK tumors. Mice that controlled the growth of the CMAS KO tumors were rechallenged after 21 days by subcutaneous injection of the B16OVA cell line in the opposite flank, which showed also delayed growth, indicating that the initial lack of sialic acid on tumor cells stimulated the development of a memory immune response. To alter sialic acid expression *in situ* we injected sialylation inhibitors close to the subcutaneous tumors. We could reduce tumor growth at early time points, however this effect was lost over time. Local tumor treatments with sialylation inhibitors showed a clear increase in CD8+ T effector memory cells in the TME, which expressed higher levels of PD1.

Our work shows that the sialic acid-Siglec facilitates CD8 T cell accumulation in the TME, and can be combined with anti-PD1 therapy. We are currently focusing in the study of this axis on myeloid cells.

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912 – P1.09.21

Assessment of the efficacy of a monoclonal antibody against pancreatic tumour cells by complement-dependent cytotoxicity assay

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Introduction: Pancreatic cancer is one of the most difficult malignancies to treat, with dismal survival rates and limited therapeutic options. Despite advances in conventional therapies such as surgery, chemotherapy and radiotherapy, the prognosis for pancreatic cancer patients remains poor. In recent years, immunotherapy has emerged as a promising approach to combat this aggressive disease and monoclonal antibodies (mAbs) are essential for the success of targeted therapies.

Methods: In this study, the effectiveness of a human HLA-specific mAb in targeting tumor cells was evaluated in pancreatic ductal carcinoma cells (PANC-1) by the complement-dependent cytotoxicity assay (CDC). The human HLA-specific mAb was generated through recombinant techniques and tested for cytotoxicity against PANC-1 cells and PANC-1 cells transfected to express the target HLA molecule by CDC. Each cell line was incubated separately with the human HLA-specific mAb, a human mAb of different specificity (negative control) or a hyper-immunized serum (positive control), afterwards 5 µl of rabbit complement was added and left to incubate for 2 hours at room temperature. Finally, a fluorescent dye was added to each well and cell death was observed under a fluorescence microscope.

Results: Our findings reveal a significant reduction in tumor cell viability following treatment with the hyper-immunized serum. Furthermore, we observed higher cell death in the transfected lines incubated with the hyper-immunized serum and the mAb compared to negative control Ab.

Conclusion: Our results demonstrate a significant reduction in cell viability following treatment with the mAb in the case of transfected lines, indicating its potential as a therapeutic agent for targeted therapy. These findings highlight the importance of targeted immunotherapeutic approaches in the management of pancreatic malignancies.

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928 – P1.09.22

DSP502 – A novel approach for targeting TIGIT and PD-1 for cancer immunotherapy

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Purpose: Dual Signaling Protein 502 (DSP502), a novel, multi-functional IgG1-Fc-fusion protein targets the PD1 and TIGIT molecular pathways in a unique way. DSP502, comprises the extracellular domains of TIGIT and PD-1, is designed to simultaneously bind its two respective ligands, PVR and PD-L1, expressed on cancer and myeloid cells in the tumor microenvironment. Here we describe the effect of DSP502 on anti-tumor immunity in vitro and in vivo.

Methods: Using human PBMCs, purified T-cells, purified NK cells and Macrophages of healthy donors and NSCLC patients, we evaluated DSP502-mediated activation of immune cells against human K562 CML cells wild type or the same cells overexpressing PVR, PD-L1 or both. PBMC, T-cell & NK cytotoxicity, T-cell, NK & macrophage activation, and phagocytosis induction methods were used. DSP502 anticancer activity was further evaluated in an AB12 mesothelioma syngeneic mouse model.

Results: DSP502 significantly augmented PBMC and T-cell mediated killing of tumor cells, which was dependent on the ligand's expression. DSP502 significantly enhanced NSCLC patients derived PBMC's killing of tumor cells. In a co-culture system, DSP502 significantly increased complex formation of NK cells with the target tumor cells, when both ligands were expressed on the cancer cells. In addition, NK-mediated cytotoxicity over tumor cells was increased following treatment with DSP502. Macrophages activity was also affected by DSP502 evident by reduction of M2 macrophages expressing $CD11b^{+}CD163^{+}$, increase in M1 macrophages expressing $CD11b^{+}CD86^{+}$ and the increasesd phagocytic activity over tumor cells. Importantly, DSP502 significantly prolonged the survival of AB12 mesothelioma tumor bearing mice.

Conclusions: DSP502 presents numerous functionalities that can work together in synchrony and synergy to enhance anti-tumor immunity. Beyond targeting PVR and PD-L1, DSP502 has the potential to further affect TIGIT pathway via its effects on CD96 and DNAM-1.

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934 – P1.09.23

A new pathway towards immunotherapy-supported radiology

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Purpose: Immunology has revolutionized the treatment of blood-borne cancer over the past few decades. Nonetheless, the core challenge remains to apply its proficiency to solid tumors due to their peculiarity, which, among others, is characterized by the absence of unique biomarkers.

Methods: Herein, we present the new approach of immunotherapy-supported radiology to augment conventional Boron Neutron Capture Therapy (BNCT). This fledgling radiologic treatment modality is in need of efficient delivery methods, as depicted herein. Knowing about the ability of solid cancers to 'chemically hide' in a gamut of surrounding fibroblast, thus preventing the immunocompetent cells from releasing their cytotoxins, we use selected immunocompetent cells instead as a new class of highly selective delivery agents (nanorobots) for nanoparticles to place high concentrations of radiotherapeutic 10-boron payloads. After delivery into the cancerous cells or in their close vicinity, the non-radioactive, stable 10-boron nanoparticles can be activated with the highest controllability by exposure to a beam of low energetic (epithermal) neutrons provided by an accelerator, thus paving the way to overcome the cancer-induced cytotoxin revocation by simple radiology. In detail, as high payloads of several ug/g in the malignant cells that form a tumor can be achieved, the FDA-approved BNCT uses the neutron-induced decay of 10-boron in an alpha-particle and a lithium-ion to destroy the cancerous cell. The incident thermal neutron dose is patient-save but leads to several Gy of treatment dose triggered by the steered nuclear disintegration of 10-boron in a few hour-long exposures with a neutron flux of 10^8 neutrons/(cm^2s). As the cancer-killing linear electron transfer (LET) induced by BNCT is physically curtailed to under ten micro-meters, roughly the radial dimension of one cell, our method of high-yield ultra-precise boron delivery destroys mainly the impaired cells, sparing surrounding healthy tissue, an advantage compared to existing ion- and gamma-radiation based treatments.

Results/Conclusions: Our in-situ loading mechanism allows a high upload of nanoparticles in the selected immunocompetent cells so that the treatment of solid cancers at a depth of up to several cm inside a patient's body can be envisaged. The presentation will summarize the status quo of our current research.

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951 – P1.09.24

Metformin promotes antitumor activity of NKT cells via overexpression of NFAT and STAT4

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Purpose: Over the past few decades, metformin, an anti-glycemic drug, has emerged as a potent activator of the immune system, offering promising prospects in the fight against tumors. While prior research has provided some insights into the impact of metformin on various immune cells, there remains much to uncover. Our study aimed to investigate the potential effects of metformin on the functional phenotype of NKT cells, recognizing their potential as an antitumor weapon through modulation of the tumor microenvironment.

Methods: 4T1 breast cancer was induced in BALB/C wild-type (WT) mice, subsequently treated with metformin. The functional phenotype of splenocytes and tumor-infiltrating leukocytes were investigated.

Results: Metformin exhibited significant inhibition of 4T1 breast carcinoma appearance and growth in WT mice. Furthermore, metformin increased the percentage of interferon (IFN)-γ^+, and CD107a MFI$^+$ CD3$^+$CD49b$^+$ NKT cells while decreased the percentage of PD-1$^+$, FoxP3$^+$, and interleukin (IL)-10$^+$ in spleens of metformin-treated mice. In primary tumors, metformin significantly increased percentages of NKp46$^+$ and FasL MFI$^+$ NKT cells and reduced the percentages of FoxP3$^+$, PD-1$^+$, KLRG1 MFI$^+$, and IL-10-producing NKT cells. Additionally, a significantly elevated percentage of FasL$^+$ and a decreased percentage of PD-1$^+$ and KLRG1$^+$ T cells were measured in spleens of metformin-treated mice. Metformin increased the percentage of FasL$^+$, NKp46$^+$, IL-17$^+$, and STAT3$^+$ T cells while decreased the percentage of PD-1$^+$, FoxP3$^+$, and KLRG1$^+$ T cells in tumor tissue. Furthermore, metformin decreased the accumulation of IL-10$^+$ and FoxP3$^+$ Tregs, as well as Gr-1$^+$ myeloid-derived suppressor cells (MDSCs) in the spleens of metformin-treated mice, and IL-10$^+$ and FoxP3$^+$ Tregs, Gr-1$^+$, NFκB$^+$, and iNOS$^+$ MDSCs, and iNOS$^+$ dendritic cells (DCs) in tumor tissue, while increasing the quantity of DCs. Notably, metformin administration significantly upregulated the expression levels of MIP1a, STAT4, and NFAT in splenocytes isolated from metformin-treated mice, suggesting increased NKT cell activity.

Conclusion: Metformin administration directly altered the phenotype of NKT cells, further stimulating T cells either directly or through dendritic cells, while also inhibiting the immunosuppressive effects of MDSCs and Tregs. Future studies should aim to clarify the precise mechanism of NKT cells in the antitumor immune response.

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Phenotypic and Functional Analysis of Cytokine-Induced Memory-Like Natural Killer Cells

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Introduction: Natural Killer (NK) cells play a crucial role in innate immunity against virus-infected and tumor cells. Interleukin-12/15/18 exposure in vitro induces a memory-like phenotype in NK cells, termed cytokine-induced memory-like (CIML) NK cells, characterized by heightened cytotoxicity and prolonged survival. This study focuses on exploring the expression patterns of activating and inhibitory receptors in CIML NK cells and evaluate its cytotoxic capacity against target cell lines, that is crucial for understanding their therapeutic potential.

Methods: To obtain human CIML cells, peripheral blood NK cells were isolated and preactivated with IL-12/15/18 overnight and expanded during 7 days with IL-15. Multiparametric flow cytometry analysis to assess phenotypic profiles was performed on day 0 (before stimulation), at 16 hours and at 7 days of incubation. Functional assays, including CD107a degranulation and IFN-γ and TNF-α production, were conducted to evaluate CIML NK cell cytotoxicity against target cells K562 and 721.221.

Results: CIML NK cells exhibited increased expression of NKG2A, NKG2D, NKp30, NKp46, CD25, and CD69 compared to control NK cells stimulated with IL-15, highlighting the potency of IL-12/15/18 in stimulating a functionally enhanced phenotype. Additionally, preactivation with IL-12/15/18 significantly increased CD107a expression in CIML NK cells after stimulation with target cells compared to control NK cells, indicating higher cytotoxicity against tumor cells. Moreover, CIML NK cells demonstrated elevated intracellular IFN-γ and TNF-α production when stimulated with IL-12/15/18, underscoring their enhanced antitumor functionality.

Conclusion: This study underscores the potential use of CIML NK cells in cancer immunotherapy. Enhanced expression of activating receptors and increased functional capacities, including degranulation and cytokine production, suggest CIML NK cells as promising candidates as antitumor agents. Further investigations into refining CIML NK cell expansion protocols and understanding their mechanisms of action are needed to advance their clinical translation and improve cancer treatment outcomes.

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Sex differences in immune checkpoint receptor expression in patients with upper gastrointestinal malignancies: potential implications for patient response to immune checkpoint inhibitor therapy

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Incidence of upper gastrointestinal malignancies continues to increase and 5-year survival rates remain dismal at ~20%. This is due to an advanced stage at diagnosis and a lack of response to conventional therapies. The intention to treat standard-of-care is surgery with peri-operative chemotherapy or neoadjuvant chemoradiotherapy. Recently immune checkpoint (IC) inhibitor therapy has been added to this arsenal of treatment in certain clinical settings. However, less than 30% of patients treated achieve a meaningful response. Our group has previously shown in a cohort of oesophageal adenocarcinoma (OAC) patients that expression of certain immune checkpoint receptors on circulating T cells and tumour infiltrating lymphocytes positively correlated with a higher grade of tumour and a poor response to neoadjuvant treatment. Response rates in upper GI patient cohorts treated with IC inhibitors remain low.

As part of this study, peripheral blood T cells from treatment naïve patients with upper gastrointestinal malignancies (n=16) were profiled by flow cytometry. Patients with gastric adenocarcinoma, adenocarcinoma of the oesophagus/gastro-oesophageal junction or oesophageal squamous cell carcinoma were included in this study. Cell surface expression of key IC receptors (PD-1, TIGIT, TIM-3, CTLA-4 and LAG-3) were measured on CD4 and CD8 T cells. The expression of these receptors was measured again after 48 hours during which T cells were activated or rested.

We correlated this data with clinical parameters and discovered significant differences in expression of key IC receptors based on sex both basally and following T cell activation. The expression of these ICRs on peripheral T cells is also influenced by the anatomical location of the tumour (oesophagus/gastro-oesophageal junction vs. gastric tumours).

These preliminary results suggest that patient sex may have implications for response to certain IC inhibitors. They also suggest differential T cell phenotype based on tumour location within the upper GI tract. Our results may improve tailored selection of patients to receive IC inhibitor therapy with the ultimate goal of expanding the relatively limited cohort of patients who currently benefit from IC blockade.

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Role of ion channels in CAR T cells

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Cancer immunotherapy partly depends on the reprogramming of host immune cells to recognize and eliminate cancerous cells. Genetic modification of T cells to express chimeric antigen receptors (CARs) has shown great results in treating hematological malignancies. However, despite its success, many challenges remain to be overcome improving the efficacy and safety of this therapy. Ion channels in T-cells participate in the regulation of activation via modulation of the Ca^{2+}-dependent pathway. Moreover, ion channels play a role in multiple effector functions that are inevitable for target cell abolition. Consequently, modification of ion channels' function can contribute to successful immune therapy. However, no study has been reported about the expression and functional role of CAR T-cell ion channels so far.

In the present study, we established a 3rd-generation CAR-expressing cell line (targeting CD19, CD19-CAR cells) from Jurkat E6-1 cells. We used the whole-cell patch-clamp technique and FURA-2-based Ca^{2+}-imaging to determine the biophysical properties of Kv1.3 and Ca^{2+}-response of CD19-CAR cells, respectively. We used a Calcein Red-based killing assay to test CD19-CAR cells' target killing cytotoxicity. By immunocytochemistry, we evaluated the localization of Kv1.3 in standalone and in the CAR-synapse-engaged CD19-CAR cells.

We demonstrated that Kv1.3 activation and inactivation kinetics are the same in Jurkat non-transfected (control) and CD19-CAR Jurkat cells, while voltage-dependent equilibrium activation was different. Thapsigargin-induced Ca^{2+}-response of CD19-CAR cells was lower as compared to the non-transduced cells. We could show that Kv1.3 channels are colocalized with CARs in standalone CD19-CAR cells, and they redistribute the contact region between a CD19-CAR cell and a target cell (Raji B cell line). To test the effect of Kv1.3 blocking, we used Vm24 (specific Kv1.3 inhibitor, 1 nM), and surprisingly the target killing potential of CD19-CAR cells was impaired. Based on these results, we suppose that ion channels can affect the outcome of immunotherapy, and further experiments are needed to clarify their functional role.

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Miniaturization of T cell cultivation and utilization for process development

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The use of T-lymphocytes in adoptive cell therapy shows great promise for treatment of cancers, and there is an increasing number of cell therapies being developed. The rapid increase in novel immunotherapies necessitates alternative approaches in process development, to increase capacity and provide opportunities for flexible and personalized production of a large variety of products. Good therapeutic cell manufacturing needs to encompass rapid production of the right type of cells, with optimal activity. Both T-cell quality and cellular subsets can have large impacts on therapeutic efficacy.

With in vitro cell expansion, the resulting T-cell populations are heterogeneous with many subsets. It has been demonstrated that the less differentiated populations of T-cells lead to a more persistent anti-tumour response. In particular, the memory stem-like T-cells (T_{SCM}) have higher renewal properties and the ability to reconstitute the entire heterogeneity of memory T-cell subsets. However, we currently lack a complete understanding of how cultivation parameters impact the resulting T-cell populations, and current cultivation approaches provide for limited throughput and side-by-side comparisons of such conditions.

Here, we asked whether miniaturized high-throughput systems could be adapted to achieve screening of T-cell cultivation processes. Furthermore, we asked how process parameters, traditionally measured manually (e.g. cell culture density), could be replaced by high-throughput-compatible analyses and automatic read-outs.

T-cell cultivation was optimized in microtiter plates, and the set-up was used to compare the impact of culture media, signalling molecules and metabolite additions on T-cell proliferation and early memory markers. Robotic methods for cell processing and image-based high-throughput methods were developed for quantification of cell culture density, viability and specific T-cell markers. The established platform is generic and readily translatable for screening of a large number of process parameters and media components.

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1118 – P1.09.29

Polymorphonuclear myeloid-derived suppressor cells impair the anti-tumor efficacy of GD2. CAR T-cells in patients with neuroblastoma

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The outcome of patients affected by high-risk or metastatic neuroblastoma (NB) remains grim, with >50% of the children experiencing relapse or progression of the disease despite multimodal, intensive treatment. To identify new strategies to improve the overall survival and the quality of life of these children, we recently developed and optimized a third-generation GD2-specific chimeric antigen receptor (CAR) T-cells (NCT03373097) for the treatment of patients with relapsed/refractory NB. We found that our CAR T-cells can induce marked tumor reduction and even achieve complete remission more efficiently than previous studies. However, often responses are not sustained and relapses occur. Here, we demonstrate a mechanism of resistance to GD2 for the first time.CAR T-cell treatment, showing how polymorphonuclear myeloid-derived suppressor cells (PMN-MDSC) increase in the peripheral blood (PB) of NB patients after GD2.CAR T-cell treatment in case of relapse or loss of response. *In vitro*, isolated PMN-MDSC inhibited the anti-tumor cytotoxicity of different generations of GD2.CAR T-cells. Gene-expression profiling of GD2.CAR T-cells "conditioned" with PMN-MDSC show downregulation of genes involved in cell activation, signal transduction, inflammation, and cytokine/chemokine secretion. Analysis of NB gene-expression dataset confirms a correlation between the expression of these genes and patient outcome. Moreover, in patients treated with GD2.CAR T-cells, the frequency of circulating PMN-MDSC inversely correlated with the levels of GD2.CAR T-cells, resulting more elevated in patients who did not respond or lost response to the treatment.

The evaluation of PMN-MDSC in PB of high-risk or metastatic NB represent novel useful prognostic markers to predict the response to GD2.CAR T-cells or other adoptive immunotherapies. This study underlines the importance of further optimizing both CAR T-cells and the design of clinical trials by targeting inhibitory cells that compromise the clinical outcome.

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Development of Novel Anti BCMA (B Cell Maturation Antigen) Antibody as a live cell therapy candidate in Multiple Myeloma

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Purpose: Multiple myeloma (MM) is characterized by the expansion of malignant plasma cells (PCs) in the bone marrow (BM) which is associated with excessive production of monoclonal immunoglobulins in blood and urine in patients. The addition of monoclonal antibodies (MoAbs) as immunotherapies in MM has further improved patient outcome. However, MM remains incurable for most patients, since drug-resistant clones constantly emerge and evolve. BCMA (B Cell Maturation Antigen) has been found to be overexpressed in MM and is being explored as a target for both antibody based and live cell therapy.

Method: BCMA expression in MM patients was quantified using RQ PCR as well as soluble BCMA was quantified using ELISA. The consensus BCMA ECD sequence was cloned in the vector which was sequenced and Protein expression was carried out using Expi293 system which were characterized using SDS page. Mice immunization was carried out and hybridoma cells were generated for production of Anti BCMA ECD antibodies which were further purified and characterized using western blotting and other assays. Best binders were identified and ScFv sequence was incorporated to novel second generation CAR (Chimeric antigen receptor) T cells which screened using flow cytometry.

Results: We characterized the expression of BCMA in cohort of MM patients and found it be elevated at mRNA level furthermore we found serum BCMA or soluble BCMA levels were also elevated which is the predictor of inferior therapeutic outcome in patients with MM. We further Validated BCMA as a candidate for development of Anti BCMA therapy and achieved expression of BCMA ECD protein in eukaryotic system which was further used to generate hybridomas, Overall we generated twenty different clones which were further screened for best binders which were characterized for bind with BCMA ECD protein using ELISA. The best binder (9C4) was further used for deciphering ScFv which was used for development of novel second generation CAR T cells. which were further characterized for expression of this Novel Anti BCMA CAR.

Conclusion: The current study has led to characterization of BCMA expression and variation in Indian MM patients as well as development of Anti BCMA second generation CAR.

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Continuous B cell destruction and regeneration is crucial for effective response to anti CD19 CAR-T cell therapy

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Purpose: Chimeric Antigen Receptor (CAR)-T cell therapy targeting CD19, whereby genetically engineered T cells target CD19+ malignant cells, is a promising immunotherapy. The resulting interactions induce an inflammatory response that destroys the cancer cells and further activates CAR-T cells. However, patients with low tumor burden are more likely to respond, raising the question: who activates the CAR-T cells? We hypothesized that the outcome relies on early interactions between CD19+ normal B cells and CAR-T cells that stimulate B lymphopoiesis in the bone-marrow (BM). Accordingly, we explored the elimination of B cells and the resulting BM B lymphopoiesis post CAR-T infusion. **Methods:** 39 patients treated with anti CD19 CAR-T cells at Rambam hospital were enrolled. Plasma proteins' levels were measured by high throughput multiplex assay. scRNASeq data of healthy humans' BM samples was analyzed to in-depth study the B lymphopoiesis process.

Results: To study the destruction of normal B cells following infusion, we analyzed the reduction in IgG, the main product of B cells. Unsurprisingly, 30 days post CAR-T infusion IgG levels decrease. Importantly, in multivariable analysis the percentages of IgG drop significantly correlate with response. It has been demonstrated that depletion of B cells stimulates BM B lymphopoiesis. We therefore tracked the levels of CXCL12, a regulator of nascent B cells in the BM, that has been shown to fluctuate post B cell depletion. Importantly, we found that within day 10 and day 20 post-infusion, following the average peak concentration of CAR-T cells and nearing its elimination from peripheral blood, the increase in CXCL12 is significantly higher in responders. Beyond that we revealed a significant increase in VEGFA, MIP1, MCP1 and Eotaxin within responders. By exploring the cell-cell interactions in the BM and analyzing scRNAseq data of BM samples, we identified that they are B lymphopoiesis associated proteins since they interact with progenitor B cells subpopulations along their developmental trajectory in the BM.

Conclusion: Early interactions between normal B cells and CAR-T cells that further stimulate BM B cell regeneration correlate with response to therapy. This may be leveraged to infer early predictors and improve outcomes.

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Local repeated delivery of checkpoint blockade therapy through an actuatable reservoir to treat Ovarian Cancer.

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Background: Ovarian cancer is the most lethal gynaecological cancer with a global 5-year survival rate of 30-50%. Treatment with chemotherapy is effective initially, however most women ultimately relapse and develop chemotherapy-resistant tumours. Delivering chemotherapy directly to the abdomen through an implanted catheter significantly increases patient survival as compared to standard intravenous infusion. However, complications involving fibrosis and impaired therapy distribution prevents the use of this strategy. Therefore, there is a pressing need to develop novel therapies and strategies to prevent cancer recurrence and increase patient survival. We have developed an implantable mechanotherapeutic platform to deliver immunotherapy to the abdomen to treat Ovarian Cancer. Our strategy enables the repeated delivery of therapy directly to the tumour site, without causing fibrosis around the implanted catheter.

Methods / Results: We previously demonstrated that cyclic inflation and deflation (intermittent actuation (IA)) of an implanted device modulates the foreign body response (FBR). Briefly, devices were implanted subcutaneously in rats or mice and cyclically actuated for 5min every 12hrs. IA interfered with the development of a fibrotic capsule surrounding the device and consequently improved drug delivery for up to 8-weeks post implantation. This was compared to non-actuated devices which developed a thick surrounding fibrous capsule and demonstrated a near complete loss of drug delivery due to the FBR. Furthermore, we showed that rapid, on-demand actuation can be used to accelerate the transport and distribution of therapy from the device into the surrounding tissues, enabling temporally controlled drug delivery[4]. Using mouse models of Ovarian Cancer, we have shown that intraperitoneal implantation of our device does not interfere with tumour growth, as measured by bioluminescent imaging. We will deliver anti-TIGIT monoclonal antibodies directly to the peritoneal cavity of Ovarian Cancer bearing mice through our custom-made actuatable reservoir. TIGIT blockade promotes potent Natural Killer and CD8 T cell cytotoxic responses by increasing IFNγ and GranzymeB production in multiple cancer models.

Conclusion: Our strategy aims to overcome the current challenges in treating Ovarian Cancer, by enabling the rapid delivery of repeated doses of immunotherapy directly to the tumour site, without causing patient discomfort resulting from fibrosis around an implanted catheter.

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Adeno-associated virus-mediated IL-10 delivery reduces the infiltration of tumor-associated macrophages and suppresses tumor growth in hepatocellular carcinoma

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Purpose: Hepatocellular carcinoma (HCC) is the most common primary liver cancer in adults. Previous research has identified interleukin-10 (IL-10) as a factor inducing the transformation of macrophages into M2 macrophages, thereby promoting tumor growth. IL-10 has also been associated with reducing macrophage activation and pro-inflammatory functions. However, our previous study demonstrated that the administration of exogenous IL-10 delivered by adeno-associated virus (AAV-IL-10) suppressed tumor growth and improved survival in a syngeneic orthotopic HCC mouse model. In this study, we aim to investigate the effect of AAV-IL-10 treatment on macrophages, including Kupffer cells in the liver and tumor-associated macrophages (TAMs) in HCC.

Methods: The orthotopic HCC mouse model was established by intrahepatic injection of Hep55.1c into male C57BL/6 mice. Mice received AAV-IL-10 or control virus via tail vein injection after 5 days of tumor implantation. Tumors were harvested fourteen days after tumor implantation. Serum was collected on Day 14 to confirm the IL-10 concentration. We used immunohistochemistry, immunofluorescence, and flow cytometry to assess the distribution and infiltration number of TAMs in HCC tumors and non-tumor liver tissue.

Results: Our findings revealed a pronounced infiltration of TAMs within the Hep55.1c-inoculated HCC model, predominantly comprising M2-like TAMs. Notably, treatment with AAV-IL-10 led to suppressed tumor growth in HCC mice, concomitant with a decreased number of TAMs. Specifically, there was a marked decline in M2 macrophages, whereas M1 macrophages exhibited no significant alteration, resulting in a reduced M2/M1 macrophage ratio. Regarding non-tumor liver tissue in HCC mice receiving IL-10 treatment versus controls, the percentage of Kupffer cells remained unchanged, while the percentage of M1 macrophages increased and that of M2 macrophages decreased. These observations suggest that AAV-IL-10 therapy diminishes TAM numbers in HCC, particularly M2 macrophages, consequently altering the M2/M1 macrophage ratio.

Conclusion: AAV-IL-10 can suppress tumor growth and reduce the infiltration of TAMs in HCC. Additionally, it can decrease the M2/M1 macrophage ratio in both tumor and non-tumor liver tissue.

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The impact of antigen-binding domains on antigen-directed and tonic chimeric antigen receptor (CAR) signaling

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CAR T cell therapies have transformed the treatment of hematological malignancies. However, the targeting of solid tumors remains hindered by their complex tumor environment. Additionally, potential targets often comprise proteins which are overexpressed by cancer cells but are also present on healthy cells, such as the human epidermal growth factor receptor 2 (HER2) in a number of carcinoma or the carcinoembryonic antigen (CEA) in adenocarcinoma. Thus, optimizing affinity for the cancer specific target is crucial and strongly dependent on the antigen-recognition domain of the CAR, the single-chain variable fragment (scFv). Binding of the scFv initiates activation of the CAR T cell; however, excessive high affinity or non-specific binding may reduce response rates due to accelerated exhaustion and antigen-independent activation, so-called tonic signaling.

In this study, our objective was to elucidate how structural properties of the scFvs are linked to antigen-dependent and independent CAR T cell properties through examination of their biophysical and binding characteristics at the molecular level. Therefore, panels of low to high affinity scFvs against CEA and HER2 were designed using structure-based and machine learning methods and compared to a previously published panel. Subsequently, all scFvs were analyzed with regard to their stability, specific and non-specific binding, as well as their binding kinetics and self-binding. Initial experiments showed major differences in protein stability and antigen-specific binding, as well as non-specific binding of the scFv caused by single amino acid substitutions.

To investigate the correlation between biophysical properties at the molecular level and the anti-tumor behavior of CAR T cells, these affinity panels were also analyzed at the cellular level. Overall, activation of the cells seems to follow the affinity hierarchy measured by NFAT activity. Additionally, prolonged survival without the target antigen was observed in some high affinity variants.

In summary, we highlight that the efficacy of CAR T cells for solid tumors is strongly affected by the biophysical properties of the antigen-binding domain and that affinity tuning modulates the antigen-dependent but also independent CAR signaling. Fully understanding these properties will be the key to the design of next generation CARs, overcoming the current challenges in solid tumor targeting.

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Identification of novel ICOSL isoforms recurrently expressed in Sezary syndrome

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Sezary syndrome (SS), is an aggressive T-cell lymphoma with poor prognosis and no efficient therapy, characterized by a clonal proliferation of neoplastic $CD4^+$ T cells, named Sezary cells (CS), in the blood, the skin, and lymph nodes. These cells co-express different markers and their ligands such as PD1/PDL1/2 and OX40/OX40L. In this study, we have shown that besides ICOS, a TCR-costimulatory receptor of the CD28 family, SC do also express its ligand (ICOSL), known to be mainly expressed by antigen presenting cells. This expression was validated on different clinical samples and cell lines of SS, as well as other subtypes of T-cell lymphoma. Interestingly, we have identified in CS two long isoforms of ICOSL, ICOSL4.1 and ICOSL4.2, containing one or two intra-cytoplasmic proline-rich domain(s), which may have a distinct signalling function in CS. Furthermore, we have demonstrated that blocking the ICOS/ICOSL axis significantly increases tumor cell death and caspase-3 cleavage, indicating its role in the maintenance of tumor cell survival. To scrutinize the molecular mechanisms underlying this axis, we have performed a phospho-proteome profiling in a SS cell line after blocking the ICOS-ICOSL interaction. Results revealed that ICOS-ICOSL axis is implicated in the activation of AKT/AMPK signalling. We further demonstrated that targeting ICOSL in SS using an ICOS-Fc construct increases significantly tumor death through the recruitment of NK cells and the induction of antibody dependent cell cytotoxicity. Hence, targeting ICOSL in SS could open potentially a new avenue for the treatment of this disease.

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1401 – P1.09.36

Leveraging single B cell technologies in the development of anti-PD-L1 antibodies

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Purpose: The novel single B cell technologies offer a convenient discovery platform for antibody research. Especially, direct staining of IgG-switched memory B cell receptors with fluorophore-conjugated antigens allows functional evaluation of each antibody clone at the earliest stages of antibody discovery. Herein, we report our ongoing studies on developing novel anti-PD-L1 antibodies by employing this technology.

Methods: First, a full-length extracellular domain of human PD-L1 protein was produced in CHODG44 cells in-house. New Zealand White rabbits were injected s.c. with the recombinant PD-L1 in a mixture with Freund's complete and incomplete adjuvants for primary and booster immunizations, respectively. Blood was sampled on days 0, 14, 28, 42, 56, 70 and serum anti-PDL1 IgG titers were evaluated by ELISA. 14 days after the second injection, PBMCs were isolated by Ficoll density separation and stained for flow cytometry based single cell sorting. PD-L1 recognizing IgG^+ viable cells were sorted one cell per well using BD FACSAria III Cell Sorter. Expression cassettes were developed by cloning the IgG gene from naive rabbit splenocytes between the CMV promoter and poly(A) tail by overlap extension PCR. We transiently expressed recombinant rabbit IgG molecules in HEK293 cells and the resulting antibody titers were determined by ELISA.

Results: Anti-PD-L1 titers were found to be steady after a peak increase following the second immunization. In the PBMCs from the same day, approximately 10% of the IgG^+ cells displayed binding activity to PD-L1. We observed distinct binding patterns for each single cell when we normalized the PD-L1 fluorescence signal to surface IgG expression in index sorting analysis. This can be attributed to the diversity in antigen binding affinities of different IgG clones. The expression cassettes were successfully developed by overlap extension PCR for each rabbit IgG heavy and light chains and accomplished to produce recombinant rabbit IgG. Right now, we are working towards obtaining amino acid sequences of these anti-PD-L1 antibodies.

Conclusion: The high-throughput screening property of flow cytometry, together with the simultaneous analyzing capabilities at the single cell sorting step, presents an attractive approach for antibody discovery technologies.

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1453 – P1.09.37

Computational free flow cytometry for Sézary cells identification and quantification

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Purpose: The specificity and reliability of KIR3DL2 as a positive marker for Sézary cell (SC) is now established to help initial diagnosis of Mycosis fungoides (MF) and Sézary syndrome (SS). During treatment, blood tumor burden is expected to decrease and flow cytometry (FCM) monitoring of blood SCs is essential. Here, we combined the use of the internationally recognized markers CD7, CD26, together with KIR3DL2 and the surrogate of T-cell clonality, TCR-Cb1 as routine tools for SC detection.

Methods: 566 samples from 265 patients with either confirmed or suspected SS/MF diagnosis were prospectively evaluated at the hematology routine laboratory between February 2023 and September 2023. The panel included 13 markers. We used our previously published gating strategy which was refined for the purpose of this study. In addition, data files were blindly analyzed by the two other leading groups from the Sézary group of the French CytHem association (www.cythem) which is aimed at promoting hematological cytometry. SC counts were compared to the results from blood clonal expansions detected by high throughput sequencing.

Results: During the 8 months study, 70 and 76 already diagnosed SS and MF patients, respectively, were analyzed. 9 newly SS diagnosed patients were identified with B2 stage according to CD7 and CD26 expression. All SCs expressed KIR3DL2 with a monophasic distribution of TCR-Cb1.

The use of TCR-Cb1 during the monitoring of treated SS, pre-SS or MF patients was particularly helpful in distinguishing non-malignant from malignant cells and in detecting low frequencies of SCs

Conclusion: This prospective study investigated the added value of both KIRDL2 and TCR-Cb1 to the recommended FCM markers for SS/MF initial diagnosis and blood tumor burden follow-up. Using a 13 colors panel, SC detection and quantification was improved using an indirect marker of T cell clonality, leading to a better assessment of blood stage at initial diagnosis and during therapy. This strategy is a simple, no time-consuming methodology which does not need high skills in FCM and computational analysis.

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1454 – P1.09.38

The MHC MACSimer technology combines flexibility, quality and releasabilty for the detection of antigen specific T cells

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Antigen (Ag)-specific T cell responses play an essential role in monitoring and combating cancer, infectious diseases and autoimmunity and the precise analysis, isolation and characterization of rare Ag-specific T cells is crucial for the development of cellular therapies and cancer- or virus-specific vaccinations. Given the strong personal component of Ag-specific T cell responses ("MHC/HLA" restriction), innovative tools must provide researchers with full flexibility and high quality enabling integration into translational workflows.

We have therefore designed a new reagent, the MHC MACSimer. MHC MACSimers feature superior specificity and fluorescence brightness through an optimally balanced amount and ratio of MHC molecules and fluorophores, enabling the detection of even very rare T cell subsets with highest reliability, stability and robustness.

To further satisfy the demand for flexibility and compatibility of our MHC MACSimer with translation workflows, we have additionally integrated

(i) A peptide-loading technology into our peptide-loadable **MHC MACSimer FleX Kits** allowing for extensive T cell epitope screening.
(ii) The REAlease® technology into our MHC MACSimer FLeX Kits and **peptide-loaded MHC MACSimers** for label-free T cells after sorting, e.g. for multiple sequential sorting steps or functional downstream assays.
(iii) A broad collection of **MACSpep** single peptides covering a broad variety of T cell epitopes related to human diseases, such as cancer, infection, and autoimmunity and the ideal comparability with our MHC MACSimer FleX kits.

We show that our MHC MACSimers feature superior performance when staining and analysing shared cancer antigens, Neo-Ags or virus-specific T cells of different origins (PBMCs, whole bloods samples or dissociated organs) and comparing them to other state-of-the art MHC multimer reagents.

They perfectly integrate into secondary antibody staining panels with multimer staining approved Abs, e.g. T cell exhaustion or differentiation panels, allowing to get the most information out of the rarest T cells. Combining them with fluorochrome-specific magnetic beads using MACS technology leads to highly pure (>>90%) and high-yield-enriched T cell populations, displaying the "expected" T cell phenotype.

In conclusion, our three reagent classes improve Ag-specific T cell analysis, enumeration and isolation and cover the need for flexibility, stability and standardization.

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1474 – P1.09.39

Preparación de nanopartículas para evaluación del efecto antitumoral en un modelo de melanoma

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Purpose: Prepare nanoparticles with the simple emulsion and ianotropic gelation technique, to be applied in an in vivo melanoma model.

Methods: The preparation of the nanospheres was carried out using the method of simple emulsion and cross-linking, with 2% chitosan and 0.1% albumin in distilled water for 24 hours. Subsequently, 100 ml of oil were mixed mineral with 1% SPAN 80, stirred at 700 rpm for three minutes and washed with 25% glutaraldehyde, subsequently through Scanning electron microscopy evaluated its morphology.

The weight of a dry and clean Eppendorf tube was calculated, then the solution with the nanoparticles was placed in the dry tube and left in the incubator to dry the water. They were then weighed again and the initial weight was subtracted to infer the mass of the nanoparticles.

For in vivo experiments, six mice were used for each experimental group.

male C57BL/6 with H2K b from 6 to 8 weeks of age, which were maintained in light-dark conditions, controlled temperatures, and fed ad libitum in the Bioterio of the Department of Cellular and Tissue Biology of the Faculty of Medicine of the UNAM.

Results: In relation to survival and tumor size. Mice without treatment showed a notable increase in tumor volume, reaching a maximum on day 26 (22.38 cm 2). While tumor development was very similar in mice treated with the nanoparticles alone or coupled to CpG, MAGE-AX, MAGE-AX-CpG and MAGE-AX-CpGs- ClecA9. On day 28, a considerable change was noted in the mice treated with the

chitosan nanoparticles coupled to MAGE-AX-CpGs-ClecA9, since the volume was increased widely, reaching a maximum on day 30 after inoculation of melanoma.

Conclusion: The best method to make nano-sized microparticles is to ionic gelation. It was possible to effectively load tumor lysates from melanoma in nanoparticles.

The CLEC-9 group had a lower tumor growth rate compared to the others.

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1510 – P1.09.40

Enabling Fast and Convenient Immune Profiling of Fresh and Long-Term Stabilized Human Whole Blood Samples With CyTOF

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Purpose: Accurate immune cell phenotyping in cancer patients' whole blood (WB) is crucial for disease prognosis and monitoring immunotherapy efficacy. Fresh WB analysis within 24 hours is vital to preserve cellular composition. However, logistical challenges arise when WB collection and cytometric analysis occur at different sites, leading to processing delays. To mitigate this, various WB preservation reagents like PROT1 and Cytodelics have been introduced. Nonetheless, not all antibody panels are compatible with these reagents. To address this issue, a CyTOF® panel compatible with commercial WB stabilizers has been developed for pharmaceutical and clinical research purposes.

Method and Material: CyTOF flow cytometry utilizes metal-tagged antibodies for cellular and functional phenotype identification, enabling rapid design and application of 50-plus-marker panels. Its low signal spillover and absence of autofluorescence eliminate the need for compensation. Additionally, antibody cocktails and stained samples can be frozen for future use, offering a streamlined and flexible workflow in clinical research, overcoming limitations of fluorescence-based cytometry. The CyTOF panel contains 20 antibodies to identify over 30 immune cell populations. For easy customization, there are more than 30 additional open channels to analyze markers of interest. The panel works with fresh and stabilized WB samples and is amenable to different staining and acquisition workflows. To show the flexibility of sample staining and stabilization, WB from three healthy donors was assessed using two stabilization workflows. First, fresh WB samples were stained with the antibody panel, followed by PROT1 or Cytodelics stabilization and storage at –80 °C. The second workflow involved immediate stabilization/fixation of WB with PROT1 or Cytodelics and storage at –80 °C. Subsequently, the samples for the second workflow were thawed, surface stained, and acquired. To reduce technical variability from staining, the antibodies were pooled together and frozen at –80 °C as single-use aliquots. Furthermore, all samples were barcoded and acquired as a single tube to reduce variability from sample acquisition.

Conclusion: The CyTOF panel, compatible with WB stabilizers, tackles traditional challenges in WB processing and acquisition. Freezing antibody cocktails ensures batch-to-batch consistency in clinical research, facilitating swift and convenient WB sample analysis through CyTOF workflows.

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1519 – P1.09.41

Exposure to cancer spheroids leads to human NK cell degranulation and anti-tumor activity, which is enhanced with cytokine preactivation and/or cetuximab

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Adoptive natural killer (NK) cell-based immunotherapy is a promising treatment approach in cancer that is showing notable efficacy against hematological malignancies. However, the success of NK cell immunotherapy in patients with solid tumors is limited due to several barriers, which include the immunosuppressive tumor microenvironment, poor NK cell infiltration into the tumor and heterogeneity of tumor cells. Advances in 3D *in vitro* culture technologies have enabled the development of more physiological human cancer models that mimic important tumor features absent in 2D cultures, which may be essential for designing improved immunotherapies against solid tumors.

This study aims to analyze the interaction of NK cells with 3D tumor models and the enhancement of NK cell anti-tumor activity. In order to do so, we have developed tumor spheroids from a colorectal cancer cell line (HCT-116) and from a lung adenocarcinoma cell line (A549). We have established co-cultures of the tumor spheroids with NK cells isolated from healthy donors and we have observed that the integrity of the spheroids is notably affected when exposed to NK cells. Our flow cytometry-based functional analyses evidence an increased degranulation, as measured by the CD107a marker, and a higher production of IFN-γ and TNF-α cytokines in NK cells exposed to tumor spheroids. Our study has shown that NK cell degranulation and production of IFN-γ and TNF-α can be enhanced by different mechanisms. These include the *in vitro* priming of NK cells by exposure to pro-inflammatory cytokines (IL-12, IL-15 and IL-18) and/or the induction of NK cell antibody-dependent cell-mediated cytotoxicity (ADCC) in the presence of the monoclonal antibody cetuximab. Additionally, confocal microscopy allows us to both analyze NK cell-induced apoptosis of spheroids and infiltration of the NK cells into the spheroids. Currently, we are working with cancer organoids, as another 3D tumor model, to evaluate their interaction with human NK cells. The results from this project could potentially provide very valuable information to define effective combination therapies able to generate NK cells with high cytotoxic potential that could lead to more successful adoptive NK cell-based therapy for the treatment of solid tumors.

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1532 – P1.09.42

The impact of Fc receptors and host characteristics on myeloid phagocytic response to rituximab-treated 3D-cultured B-cell lymphoma

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Antibody-based immunotherapy is successful in treating cancer, however its effectiveness varies among patients. To improve treatment outcome in each patient we need to understand what factors affect biological activities of therapeutic antibodies. Antibodies depend very much on the functional properties of immune cells that express Fc receptors (FcR). In this study, we explored FcR expression as well as host characteristics of monocytes in the capacity to phagocyte 3D-cultured human $CD20^+$ B-cell lymphoma (spheroids) treated with isotype variants of anti-CD20 rituximab (RTX) monoclonal antibody. The monocytes were obtained from healthy donors of different ages and sexes and their FcR for IgG (FcγRI, FcγRIIa, FcγRIIIa) and IgA (FcαRI) were determined, as well as FcR gene polymorphisms. Antibody-dependent phagocytosis was assessed using flow cytometry, confocal imaging, and Fc receptor blocking. Different RTX isotypes showed varying efficacy in stimulating phagocytosis of lymphoma spheroids. RTX-IgG3 proved to be most efficient, followed by RTX-IgG1, while moderate efficacy was observed by RTX-IgA1, RTX-IgA2, RTX-IgG4, and RTX-IgG2 had minor effect. RTX-stimulated monocytes infiltrated lymphoma spheroids predominantly at the periphery, but monocytes could also be identified in the spheroid core. Blocking FcγRI or FcγRIIa, but not FcγRIIIa, with antibodies inhibited RTX-IgG1 and RTX-IgG3-mediated phagocytosis. Monocytes derived from younger women exhibited elevated levels of FcγRI and FcγRIIa in comparison to their older counterparts. Conversely, in older men, there was a notable rise in FcγRI and FcγRIIIa levels when contrasted with younger men. This pattern was further supported by the observation that monocytes isolated from younger women displayed heightened phagocytic activity compared to those from older women. Similarly, older men demonstrated superior IgG-mediated phagocytosis relative to younger men. Single Fc receptor levels, or FcγRIIa and FcγRIIIa genetic variants, had low correlation with phagocytic intensity, possibly due to the involvement of multiple Fc receptors in IgG-mediated phagocytosis. In conclusion, the interplay of antibody isotype, Fc receptors, age, and sex significantly affects tumor phagocytosis. This study unveils a critical connection between host characteristics and the effectiveness of therapeutic antibodies, offering invaluable insights for advancing cancer immunotherapy treatments.

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1580 – P1.09.43

Deciphering the role of immune microenvironment during CD19 CAR-T treatment in leukemia

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Background: CD19 CAR-T therapy has shown remarkable clinical responses in patients with B-cell acute lymphoblastic leukemia or B-cell non-Hodgkin's lymphoma. However, many parameters such as cytokine release syndrome (CRS), neurological resistance, modest anti-tumor activity of CAR-T cells after infusion, loss of the CD19 antigen by the tumor cells can limit the therapeutic efficacy.

Objectives: Here, we explore the interactions between the tumor microenvironment (i.e. patient's immune system) and CAR-T cells, to determine if the immune composition constitute a predictive biomarkers for CAR-T efficacy and side effect.

Methods: We used mass-cytometry approach to characterize the immune cells compartment pre- and post-treatment (1 week, 2 weeks, 1 month, 2 months, 6 months).

Results: Our data shows much greater complexity in the CAR-T, conventional CD4 and CD8 T cell population than previously appreciated. Moreover, we observed a non-uniform pattern of variations across patients tested, which highlights a broad diversity of these cells as previously described. These observations, particularly with respect to markers associated with T cells exhaustion, may help to explain heterogeneity in clinical outcomes.

Conclusion: Great diversity of CAR-T, CD4 and CD8 T cells between patients and across time-point. Next step: investigate the antigen specificity of these T cells using an MHC class I tetramer screening approach.

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1593 – P1.09.44

Complexes of IL-2 and anti-IL-2 mAb selectively stimulating CD25+ T cells in combination with immune checkpoint inhibitors possess potent antitumor activity but the timing is crucial

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Purpose: Complexes of IL-2 and anti-IL-2 mAb (IL-2co) were shown previously to possess high biological activity *in vivo*. IL-2co exert selective stimulatory activity for either intermediate-affinity dimeric or high-affinity trimeric IL-2R expressing cells depending on the clone of anti-IL-2 mAb used. IL-2/anti-IL-2 mAb S4B6 complexes (IL-2/S4B6) are highly stimulatory for $CD122^{high}$ populations (memory $CD8^+$ T and NK cells), while only moderate stimulatory activity for T_{reg} cells was found. Conversely, IL-2/anti-IL-2 mAb JES6-1A12 complexes (IL-2/JES6) highly selectively and potently stimulate the expansion of $CD25^+$ cell population (Treg cells). Thus, it has been considered that IL-2/S4B6 are suitable for tumor immunotherapy while IL-2/JES6 are convenient for the treatment of autoimmune diseases or to facilitate long-term allograft acceptance. However, we recently found that IL-2/JES6 surprisingly possess antitumor activity, particularly in combination with immune-checkpoint inhibitors (anti-CTLA-4 plus anti-PD-1 mAb; ICIs henceforth). Thus, the purpose of this study was to investigate the potential of immunotherapy through combination of ICIs and IL-2co and to determine whether CD122-biased IL-2/S4B6 or CD25-biased IL-2/JES6 are more efficient.

Methods: We investigated the stimulatory activity of IL-2co for primed $CD8^+$ and $CD4^+$ T cells *in vitro* via (^{3}H)-thymidine incorporation and *in vivo* via adoptive transfer experiments. Phenotype of T cells and other immune cell population was evaluated via flow cytometry and *in vivo* antitumor activity of ICIs plus IL-2co was studied in CT26 tumor model.

Results: IL-2/JES6 possess antitumor activity comparable to IL-2/S4B6 at equimolar dosage when combined with ICIs. However, the timing of ICIs and IL-2/JES6 administration is crucial since IL-2/JES6 administered before ICIs do not improve the antitumor activity of ICIs, but IL-2/JES6 given after ICIs significantly potentiate it. Moreover, IL-2/JES6 possess much lower toxicity than IL-2/S4B6 thus enabling high-dosage treatment leading to superior antitumor effect.

Conclusion: IL-2/JES6 or similar $CD25^+$ T cell-biased IL-2 therapeutics may be efficient tool for cancer immunotherapy particularly in combination with ICIs due to their low toxicity and high stimulatory activity for activated $CD8^+$ T cells.

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1598 – P1.09.45

CD25-biased IL-2-based immunocytokines in combination with immune-checkpoint inhibitors completely eradicate large established tumors in CD8+ T cell-dependent manner

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Purpose: We found recently that complexes of IL-2 and anti-IL-2 mAb selectively stimulating $CD25^+$ cells (i.e. CD25-biased IL-2co) surprisingly possess antitumor activity, particularly in combination with immune checkpoint inhibitors (ICIs) despite robust Treg cell expansion. Thus, we decided to evaluate a panel of CD25-biased IL-2-based immunocytokines (IC) with different requirements for CD25 expression levels to stimulate IL-2-responsive cells. ICs consist of IL-2 linked through flexible oligopeptide $(Gly_4Ser)_7$ to the light chain of anti-IL-2 mAb (or irrelevant anti-FITC mAb representing a control IC) thus mimicking IL-2co both structurally and functionally but without drawbacks like excess of either IL-2 or Ab and potential dissociation leading to off-target effects. The purpose of this study was to investigate the stimulatory activity of various ICs for antigen-primed $CD8^+$ T cells and other immune cell subsets and their antitumor activity in combination with ICIs.

Methods: Stimulatory activity of CD25-biased IL-2-based ICs for antigen-primed $CD8^+$ T cells was investigated via OT-I $CD8^+$ T cell adoptive transfer experiments. The expansion of various immune cell populations and T-cell phenotypes were evaluated via flow cytometry. Antitumor activity of IL-2-based ICs in combination with ICIs (anti-PD-1 and anti-CTLA-4 mAbs) was studied in CT26 and MC38 tumor models.

Results: Y33 IC demonstrated remarkable potential to drive the expansion of antigen-primed $CD8^+$ T cells though a weaker than control IC. However, Y33 IC showed superior activity to induce the expression of effector molecules in antigen-primed $CD8^+$ T cells with limited stimulation of Treg cells. Y33 IC plus ICIs significantly prolonged survival and completely cured most CT26 or MC38 tumor-bearing mice without any sign of toxicity (body weight and temperature, pulmonary oedema). Depletion of $CD8^+$ T cells considerably reduced the antitumor activity of Y33 plus ICIs, indicating that $CD8^+$ T cells were the predominant immune population for therapeutic effect.

Conclusion: CD25-biased IL-2-based ICs may represent a promising tool for cancer immunotherapy, particularly in combination with ICIs.

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1660 – P1.09.46

Introducing SequiMACS technology: pioneering multi-cell isolation in single complex blood samples

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Purpose: Immunomagnetic cell isolation plays a pivotal role in studying specific cell populations of the innate and adaptive immune system which are crucial in elucidating immunological mechanisms. However, existing methods are restricted to isolate one target population at a time, necessitating sample splitting and thus compromising mainly on cell yield and recovery and limiting studies on autologous derived cells. At Miltenyi Biotec, we address this gap by introducing a novel technology, '*SequiMACS*®', which allows for the simultaneous labeling and isolation of multiple cell types from a single complex sample to optimize cell isolation workflows, target cell yields, and cell recoveries.

Methods: SequiMACS® technology is an extension from the previously developed StraightFrom®REAlease® approach, where now multiple release systems can be combined to label and isolate target cells from complex samples thanks to Miltenyi's unique MACS columns. In this study, we demonstrate the efficacy of SequiMACS® by co-isolating CD3+ T cells and CD19+ B cells from human whole blood. The isolated cells were subjected to various downstream applications including cell culture, activation, and expansion. Furthermore, single-cell sequencing of TCR and BCR, was conducted to illustrate how co-enriching multiple cell populations from the same cell sample could optimize the immune profiling of cell subsets.

Results: Our results showcase high purities and recoveries achieved through the co-isolation of CD3+ T cells and CD19+ B cells from human whole blood using SequiMACS® technology. By sequentially eluting the target cells from a single MACS® column, we were able to maintain the integrity and functionality of the isolated cells, enabling robust downstream applications.

Conclusion: The SequiMACS® technology represents a significant advancement in immunomagnetic cell isolation, offering a smart workflow solution for classical multi-cell isolation from single complex specially in quantity-limited blood samples. By optimizing the use of precious samples and streamlining the isolation process, SequiMACS® holds promise for accelerating research in cancer immunology and other fields reliant on limited starting materials and precise cell isolation techniques (e.g. Chimerism Analysis from Pediatric patients).

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Antitumoral activity of bacterioruberin, a rare carotenoid from haloarchaea, in solid tumor models

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Purpose: Carotenoids, extensively researched for their anticancer properties, act as antioxidants by scavenging ROS and RNS, thereby preventing DNA, lipid, and protein oxidative damage. They also modulate gene expression and signalling pathways related to cell growth, differentiation, inflammation, and immune response enhancement. Additionally, certain carotenoids induce apoptosis, inhibit angiogenesis, and disrupt cell cycle progression in cancer cells.

Bacterioruberin (BR), an unusual carotenoid sourced from extremophilic haloarchaea, among others microorganisms, exhibits significantly higher antioxidant capacity compared to well-studied classical carotenoids, suggesting antitumor potential. Previous results from our group demonstrate that BR triggers death of cells from hematological tumoral but not healthy cells. The primary objective of this research is to investigate the therapeutic potential of BR addressing solid tumors employing A549 lung carcinoma epithelial cell line. These cells are used as a model for non-small cell lung cancer (NSCLC), which constitute about 80% to 85% of lung cancers.

Methods: *In vitro* experiments were conducted using incremental doses of BR (0-75 µg/ml), and its effects were assessed through various assays. These included the evaluation of cell toxicity (MTT; 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide), proliferation (CFSE-DA; (5[6]-carboxyfluorescein diacetate succinimidyl ester), apoptosis (YO-PRO-1/Propidium Iodide), and cell cycle alterations (Propidium Iodide), through flow cytometry analysis. Morphological changes associated with apoptosis were also examined using fluorescence microscopy (dual acridin orange/ethidium bromide staining).

Results: At the highest concentrations of BR, a decrease in A549 proliferation of more than 50% was observed. On the other hand, the compound induced apoptosis in a dose-dependent manner, with apoptosis rates increasing significantly from the dose of 9.375 µg/ml. The highest dead cell population was obtained after treatment with the dose of 75 µg/ml.

Conclusion: These results could indicate the potential of bacterioruberin as a promising therapeutic option, making it an attractive candidate for further research and potential development of bacterioruberin-based therapies. Further investigations are needed to elucidate the underlying mechanisms responsible for bacterioruberin's antitumoral effects.

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1719 – P1.09.48

Viral infections for cancer immunotherapy

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Purpose: Cancer is a worldwide problem that needed to be solved; therefore, many researchers have been tried to reinvigorate exhausted T cells. Despite of these efforts, the treatment of cancer has not been conquered due to the deficiency of tumor antigens and the expression of immune suppressive molecules that inhibit the anti-tumor immune responses by tumor-specific T cells. To overcome these problems, we hypothesized that tumor cells deliberately infected with viruses present viral antigens to activate memory T cells, and that these T cells then kill infected tumors.

Methods: To address this hypothesis, we administered B16F10 melanoma cells subcutaneously into naïve mice containing LCMV-specific P14 memory CD8 T cells followed by intratumoral LCMV infections. These mice were infected with LCMV intratumorally to examine the survival and immune response mounted by memory T cells.

Results: First, we found that these tumor cells were infected by intracellular staining of viral nucleoprotein, and the survival of virus-infected tumor-bearing mouse increased 3 times compared to control. Second, the frequencies and numbers of P14 cells in the spleen, tumordraining lymph nodes, and tumors increased in virus-infection group compared to controls until D10 pi, suggesting that memory T cells recognized viral infections. Finally, we investigated any possible immune escape mechanisms by measuring the levels of cytokines in tumors by using ELISA.

Conclusion: Taken together, the development of strategy of virus-specific memory CD8 T cells targeting tumor cells with viral infections would be major key of noble cancer immunotherapy.

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1722 – P1.09.49

Checkpoint molecules on Antigen-Loaded Extracellular Vesicles modulate their anti-tumoral effects in a mouse model of melanoma

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Extracellular Vesicles (EVs) have been tested in humans as cancer immunotherapy, but the treatment needs improvement. EVs secreted by bone marrow derived dendritic cells (BMDCs) have been shown to induce an antigen-specific immune response in mice and, when loaded with tumor-specific antigens, significantly delay tumor growth and recruit immune cells to the tumor site. Our previous work investigated the combination of BMDC EV treatment with checkpoint blockade therapy (anti PD1 or anti PD-L1 antibodies) and showed a synergistic effect with EVs. As the BMDC EVs contain both PD1 and PD-L1, we speculated that EVs from PD1 or PD-L1 knock out (KO) mice would induce stronger anti-tumor responses than WT EVs.

Mice were immunized with WT, PD1 KO and PD-L1 KO BMDC EVs, and all EV-injected groups showed a similar number of antigen-specific CD8 T cells detected in the spleen. However, following *ex vivo* restimulation of the splenocytes, OVA specific CD8 T cells from the PD1 and PDL-1 KO EVs were found to be more potent than the WT treatment.

In contrast, when EVs were injected as tumor therapy in a mouse melanoma model, WT EVs performed better than the two knock outs in delaying tumor growth. All the EV treatments induced a potent anti-tumor response by recruiting several immune cell types at the tumor sites, but the absence of checkpoint molecules on the EVs dampened the antigen specific response and altered the antibody response elicited against the tumor antigen. This contrasts with earlier findings in tumor-derived EVs, where exosomal PD-L1 has been shown to inhibit immune responses to the tumor.

Taken together, we have confirmed that BMDC EVs loaded with antigen are potent immune stimulators and promising tools for immunotherapy. Additionally, the checkpoint molecules present on theses EVs may play a functional role in immune activation and are important in eliciting a strong antigen specific anti-tumor response. Our results also highlight the need to further investigate the PD1/PD-L1 axis in cancer therapy.

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1763 – P1.09.50

Phenotypic analysis of cytokine-induced killer cells (CIK): identification of receptors involved in their functional capacity

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Cytokine-induced killer cells (CIK) cells are a heterogeneous group of immune effector cells exhibiting a mixed T- and natural killer (NK) cell-like phenotype, obtained by in vitro incubation of human peripheral blood mononuclear cells (PBMC) with interferon (IFN)-γ, interleukin (IL)-2, and anti-CD3 monoclonal antibody. Their cytotoxic capacity is mainly attributable to $CD3^+CD56^+$ cells and it is not restricted by the major histocompatibility complex (MHC), allowing them to recognize virus-infected or malignant cells in the absence of MHC. The regulation of CIK cell function by inhibitory and activating receptors is a crucial aspect of immune regulation and phenotype characterization is essential to understand their functional properties and optimize their use in cancer therapy. In this study, CIK cells were cultured and expanded from PMBC of healthy donors with the addition of IFN-γ, IL-2, and anti-CD3 antibody for 21 days. The phenotype of CIK cells has been characterized, before and after the expansion, to determine the expression of activating and inhibitory receptors. The results show that the majority of CIK cells obtained at the end of expansion are CD8+ and express the TCR-alpha/beta. There was a significant increase in the activating/costimulatory receptors DNAM-1 and NKG2D, while the most expressed inhibitory receptors were TIM-3 and LAG-3. Furthermore, we found a significant increase in CD6, Fas, and Perforin expression. On the contrary, a significant decrease was observed in the expression of KIR2D and NKp80. Analysis of CIK function showed that CD3 and to a lesser extent NKG2D play a role in redirected degranulation and cytokine production. Inhibitory receptors also regulate the functional capacity of these cells. In summary, the results support that advances in the phenotype and functional analysis of CIK cells are critical for the development of effective immunotherapies for cancer treatment.

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Programmed death-ligand 1 Expression on T Lymphocytes is Associated with β2-microglobulin Levels in Treatment-naïve Patients with Chronic Lymphocytic Leukaemia

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The aberrant expression of co-inhibitory proteins is well-documented in patients with chronic lymphocytic leukemia (CLL), where βeta 2 microglobulin (β2M) serves as a key prognostic marker for patients treated with chemotherapy and chemoimmunotherapy. Nevertheless, the predictive value of β2M as a biomarker in effectively identifying the efficacy of immune checkpoint inhibition therapy in untreated patients with CLL remains unexplored. This study aimed to evaluate T cell-mediated responses to immune checkpoint inhibition in untreated patients with CLL. Furthermore, we investigated correlations between immune checkpoint expression on T helper and cytotoxic T cells; and β2M status. We assessed immune checkpoint expression on T cells from patients with CLL using flow cytometry. In addition, we measured baseline β2M levels using enzyme-linked immunosorbent assay (ELISA) protocol. Soluble immune checkpoint proteins; including PD-1, PD-L1, and cytotoxic T lymphocyte antigen-4 (CTLA-4) were quantified using a multiplex assay. After adjusting for age and sex, β2M levels strongly correlated with soluble PD-1 ($r = 0.65$, $p = 0.022$), and the surface expression of PD-L1 ($r = 0.60$, $p = 0.036$) on T lymphocytes. The expression of PD-1, PD-L1, and CTLA-4 was increased following T cell activation with PMA ($p<0.05$). Our results revealed no significant differences in the expression of PD-1, PD-L1, and CTLA-4 following inhibition with PD-1/PD-L1 blocking antibody. Overall, these findings suggest that immune checkpoint profiling in patients with CLL and the association between β2M and the PD-1/PD-L1 axis may be useful in identifying patients that may benefit from PD-1/PD-L1 checkpoint-based therapies. Additionally, PD-1/PD-L1 upregulation on T cells is linked to markers of disease progression, highlighting the role of β2M in inhibiting T cell responses in CLL patients. Monitoring β2M levels in patients with CLL may provide valuable insights into patient responses to immunotherapy targeting the PD-1/PD-L1 axis.

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1827 – P1.09.52

Developing new and effective strategies for adoptive T- cell therapy in pediatric gliomas

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Tumors of the central nervous system (CNS) are the most common form of childhood malignancy, and remain the leading cause of cancer-related morbidity and mortality among children. Cancer immunotherapy holds a lot of potential as a targeted therapy designed with high affinity to locate specifically the tumor cells and act directly on them, however, its applicability in the context of gliomas seems limited, in part, by the lack of ubiquitously expressed tumor antigens. our studies identified a previously uncharacterized biomarker, a mitochondrial protein that is highly expressed in pediatric gliomas that holds potential for serving as a novel CAR target with a dual function for cancer immunotherapy in pediatric gliomas.

The aim of this project is to contribute to the state of art of immunotherapy in pediatric gliomas. This will be done in different directions, First by screening different pediatric models that potentially express our novel biomarker, second by improving the efficacy of our specific CAR-T cells enabling monitoring their pathway and biodistribution utilizing gold nanoparticles (GNPs). Lastly, by Integrating CAR-T administration with targeted inhibitors to investigate potential synergistic effects against pediatric gliomas.

We have developed elegant immunocompetent mouse models of pediatric brain tumors by cloning mutations/fusions found in human pediatric patients into Cre/loxP-inducible lentiviral vectors. We validated the expression of our novel antigen on the surface of different pediatric models by using flow cytometry analysis. Next, we generated specific CAR-T cells against this antigen and validated their specificity and functional activity. This was done by evaluating the ability to stimulate the engineered T cells and their target killing capacity. Untransduced (UT) lymphocytes were used as controls in all the experiments. Notably, while the specific CAR-T cells were able to exert their cytotoxic effect, proliferate and secrete IFN-g when co-cultured with glioma cells, UT T cells showed little to no response. Altogether, these results support the potential anti-angiogenic effect of these CARs.

We are currently assessing the efficacy of these CAR's *in vivo* using our pediatric models. In addition we will monitor the CAR-T cell migration and infiltration (biodistribution) using gold nanoparticles (GNPs) that can be tracked using CT screening.

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1909 – P1.09.54

Identification of different subsets of cytokine-induced memory-like NK cells according to their phenotypic and proteomic profile: correlation with effector function

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Purpose: Cytokine-induced memory-like (CIML) NK cells are generated by *in vitro* culture with a combination of IL-12, IL-15 and IL-18. These CIML NK cells possess optimal characteristics for cancer immunotherapy, such as longer lifespan and increased cytotoxicity. Based on CD16 expression, this study aims to characterize two subsets of CIML NK cells phenotypically and functionally.

Methods: NK cells were isolated from healthy donor buffy coats and stimulated overnight with IL-12/15/18 at concentrations of 10ng/mL, 1ng/mL, and 50ng/mL, respectively. Following stimulation, NK cells were cultured with IL-15 (1ng/mL) for 7 days. Phenotypic and functional analysis of CIML NK cell subsets based on CD16/CD56 expression was done by flow cytometry. In addition, CD16+CD56+ and CD16−CD56+ subsets were sorted via FACS for high-throughput multiplexed quantitative proteomics. Statistical comparison of FACS analyses included non-parametric Friedman and Durbin-Conover test. The Benjamini-Hochberg False Discovery Rate (FDR) correction was applied for proteomic analysis, considering peptides/proteins exhibiting FDR<0.05 as significantly different.

Results: Phenotypic analysis of CIML NK cells revealed significant differences between the CD16+CD56+ and CD16−CD56+ subsets. CD16−CD56+ cells exhibited higher expression of activating receptors, increased Granzyme B production, and reduced expression of inhibitory receptors compared to CD16+CD56+ cells. In response to melanoma cell lines, CD16−CD56+ NK cells exhibited higher degranulation (CD107a/b+) compared to CD16+CD56+ cells. Additionally, proteomic profiling identified 35 proteins out of 4750 showing differential expression between the CD16+CD56+ and CD16−CD56+ subsets. Among these, 22 were significantly decreased and 13 were significantly increased in the CD56+CD16+ NK subset (FDR<0.05). Relevant proteins such as Granzymes, CD273, CD39, CD158b, and Fc receptors were identified in this comparative analysis.

Conclusion: In summary, our research offers a thorough characterization of *in vitro*-expanded CIML NK cells in terms of CD16 expression. We found that CIML NK cells with a CD16−CD56+ phenotype exhibit a greater degranulation capacity against tumor cells. Finally, our analysis identified potential NK cell biomarkers that could enhance their clinical efficacy.

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1923 – P1.09.55

Tumor-specific, AAV-mediated anti-PD-1 therapy combined with CAR-NK-cells as experimental immunotherapy for glioma

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Purpose: Glioblastoma (GB) is the most common primary brain tumor, characterized by a highly immunosuppressive tumor microenvironment (TME). While CAR-NK cells targeted against HER2 have shown activity in several murine glioma models, effect in advanced tumors was limited. IFNγ secreted by CAR-NK cells contributes to PD-L1 upregulation and suppression of intratumoral T-cell activity. Tumor-targeted Adeno-associated viral vectors, such as HER2-AAVs, coding for immunocheckpoint inhibitors (ICIs), are a promising tool to achieve a specific intratumoral ICI therapy. This avoids the lack of therapeutically active ICI concentrations or side effects, drawbacks of systemic ICI therapy. Here, we evaluated the effects of combination immunotherapy with HER2-specific CAR-NK cells (NK-92/5.28.z) and HER2-AAVs in preclinical GB models.

Methods: The used AAVs encode an anti-PD-1 immunoadhesin (aPD-1) and are targeted against HER2 with designed ankyrin repeat proteins (DARPins). The immune response and the modulation of the TME are currently characterized with highplex multi-color flow cytometry, multispectral histology and NanoString RNAseq.

Results: HER2-expression levels correlated with HER2-AAV mediated gene transfer in GB cells. After transduction, secreted aPD-1 effectively bound its target on PD-1 expressing cells. aPD-1 efficiently disrupted the PD-1/PD-L1 axis, leading to re-activation of T-cells. CAR-NK mediated tumor cell lysis was not impaired by AAV-transduction. aPD-1 was detected at high intratumoral and low systemic concentrations after local injection of HER2-AAVs into tumor grafts in vivo. Combination therapy significantly prolonged survival in subcutaneous and orthotopic GL261 and Tu2449 immunocompetent mouse models, resulting in complete tumor control in several animals.

Conclusion: Local combination therapy with CAR-NK cells and HER2-AAVs encoding aPD-1 is a promising immunotherapeutic strategy for GB, with potentially enhanced efficacy and reduced side effects.

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European Journal of Immunology Clinical-Basic-Translational

1959 – P1.09.56

Potential of inhibition of NLRP3 inflammasome in enhancement of immunotherapy in prostate cancer

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Purpose: The NLRP3 inflammasome is a crucial innate immune pathway. However, there is expanding evidence of its role in cancer cell biology. The increased expression of the NLRP3 inflammasome has been found in several malignancies and is associated with worse prognosis, a higher rate of tumor growth, and an immunosuppressive tumor microenvironment. Moreover, increased expression of INLRP3 has been associated with resistance to several chemotherapeutics and anti-PD-1 checkpoint inhibitors. Current studies show that chemoresistance can be overcome by inhibition of NLRP3. In this study, we investigated whether a pharmacological inhibition of NLRP3 inflammasome activation could enhance anti-tumor immune responses and and decrease tumor resistance to immunotherapy.

Methods: A selective small-molecule inhibitor, MCC950, was used to inhibit the NLRP3 activation. Cancer cell lines PC3 and LNCap transfected with fluorescent protein were treated with MCC950, and subsequently co-cultivated with PBMCs or lymphocytes from prostate cancer patients. The immune rejection was analyzed by cancer cell fluorescent intensity using fluorescent microscopy. Additionally, cell surface markers and cytokine production were evaluated by flow cytometry. The immunogenicity of MCC950-treated cancer cells was also evaluated using monocyte-derived dendritic cells, prepared from peripheral blood monocytes and loaded with cancer cell lysate.

Results: Our findings reveal that inhibition of the NLRP3 inflammasome by MCC950 has the potential to enhance antitumor immune responses by promoting cancer cell immunogenicity. Furthermore, NLRP3 inhibition leads to reduced immunosuppressive signalling and cytokine production, such as PD-L1 downregulation and decreased release of IL-8, thereby decreasing the cancer cell resistance to antitumor immune responses. Additionally, we demonstrated that dendritic cells loaded with lysate from MCC950-treated cancer cells promoted the proliferation of $CD8^+$ T cells, suggesting its potential as a booster of dendritic cell-based vaccines.

Conclusion: Our findings revealed that targeting NLRP3 inflammasome could sensitize tumor cells to immunotherapy and might enhance antitumor effector functions of ex vivo-produced dendritic cells for cancer immunotherapy.

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1998 – P1.09.57

Towards the deciphering of immunological mechanisms of tumor immunotherapy-induced cardiotoxicity

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Immune checkpoint inhibitors (ICI) changed the landscape of cancer therapy but in 1% of the cases their use is associated with immune-related adverse events, such as fulminant myocarditis. ICIs are antibodies that block negative regulators of the immune response of T lymphocytes, including cytotoxic T lymphocyte associated protein 4 (CTLA-4), programmed cell death protein-1 (PD-1), and PD-ligand 1 (PD-L1). Seminal studies of patients with melanoma who died from fulminant myocarditis after combined treatment with anti-PD1 \ PDL1 and anti-CTLA4, identified infiltration of T cells into the myocardium suggesting that T cells are involved in ICI-associated myocarditis.

In order to investigate how T cells are linked to cardiac dysfunction in ICI myocarditis, we have set off to characterize the immune-phenotype of these patients. We set up and optimized a 34 parameter-panel for flow cytometry on peripheral blood samples (PBMC) enabling us to assess the activation state of T cell subsets, especially CD8.

For all patients we perform high sensitivity enzyme-linked immunosorbent assays (ELISA) in order to evaluate biochemical markers that are indicators of myocardial injury, such as ST2, atrial natriuretic peptide (ANP), NT- proBNP and Cardiac Troponin I (cTnI).
Our study could shed light into the immunological basis of ICI-induced cardiovascular toxicities.

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2011 – P1.09.58

the Effect of aberrant N-glycosylation of PD-L1 on the potency of anti-PD-1 treatments

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Immune checkpoint inhibitors (ICIs) have transformed the landscape of cancer therapy, yet the variability in patient response underscores the need for deeper mechanistic insights. This study investigates the impact of PD-L1 glycosylation on the efficacy of anti-PD1/PDL1 ICIs and introduces the Immune-Checkpoint Artificial Reporter (IcAR) technology for assessing ICI potency.

Cell lines were engineered to express wildtype PD-L1 and variants with single glycosylation site mutations (N35A, N192A, N200A, N219A), as well as a quadruple mutant (Nx4). Functional binding assays using IcAR-PD1 were conducted to evaluate the interaction between PD-L1 mutants and clinically relevant PD1/PDL1 ICIs.

While single glycosylation mutations showed comparable binding affinity to wildtype PD-L1, complete N-glycosylation site removal significantly diminished both staining intensity and functional binding. Notably, the N35A mutation exhibited a pronounced impact on the blockade efficacy of ICIs, particularly evident in Nx4 mutants where inhibition was absolute across varying concentrations (2.5 to 40 μg/mL).

Anti-PDL1 ICIs demonstrated consistent blockade of PD-1/PD-L1 interaction across all mutants, while anti-PD1 ICIs showed compromised efficacy, especially against glycosylated PD-L1, notably at the N35 site.

These findings elucidate the intricate relationship between PD-L1 glycosylation and ICI response, providing valuable insights into mechanisms of immunotherapy resistance and potential strategies for patient stratification. The introduction of IcAR technology represents a novel approach for assessing ICI potency in clinical settings, promising to advance precision immunotherapy strategies.

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2048 – P1.09.59

The influence of the metabolic phenotype of CD8+ T cells on immunotherapy response in patients with non-small cell lung cancer (NSCLC)

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Non-small cell lung cancer (NSCLC) accounts for nearly 85% of lung cancer cases and is the leading cause of cancer-related death worldwide. Immune checkpoint blockade (ICB) inhibitors have shown promising results in cancer treatment. Yet, a significant proportion of patients with NSCLC do not respond optimally to these therapies due to factors such as individual differences in immune response, tumour characteristics, and the tumour microenvironment (TME), which can influence how the immune system recognises and targets cancer cells.

Within the harsh TME, $CD8^+$ T cells are forced to undergo metabolic adaptations to sustain their survival and functionality. Therefore, the TME is recognised as a metabolic barrier to $CD8^+$ T cells, limiting their effector functions. It has been demonstrated that the metabolic mechanisms of $CD8^+$ T cells have important implications for the therapeutic efficacy of ICB. However, the specific metabolic changes within $CD8^+$ T cells that drive differential ICB response in NSCLC remain largely unknown. In this study, we aim to examine the metabolic profile of $CD8^+$ T cells isolated from patients pre and post ICB treatment.

Single Cell ENergetIc metabolism by profiling Translation inHibition (SCENITH) is a newly developed flow cytometry-based method to functionally profile single cell metabolism *ex vivo* and *in vitro*. By using SCENITH, imaging, and CRISPR, we aim to identify metabolic vulnerabilities that hamper $CD8^+$ T cell anti-tumour immunity and ultimately influence ICB response.

In short, the findings from this research will reveal the underlying metabolic mechanisms that influence treatment response variability and potentially paving the way for personalised immunotherapeutic strategies. Insights into these metabolic alterations could also lead to the development of targeted interventions, such as metabolic modulators or combination therapies, to augment the efficacy of ICB treatment in patients with NSCLC. This study signifies a critical step towards advancing precision medicine in cancer immunotherapy, offering new avenues to optimise ICB treatments and improve clinical outcomes for patients with NSCLC.

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2068 – P1.09.60

Immunomodulatory effect of ferric/nickel nanoparticles in vitro

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Introduction: Ferric nanoparticles are small carriers composed of iron oxide (Fe_2O_3). Due to their unique properties, they have been exploited for biomedical applications, including magnetic resonance imaging (MRI), hyperthermia, immunomodulatory treatments for cancer and many others. However, their cytotoxic and immunomodulatory therapeutic effects deserve to be explored further.

Methods: In our study, we evaluated the direct toxic effect of FN (ferric - nickel) NPs (nanoparticles) on CT26 (murine colorectal adenocarcinoma) and 3T3 (murine fibroblast) cell lines *in vitro*. Next, we tested the immunomodulatory potential of the NPs, assessing if pre-treatment of CT26 with FN NPs could affect the response (activation/ maturation/ polarization) of various immune cells *in vitro*.

Results: In our experiments, FN nanoparticles showed dose-dependent toxic effect and increased apoptotic rate in the CT26 cancer cell line, while the effect was lower in the 3T3 fibroblast cell line. According to the preliminary data from the immunomodulatory evaluation - where bone marrow derived dendritic cells (BMDCs) were pulsed and maturated with the lysates of CT26 or CT26 pre-treated with FN NPs – no change was observed in the expression of BMDCs´ maturation markers regardless of the FN NPs pre-treatment. Yet, when these BMDCs were mixed with T cells and re-stimulated with live CT26 cells later, the production of IFN-γ and TNF-α by the T cells was increased in the FN NPs pre-treated group (BMDCs pulsed with FN NPs pre-treated CT26 lysate).

Conclusions: FN NPs were able to produce significant direct cytotoxic effect on murine CT26 cell line, while this effect was lower in the 3T3 fibroblast cell line. Additionally, preliminary experiments showed that pre-treatment of CT26 cells with FN NPs improved the T cell immune response against the CT26 cell line suggesting an enhancement of their antigenicity.

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2136 – P1.09.61

Expression relationship of Nectin-2 and immune checkpoint molecules in pancreatic cancer patients

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Purpose: Checkpoint inhibitors such as anti-CTLA-4, anti-PD1 and anti-PDL-1 are used in the treatment of various cancers. However, they cannot be used in pancreatic cancer due to the lack of PDL-1 expression. Nectin-2 is a molecule that acts as a ligand for TIGIT, PVRIG and DNAM-1, which are checkpoint molecules of immune cells. Its role and targetability in pancreatic cancer are not fully known.The aim of this study is to cross-sectionally analyze the expression levels of Nectin-2 and its ligand receptors TIGIT, PVRIG, DNAM-1.

Methods: In our study, the expressions of Nectin-2, CD8, TIGIT, PVRIG, DNAM-1, PD-1, PD-L1, LAG3, TIM3, VISTA, KI-67 molecules were examined in tumor tissues of 131 patients diagnosed with pancreatic cancer.

Results: Nectin-2 was not expressed in undiseased pancreatic tissue, whereas it was expressed in 30.5% of tumor tissues. PD-1 and PD-L1 expression was not observed in any of the patients. In the group with low density of CD8 TILs, the number of patients with negative Nectin-2 expression was higher than the number of patients with positive expression. In 32 patients with positive expression of Nectin-2, KI-67 proliferation marker was highly expressed. In the patient group where Nectin-2 was not expressed, the number of patients showing TIGIT expression increased. PVRIG and DNAM-1 were also not observed in the absence of Nectin-2. Patients without Nectin-2 expression had a higher number of patients without LAG3 and TIM3 expression. Nectin-2 was observed to be significantly underexpressed in stages 3 and 4 of pancreatic cancer. When the relationship between TIGIT receptor and the expression of PVRIG and DNAM-1 molecules was analyzed, a negative relationship was found and it was statistically significant. There was a positive correlation between LAG3 expression and TIM3 expression. Lenf node metastasis was also significantly increased in patients with positive LAG3, TIM3 and VISTA expressions. Expression levels of VISTA molecule showed a significant difference in clinical stage 3.

Conclusion: These data suggest that there may be a close relationship between Nectin-2 expression and the function of CD8+ cells. Further studies are needed to explain the mechanism of this relationship and to demonstrate the targetability of Nectin-2.

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2186 – P1.09.62

Assessing immune checkpoints on NK cells and the potential for immune checkpoint inhibition to reverse NK cell exhaustion in Metastatic Melanoma Patients

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Introduction: Metastatic melanoma (MetM) is an aggressive form of skin cancer that unlike early stage melanoma, cannot be treated with surgical resection. The discovery of immune checkpoints inhibitors (ICIs) have become a new standard of care for treating patients with MetM. The expression of many immune checkpoints on NK cells have been confirmed. We set out to assess the expression of ICIs on NK cells of MetM patients and propose that targeting such ICIs for blockade could restore NK cells cytotoxic activity and cytokine production, thus further potentiating the overall anti-tumour response.

Methods: Peripheral mononuclear blood cells were isolated from healthy donors and MetM patients. The ICIs TIM-3 PD-1, TIGIT and LAG-3, metabolic markers and components of the tumour driving TGFβ-GARP signalling axis where fluorescently labelled and assessed on NK cells ex vivo by flow cytometry. Similarly, interferon-γ and granzyme B where assessed in vitro. NK cell cytokine production and cytotoxicity from MetM patients were assessed post-blockade of the ICIs TIM-3, PD-1, TIGIT and LAG-3 on NK cells.

Results: NK cells from MetM patients appear to have increased TIM-3, LAG-3 and PD-1 expression in comparison with healthy donors. MetM patients also appear to show decreased Interferon-γ and granzyme B production in comparison with healthy donors post overnight treatment with IL-12/15. CD69 expression is also significantly increased in MetM patients compared to healthy donors. Expression of TIGIT in MetM appears to have varying expression amongst healthy donors and patients.

Conclusions: Our data shows that NK cells of MetM patients exhibit some characteristics of immune exhaustion with TIM-3, PD-1 and LAG-3 expression increased and interferon-γ and Granzyme B production decreased. NK cells from MetM patients also have greater activation in comparison with healthy donor. We set out to further assess if immune checkpoint inhibition can restore patient Interferon-γ and Granzyme B expression to a similar level seen in healthy donors and reverse NK cell exhaustion which could elucidate its potential as a targeted checkpoint for MetM.

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2191 – P1.09.63

Identifying vulnerabilities to immune checkpoint inhibitors of oncogene-addicted non-small cell lung cancer subgroups

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Purpose: Even though the treatment with PD-(L)1 axis blockers induces tumor response in approximately 20% of unselected patients with advanced NSCLC, patients harboring EGFR alterations have poor outcomes with anti-PD-(L)1 antibody. Targeted therapy with tyrosine kinase inhibitors is the standard of care for patients with advanced-stage EGFR-mutant. However, resistance to kinase inhibitors is almost inevitable.

EGFR-mutant NSCLCs typically have a low TMB, low levels of $CD8^+$ T cells and natural killer cell infiltration, a limited T cell receptor repertoire and abundant infiltration of Treg cells, which might account for the poor efficacy of ICIs of this genomic subtype. We propose to investigate potential vulnerabilities and opportunities to overcome primary resistance to ICIs conferred by oncogene addiction in these patients.

Methods: In order to identify mechanisms involved in the restricted immune response of this subgroup we are performing a sub-genome-scale *in vivo* CRISPR/Cas9 screening. Murine cell lines were derived from the genetically-engineered mouse model of EGFR mutant lung cancer bearing exon 19 deletions or the L860R missense mutation. Genetically modified cell lines with the CRISPR/Cas9 library will be implanted subcutaneously into the flank of immunocompetent mice. A group of mice will then receive ICI treatment at different days post-tumor challenge and sgRNA abundances between the different mouse groups will be compared.

Results: Both oncogenic EGFR mouse cell lines were sensitive to an EGFR-specific TKI, showing a decrease in phospho-Y1068-EGFR. These results demonstrate the oncogenic dependence on EGFR signaling for growth.

In addition, EGFRdel19 or EGFR-L860R tumors injected subcutaneously were resistant to anti-PD1 and/or anti-CTLA-4 blocking antibodies, similar to the lack of response to human EGFR mutant NSCLC to immunotherapy.

Nowadays, we are conducting the *in vivo* sub-genome-scale screens and quality control analyses indicated excellent screen performance. Indeed, the vast majority of sgRNAs were well represented in all conditions and recovered within replicates.

Conclusion: We expect to identify new resistance mechanisms that could result in novel treatment strategies for these NSCLC subgroups. The identification of these targets will provide the opportunity to reprogram the tumor microenvironment and to improve the efficacy of immunotherapy in a subset of patients with lung adenocarcinoma.

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2220 – P1.09.64

Signatures of response to CD19 CAR-T-cells in diffuse large B-cell lymphoma

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Diffuse large B-cell lymphoma (DLBCL) is the most common aggressive lymphoma and is clinically and molecularly a heterogeneous disease. Using whole exome sequencing, we recently identified 5 genetic subtypes of DLBCL that provided novel insight into the biology and prediction of prognosis and identification of combination targeted therapies (Chapuy et al. Nat Med 2018). CD19-directed adoptive T-cell therapy with chimeric antigen receptor (CAR)-T cells is the standard of care treatment for refractory and or early relapsed DLBCL. While the advent of CAR-T-cells significantly improved overall survival of patients, still approximately 50% of patients relapse and eventually succumb to their disease. Hence, understanding the mechanisms of resistance to CART-cell therapy reflects an unmet medical need. Here, we explore the role of the molecular heterogeneity of DLBCL towards response to CD19-CAR T-therapy. To capture the molecular heterogeneity, we selected a representative panel of 13 DLBCL cell lines reflecting the genetic subtypes. CD19 as an important biomarker for interaction of CD19-CAR-T with the target cell, was shown to be highly varying in protein and surface expression between the different cell lines. A co-culture assay with CD19-CAR-T cells revealed strong differences in cytotoxicity and T-cell activation, ranging from 10 to 90 % killing. Overall, we noted that the strongest biomarker of response is the CD19 expression. Indeed, we noted a correlation of CD19 epitope expression on the surface to sensitivity of CAR-T-response. Currently, we are performing additional mechanistic experiments to understand additional signatures of response and resistance.

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P1.10 CANCER VACCINES

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14 – P1.10.01

Development of ProsVac-INCAN: an mRNA vaccine against prostate cancer

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Background: In 2020, The World Health Organization (WHO) reported that in men, prostate cancer (PCa) was the second most common type of cancer and fifth cause of death from cancer in the world, and its incidence is expected to increase by at least 50% in the next decades. In the last three years, the COVID-19 pandemic promoted the development of mRNA-based vaccines. This technology can also drive the development of novel treatments for other diseases due to its flexibility, productivity, non-genomic integration and low costs, along with the potential to induce both humoral and cell-mediated immunity.

Purpose: Our goal was to identify immunogenic epitopes from tumor associated antigens (TAAs) reported in the literature as related to the development and progression of prostate tumors to generate a therapeutic vaccine.

Methods: We used a 3-step, *in silico/in vitro/in vivo* approach to identify HLA-restricted, immunogenic epitopes derived from tumor-associated antigens (TAAs) previously reported as overexpressed in PCa, such as the prostatic acid phosphatase (PAP), prostate-specific membrane antigen (PSMA), Anterior Gradient 2 (AGR2), New Gene Expressed in Prostate (NGEP) and ERG, a component of the TMPRSS2:ERG gene fusion that occurs in about 50% of PCa cases. We designed longer epitopes that exhibited *in silico* cytotoxic and helper function through activation of CD8/CD4 T lymphocytes. Currently, predicted peptides are being tested in 9-week-old C57BL/6 mice for immunogenicity. The anti-tumor effects of epitope-specific cytotoxic lymphocytes are tested against human prostate tumor cell lines. The recognition capacity of the antibodies generated in mice will be tested on human tumor samples taken by biopsy.

Results: Immunogenic peptides will be chosen as vaccine mRNA molecules and injected into lipid nanoparticles in mouse models that develop prostate cancer. We propose to combine the vaccine with immune-checkpoint inhibitors to evaluate synergy.

Conclusion: Our findings will provide proof of concept for mRNA-based vaccines that are tailored to the tumor's molecular profile, and lay the ground for future development of mRNA cancer vaccines for clinical use.

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101 – P1.10.02

Dendritic cell targeting using a DNA multiepitope vaccine against EGFR for active breast cancer immunotherapy

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Purpose: DNA vaccines are a promising approach to cancer treatment. Active specific immunotherapy is a pivotal strategy to treat EGFR-positive tumor cells. EGFR overexpression appears to be associated with reduced overall survival and disease-free survival in breast cancer patients. The low immunogenicity of DNA vaccines in humans can be overcome by optimizing constructs. Previous works have shown that DNA vaccine targeting dendritic cells induced an immune response to the targeted antigen.

Methods: For this reason, we aimed to generate a highly optimized DC- targeting DNA multiepitope breast cancer vaccine against the overexpressed target EGFR. In silico construction included several CTL, CD4+ e CD8+ T lymphocytes epitopes. To enhance immunogenicity and increasing antigen presentation, sequences of a single chain Fv antibody (scFv) specific for the DC endocytic receptor DEC205 were attached with a linker to the N-terminal of the EGFR epitopes. Computational assessments were performed on scDEC205-EGFR fusion protein for antigenicity, allergenicity and physicochemical properties. Refined 3D constructs were subjected to structural B-cell epitope prediction. Validation through in vitro and in vivo experiments were done. BALB/c mice were intramuscularly immunized three times with 100ug of plasmid DNA encoding either scDEC205-EGFR; after first dose they were challenged with murine mammary adenocarcinoma 4T1 cells. Immune response assessment were accomplished using flow cytometry.

Results: Overall, the results suggest therapeutic potential for scDEC205-EGFR DNA vaccine against triple negative breast cancer. The vaccine constructs was sequenced and showed 100% identity in the nucleotide sequence aligned with the murine EGFR cDNA. This vaccine displayed the capacity to control primary tumor development, increase DC frequency and expression of MHC II molecules after specific-stimulation with EGFR peptide pool, decrease systemic levels of TNFα, and increase frequency of TIL.

Conclusion: In this research, a new active immunotherapy strategy for triple negative breast cancer treatment were constructed by linking specific EGFR protein epitopes for helper and cytotoxic T lymphocytes to sequences of a scFv antibody targeting DC endocytic receptor DEC205. Its capacity to modulate immune responses in vivo indicating potential efficacy. Further studies are recommended to study the scDEC205-EGFR DNA vaccine effect in solid tumors overexppressing EGFR.

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European Journal of Immunology Clinical-Basic-Translational

277 – P1.10.03

Characterization of hypoxanthine phosphoribosyltransferase 1 (HPRT1) mutated Hodgkin lymphoma cells

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Purpose: hypoxanthine-aminopterin-thymidine (HAT)-sensitive tumour cells can be used for delivery of tumour associated antigens to antigen presenting cells. For this purpose we established a HAT-sensitive variant of the Hodgkin lymphoma cell line L-428 and characterized the hypoxanthine phosphoribosyltransferase 1 (HPRT1) sequence from these cells.

Methods: by repeated treatment with 8‘-azaguanine (8AG), we established a HAT-sensitive variant of the Hodgkin lymphoma cell line L-428. Gene expression analysis of wild type (L-428wt) and 8AG resistant (L-428agr) cells was assessed by RNAseq. The HPRT1 cDNA sequences from L-428wt cells and L-428agr cells was cloned into mammalian expression vectors and used for transfection of the HPRT-deficient fibroblast cell line 1306. HPRT1 expression was tested by quantitative reverse transcription-polymerase chain reaction (qRT-PCR). Viability of cells in the presence or absence of 8AG or HAT was assessed by flow cytometry and ATP assays. Mixed lymphocyte/tumour cell cultures (MLTC) were used for analysing the effect of HAT on the lymphoma cells in the context of immune stimulation.

Results: L-428agr cells showed resistance against 8AG but high sensitivity for treatment with HAT, whereas L-428wt cells were resistant to HAT and sensitive for 8AG. By RNAseq analysis we identified a new mutation in the HPRT1 gene of L428agr cells. Transfection of the HPRT1 cDNA from L-428wt cells into 1306 cells reverted the sensitivity profile of these cells from 8AG resistance to HAT resistance. In contrast, transfection of the HPRT1 variant from L-428agr cells had no effect on drug sensitivity, indicating that this mutant is enzymatically inactive. Treatment of MLTC with HAT resulted in suppression of L-428agr cell growth whereas lymphocyte growth was not suppressed, resulting in net increase of T cells in these MLTC.

Conclusion: we identified a new mutant of the HPRT1 gene in 8AG resistant Hodgkin lymphoma cells. The established HAT sensitive lymphoma cells might be an interesting tool for immunotherapeutic applications, e.g. for in vitro stimulation of lymphoma-reactive T cells or for fusion with dendritic cells.

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1186 – P1.10.04

Induction of mucosal immunity by different therapeutic HPV16 vaccination approaches

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Persistent infection with high-risk types of the human papillomavirus (HPV) causes cancer in both women and men and accounts for approximately 5% of cancer cases worldwide. HPV16 is by far the most important cancer-causing high-risk type. Many therapeutic vaccines targeting HPV16-associated malignancies have shown to be highly effective in preclinical studies but lacked effectiveness when tested in human patients. To overcome this discrepancy, our group developed model systems that resemble the human system more closely. We established two orthotopic HPV16-dependent tumor models in MHC-humanized mice, which are located in the mucosa of the female genital tract and the base of the tongue, respectively. These models allow testing of different vaccine platforms containing clinically relevant HLA-A2-restricted epitopes against tumors at sites of natural infection. In first vaccination studies, we identified two vaccine platforms either consisting of amphiphilic constructs or silica nanoparticles (SiNP) that both successfully induced systemic $CD8^+$ T cell responses. SiNP were found to produce more reproducible results due to their robust synthesis procedure, which is why we focused on those in subsequent experiments. By comparing vaccinations with $CD8^+$ alone or in combination with $CD4^+$ T cell epitopes, it could be shown that the addition of $CD4^+$ T cell epitopes enhances vaccination efficacy. As mucosal tissues, such as in the female genital tract, are not readily accessible by systemic immune cells, we further focus on inducing a strong local immune response in the mucosa. Our approaches include different administration routes and prime-pull approaches in order to pull systemically induced T cells into the vaginal mucosa. The most promising vaccination strategies, as determined by these analyses, will be tested for efficacy by assessing orthotopic tumor shrinkage/elimination. Taken together, these experiments will be a crucial step in the preclinical assessment of new therapeutic HPV vaccine formulations. The obtained results will provide important insights for the design of clinical trials for therapeutic HPV vaccination.

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1696 – P1.10.05

Anti-tumor efficacy of a mesothelin-based nanovaccine in a KPC orthotopic mouse model of pancreatic cancer

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Immunotherapy has shown promising results in selected types of cancer, but its efficacy remains limited in pancreatic ductal adenocarcinoma (PDAC). Nanoparticles (NPs) can incorporate immunomodulating components, inducing a potent immune response. As mesothelin (MSLN) is a tumor-associated antigen overexpressed in PDAC, we evaluated the effect of MSLN nanovaccine therapy given at different time points in a syngeneic orthotopic KPC-PDAC mouse model. The NPs were successfully taken up by dendritic cells in vitro and were found in inguinal lymph nodes 24 h after subcutaneous injection into male C57BL/6 mice. Nanovaccine re-stimulation of splenocytes from vaccinated mice led to increased levels of IFN-γ in vitro when compared to splenocytes from mice treated with the NP control. Higher levels of MSLN-specific IgG antibodies were detected in the serum of mice treated with nanovaccine compared to that of control mice. Three vaccination regimens were tested, a prophylactic scheme that included vaccination before tumor induction and two therapeutic schemes, involving early and late vaccination after tumor cell inoculation. MSLN nanovaccination led to an inhibition of KPC tumor progression and metastasis and induced higher CD8+ T cell infiltration in the tumor that developed in response to prophylactic and early therapeutic schedules, but not in a later vaccination approach. Although the nanovaccine treatment elicited higher humoral and cellular antigen-specific responses in tumor-bearing mice in both vaccination strategies, the therapeutic vaccination also increased the expression of exhaustion markers in CD8+ T cells. Our results support the relevance of an MSLN-based nanovaccine as a new immunotherapy treatment for PDAC and propose an innovative way of vaccine delivery by the use of NPs. This project has received funding from the European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement No 861190–PAVE.

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P1.11 CELL COMMUNICATION AND SIGNALING

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275 – P1.11.01

Mapping the Effect of PD1 Immunoreceptor on T-cell Receptor Signalling

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Tumour cells can suppress immune cell activation by engaging immune checkpoints (ICs), such as PD1 on T-cells. Targeting these receptors with IC inhibitors (ICIs) can stimulate the anti-tumour immune cell activity. Clinical trials exploiting ICIs however show only poor success (response rates <15%).

While T-cell receptor (TCR) signalling is well described, little is known about IC signalling. The aim of our study is to map PD1 signalling to better understand its role in controlling T-cell activation in order to improve ICI efficacy.

Using quantitative mass spectrometry we mapped the signalling complexes formed by PD1 in T-cells under different conditions, e.g. in T-cell lines with TCR activation and TCR + PD1 activation. The proteomics analysis was complemented by phosphoproteomics, to directly look at signalling events, and transcriptomics to include the regulation on a transcriptional level. Multiomics analysis allowed us to delineate pathways that control the regulation of T-cell (de)activation.

Our analysis from TCR activated cells identified potential novel interactors of PD1, such as EZR and MSN. Proteins with similar binding profiles were assigned to clusters using Uniform Manifold Approximation and Projection analysis. Seven clusters were upregulated in PD1 expressing cells compared to control and functional analysis showed an enrichment of immune cell signalling related terms in PD1 expressing cells. Ingenuity Pathway Analysis further identified pathways related to T-cell activation including ‘RHOA Signalling’, ‘Actin Cytoskeleton Signalling’ and ‘Glycolysis’. Integrating the whole-cell proteomics and phosphoproteomics data and subsequent UMAP analysis identified a PD1 cluster enriched in immune signalling, supporting the findings of the interactomics data. Analysis of the phosphoproteomics data revealed a set of phosphorylated transcription factors (TFs). Linking these TFs to potential target genes observed in the transcriptomics data include IL3, CXCL8 and TFN, suggesting that TFs in the phosphoproteomics data from TCR stimulated T-cells regulate target genes related to signalling of immune cells.

In conclusion, our data suggests that the sheer expression of PD1 impacts TCR signalling. Stimulating PD1 with its ligand PD-L1 will give insights into how active PD1 signalling regulates T-cell (de)activation. A deeper mechanistic understanding of ICs will help to improve ICIs application in patients in the clinics.

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345 – P1.11.02

Engineered albumin as an effector negative scaffold for tailored design of long acting antibody fragment fusions

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The pharmacological effect of many protein-based biologics is limited by brief target exposure due to a short plasma half-life. A solution to this is to genetically fuse such biologics to the Fc region of IgG, which will prolong their longevity by increasing their molecular size above the kidney threshold as well as allow binding to the half-life regulator FcRn. However, Fc fusions may interact with other effector molecules and induce unwanted immune responses. Importantly, FcRn also binds albumin via a non-overlapping binding site and provides it with a long plasma half-life. While the Fc region is an N-glycosylated homodimer, albumin is a non-glycosylated single polypeptide that is effector negative. This is an advantage when the aim is to block a biological target. Here, I will discuss how knowledge about the relationship between FcRn and its ligands can be explored in the design of new formats that combine elements from antibodies with that of albumin as a strategy to achieve improved pharmacokinetics of antibody fragments or mimics.

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549 – P1.11.03

Mapping BTLA Immunoreceptor Signalling Pathways in T-cells

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Purpose: In recent years, immune checkpoints (ICs) have become major therapeutic targets in the treatment of malignancies with the successful development and approval of IC blockers (ICBs). ICBs prevent the interaction of ICs on T-cells with their ligands, therefore relieving T-cell suppression and promoting anti-tumour functions. However, responses are quite variable between cancers and patients and can be limited in many cancers. Understanding the mechanisms by which ICBs mediate their effects is key to defining ways to improve their efficacy. However, little is known about the signalling of ICs and the molecular modulations that can occur in T-cells following IC blockade. In this work, we focus on BTLA (B and T-lymphocyte attenuator), an IC that has become of interest as a novel promising target for cancer immunotherapy.

Methods: Two different commercially available anti-BTLA antibodies were used to pull-down BTLA in T-cell lines overexpressing the IC. Using quantitative mass spectrometry (qMS), we mapped the signalling pathways and inferred specific complexes formed by BTLA.

Results: The use of different anti-BTLA antibodies (binding to different BTLA epitopes) reveals distinct and overlapping populations of potential BTLA interacting proteins. Our analysis identified the BTLA known interactor PTPN11 (SHP-2) as well as other potential interactors involved in T-cell activation and cell adhesion, such as CD3E, ITGAL, ICAM3, NOTCH1 and IL-22. Reactome pathway analysis further identified pathways related to T-cell activation, including ‘PD1 signalling’, ‘Costimulation by the CD28 family’ and ‘STAT5 activation’. Other pathways related to ‘RUNX3 signalling’ and ‘Pre-NOTCH signalling’ were also identified. Complementing the interactomics data with whole and phospho-proteomics and transcriptomics delineates the pathways used by BTLA to induce T-cell suppression.

Conclusion: In conclusion, our data suggests that BTLA may block TCR activation by sequestering its downstream signalling proteins, therefore promoting T-cell suppression. A deeper mechanistic understanding of BTLA and its inhibition would help improve its application in the clinical setting.

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567 – P1.11.04

Integrating Seahorse real-time bioenergetics and click-uptake metabolic fluxes to unlock metabolic heterogeneity

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The detailed metabolic analysis that has advanced the field of immunology involve techniques that provide metabolic data from a pooled population of cells resulting in the loss of heterogeneity within the cell population. This is a major limitation of these techniques as immune cell populations usually show a high degree of heterogeneity in terms of functional outputs. Our aim is to unlock the heterogeneity of cell populations analysed for real time core metabolic flux using Seahorse technology.

This project harnesses the collective power of Seahorse XF Analyzer, click-uptake and Novocyte flow cytometer to enable complementing the bioenergetic metabolic measurements obtained using XF Analyzer of immune cell populations (cellular rates of energy production, relative contribution of glycolysis and mitochondrial activity, maximal mitochondrial bioenergetic capacity) with single-cell information related to the rate of nutrients uptake and rate of protein synthesis using flow cytometry and facilitated by the novel series of nutrient analogues obtained by post-uptake labelled using click chemistry technology. The core advance in our approach is to use biorthogonal, or 'click' chemistry to attach a fluorophore to the nutrient after it has been transported into the cell. This strategy avoids the pitfalls and flaws of other uptake assays using fluorescent-labelled nutrient-derivatives such as 2-NBDG. We introduced a number of optimizations to allow for complementary click-uptake in the Seahorse workflow, including program optimization, fixation, and cell detachment for flow cytometry. Using this approach we aim to characterize mixed populations of immune cells with reported metabolic heterogeneity, such as splenocytes and peritoneal cells of mice, challenged with LPS or PolyI:C.

Altogether, the results of this project will allow for complementary bulk and single-cell metabolic analysis in one sample, which can be employed for both basic research as well as in clinics.

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887 – P1.11.05

Inflammatory cytokines and chemokines are synergistically induced by co-culture of corneal epithelial cells and neutrophils in the presence of particulate matter

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Purpose: Ocular exposure to particulate matter (PM) causes local inflammation; however, the influence of neutrophils on PM-induced ocular inflammation is still not fully understood.

Methods: In this study, we constructed a system to investigate the role of PM in ocular inflammation using a co-culture of human corneal epithelial cells (HCE-T) and differentiation-induced neutrophils (dHL-60).

Results: To investigate whether HCE-T directly endocytosed PM, we performed holographic analysis, which showed endocytosis of PM in HCE-T. Cytokines and chemokines produced by HCE-T were measured using ELISA. HCE-T treated with PM produced IL-6, IL-8, which were inhibited by N-Acetyl-L-cysteine (NAC), suggesting the involvement of ROS. Co-culture with dHL-60 enhanced the production of IL-6, IL-8, and MCP-1. This suggests an inflammatory loop involving intraocular cor-neal epithelial cells and neutrophils. These cytokines and chemokines are mainly regulated by NF-κB. Therefore, the co-culture system was examined in the presence of an IKK inhibitor known to downregulate NF-κB activity. IKK inhibitors dramatically suppressed the production of these fac-tors in co-culture supernatants.

Conclusion: The results suggest that the inflammatory loop observed in co-culture is mediated through ROS and the transcription factor NF-κB. Thus, the co-culture system is considered a valuable tool for an-alyzing complex inflammation.

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Abstracts

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1033 – P1.11.06

Characterization of a directed-evolution–generated mutant form of alpha1-antitrypsin

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Background: Human alpha1-antitrypsin (AAT) is a 394-amino acid glycoprotein integral to the modulation of inflammation and tissue protection. Its upregulation during inflammatory states underscores its therapeutic potential for injury-driven pathologies. AAT is pivotal in epithelial barrier repair, influencing cell migration, survival, and macrophage differentiation. Recent investigations reveal multifaceted functions beyond its canonical serine-protease inhibition, implicating interactions with diverse binding partners. Compared to other species, humans have a relatively degenerate form of AAT. Using back-to-consensus and directed evolution approaches, we identified sites on its surface that appear to vary from most other animals. For example, human AAT has a lysine residue at position 243, while most other animals have either aspartate, glutamate or a serine residue at that position. By in-vitro screening of stimulated macrophage responses, functionally superior point-mutated recombinant versions of AAT were selected. Based on this multifaceted process, an alternative form of the molecule has been generated, rhAAT^{MJ6}. It includes, e.g., a K243D mutation.

Aim: Establish an experimental framework for a comprehensive comparison between rhAAT^{WT} and rhAAT^{MJ6}; gain understanding of novel structure-function properties of AAT.

Methods: Biological process directed outcomes will be compared between rhAAT^{WT} and rhAAT^{MJ6} treatments across epithelial repair models, both in-vitro and in-vivo, as well as cell migration, cell survival, and differentiation of macrophages to the M2-like phenotype. Molecular profile of rhAAT^{MJ6} will be examined in relation to its half-life and binding partners. Based on study data, modified versions of the molecule will be formulated and challenged experimentally in-vivo.

Results: According to an epithelial gap repair assay, rhAAT^{MJ6} outperformed rhAAT^{WT} in human lung and kidney cell lines. In-vivo, using a tympanic membrane perforation model, treatment rhAAT^{MJ6} has led to a faster recovery than clinical-grade AAT.

Conclusion: The preliminary findings serve as a foundation for investigating the study hypothesis. Ongoing and planned experiments are anticipated to unveil new insights into the structure-function relationship of AAT, specifically in regions beyond the protease-binding domain. These findings hold significant clinical promise for addressing various unmet medical needs, particularly for potential topical applications in conditions involving epithelial injury, such as tympanic membrane perforation and corneal injuries.

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1111 – P1.11.07

E. coli O83 EVs interact with human and mouse airway cells

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Background: *Escherichia coli* A0 34/86 (EcO83) is a commercially available probiotic bacterium used to prevent nosocomial infections in newborns. We have previously found that intranasal administration of EcO83 reduces experimental allergic airway inflammation in mice. All bacteria produce extracellular vesicles (EVs) in the form of nanoscale, bilayered structures packed with proteins, lipids and nucleic acids. EVs play a multifaceted role in bacterial communication, pathogenesis and interaction with the host immune system. Recently, we isolated EVs from EcO83 (EcO83-EVs) and showed that they reduce ovalbumin-induced allergic airway inflammation in mice. The exact mechanisms of interaction between the airways and EcO83-EVs were previously unknown.

Methods: We isolated (ultracentrifugation), purified (density gradient) and characterized (dynamic light scattering, transmission electron microscopy, cryo- electron microscopy, atomic-force microscopy, and biochemical characterization of cargo) EcO83-EVs. We investigated the interaction of EcO83-EVs with the human airways by proteomic analysis of primary nasal epithelial cultures treated with EcO83-EVs. We investigated EcO83-EVs-specific gene expression in the nasal-associated lymphoid tissue (NALT) and lungs of mice, as well as the uptake of EVs by lung cells. Finally, we investigated TLR4-induced signaling pathways and nitric oxide (NO) production in mouse macrophages activated by EcO83-EVs.

Results: Purification of EcO83-EVs resulted in the enrichment of membrane and fimbrial proteins. EcO83-EVs contain proteins, LPS and RNA, but no detectable DNA. EcO83-EVs induce the expression of proteins related to oxidative stress and inflammation in human primary nasal epithelial cultures. In mice treated intranasally with EcO83-EVs, the pattern of gene expression in the NALT and lungs was similar. In the lung, intranasal administration of EcO83-EVs leads to the recruitment of neutrophils, but only macrophages effectively take up EcO83-EVs. Mechanistic studies of the interaction between macrophages and EcO83-EVs have shown that EcO83-EVs induce NO in a TLR4-dependent manner and upregulate the NFκβ signaling pathway.

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Conclusion: In this study, we show that EcO83-EVs of the probiotic EcO83 interact with host´s airway cells and induce inflammatory and oxidative stress responses. EcO83-EVs are potent modulators of immune responses and, due to their non-viable status, offer a safe alternative to the use of whole bacteria in the treatment of allergic diseases.

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1420 – P1.11.08

The BiST of Burden: Harnessing biased STING agonists to enhance the resolution of inflammation and limit tissue fibrosis

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Stimulator of IFN Genes (STING) is a cytosolic DNA sensor that plays a central role in host protection against pathogens upon binding of DNA-derived ligands. STING primarily acts by controlling the transcription of type I interferons (IFNs) and pro-inflammatory cytokines. Notably, STING can be inhibited or activated pharmacologically to control STING-associated pathologies. 5, 6-Dimethylxanthenone-4-acetic Acid (DMXAA) is a pharmacological activator of murine STING that induces IFN-β and its affected genes. Here, we report that macrophages from DMXAA-treated mice engulfed significantly higher numbers of apoptotic cells ex vivo, and exhibited enhanced reprogramming reflected by an increased IL-10 and reduced inflammatory cytokine secretion upon LPS exposure. Macrophage reprogramming was significantly hampered in STING and IFN-β-deficient macrophages. Furthermore, we found used virtual docking and batch screening to identify biased STING agonists (BiSTs) that enhanced IL-10 and IFN-β production by splenocytes while inhibiting TNFα. One of these compounds, termed Biased STING agonist (BiST) 2.1, also induced the STING pathway in vivo and in human macrophages. Finally, we found BiST 2.1 to enhance the resolution of liver fibrosis induced by CCl_4. Thus, our findings indicate that STING can be harnessed to drive IFN-β-mediated IL-10 secretion by resolution phase macrophages and consequently shape macrophage function to enhance the resolution of inflammation and treat fibrotic disorders

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1776 – P1.11.09

Antiapoptotic and cryoprotective effect of the carotenoid Bacterioruberin on peripheral blood mononuclear cells

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Purpose: During the last few years, multiple studies have demonstrated the immunomodulatory properties of diverse natural carotenoids, highlighting their impact on the immune system, and revealing flattering results. In fact, a promising pigment known as bacterioruberin (BR), although discovered many years ago, has recently come into the spotlight showing more efficiency in its biological functions compared to those studied to date. Taking all this into account, the objective of this study was to analyze the antiapoptotic and cryoprotective effects of BR on peripheral blood mononuclear cells (PBMCs).

Methods: PBMCs were isolated from healthy donors using a density gradient centrifugation technique. Once isolated, two different experiments were performed to observe the possible antiapoptotic and cryoprotective effect of BR. First, PBMCs were cultured in 96-well plates at a concentration of 100 000 cells per well, with different concentrations of BR, ranging from 0 to 75 µg/ml, and incubated at 37°C and 5% CO_2. After 48 hours, apoptosis was induced using UV light and cells were incubated for another 24 hours. Moreover, isolated PBMCs from different healthy donors were cryopreserved at a concentration of 500 000 cells/ml with the same BR concentrations mentioned above and stored in liquid nitrogen for seven days. Acridine orange and ethidium bromide dyes were used for visualization of live, dead, and apoptotic cells by fluorescence microscopy and propidium iodide and YO-PRO-1 iodide for the analysis by flow cytometry.

Results: In the UV apoptosis-induced cultures of PBMCs, higher percentages of cells were still viable in the presence of low concentrations of BR as compared to the control (70% vs. 40%, respectively). On the other hand, increased percentages of alive cells were also observed when cryopreserved with lower concentrations of BR, compared to control conditions (90% vs. 80% respectively).

Conclusion: These results show that bacterioruberin can act as both an antiapoptotic and cryoprotective agent and may provide a basis for its use as a new therapeutic agent.

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1917 – P1.11.10

Nanotechnology-enhanced photodynamic therapy for remodeling the tumor microenvironment in colorectal cancer

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Purpose: Colorectal cancer (CRC) is a complex disease characterized by a diverse tumor microenvironment comprising immune and non-immune components, with macrophages being the most abundant tumor-infiltrating cells that exhibit distinct phenotypic states. However, owing to the remarkable plasticity of macrophages, their polarization status is dynamic and can be modified by integrating multiple signals from the neighboring milieu. The dynamic interplay between tumor cells and macrophages plays a critical role in shaping the immunomodulatory properties of the tumor microenvironment, affecting tumor progression and therapeutic responses. Moreover, the impact of both conventional and innovative therapies on the tumor microenvironment, where immune cells, including macrophages, play a crucial role, is often overlooked. This study aims to explore the nanotechnology-enhanced photodynamic therapy and drug-based strategies for targeting macrophages in the microenvironment of CRC.

Methods: A novel theranostic nanocomplex, composed of quantum dot and a photosensitizer, previously shown to accumulate in human skin mesenchymal cells, was tested for its efficacy in targeting human macrophages and CRC cell lines in co-culture. The induced transcriptomic changes, reflecting macrophage polarization state, were compared to the gene expression profile induced by several small molecule inhibitors, designed for targeting the stemness pathways.

Results: Under light excitation, the theranostic photodynamic nanocomplex effectively induced cell death across all colorectal cancer cell lines, irrespective of their molecular subtype. Importantly, the nanocomplex exhibited uniform accumulation and demonstrated no dark toxicity, while also avoiding transcriptome alterations in the CRC cells. Macrophages responded to conditioned media from nanocomplex-treated CRC cells by downregulating their M2-related gene expression and upregulating the M1-related gene expression, suggesting the potential repolarization from protumoral M2 type to the antitumoral M1 type. Stemness inhibitors demonstrated ambiguous effects on macrophages, inducing changes in gene expression associated with both M1 and M2 phenotypes, and allowing for further combination of several treatment strategies for obtaining the desired response.

Conclusion: Novel therapeutic approaches, such as theranostic photodynamic nanoparticles or stemness inhibitors, can act as immunomodulatory agents for repolarizing M2-like macrophages towards an antitumor phenotype. These findings justify further investigations to explore potential combination therapies for improved clinical outcomes in CRC treatment.

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2192 – P1.11.11

Exploring the dynamics of the surface proteins of exosomes and the repertoire of peptides presented by their human leukocyte antigen class I molecules

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Background: Exosomes (exos) are nanometer-sized extracellular vesicles released by cells and implicated in cell-to-cell signaling. Although they are often viewed primarily as transporters, and their cargo has been extensively studied, there is limited information regarding two aspects potentially related to their biological functions: (i) the influence of the microenvironment on the protein corona that covers their surface, and (ii) the characterization of the repertoire of peptides presented by their human leukocyte antigen (HLA)-class I molecules.

Objectives: Here, we aimed to analyze changes in the corona of exos under inflammatory conditions and to examine their HLA-I immunopeptidome.

Methods: Exos were isolated from a pancreatic cancer cell line and incubated with ascitic fluid (PAAF) as an inflammatory stimulus. For proteomic analysis of the corona, both untreated and PAAF-treated exos were pre-digested, 100 kDa-filtered, trypsin-digested, and analyzed by liquid chromatography coupled to mass spectrometry (LC-MS/MS). For immunopeptidomic analysis, HLA-I/peptide complexes were immunoprecipitated from both cells and exos, and peptide sequencing was performed by high-resolution LC-MS/MS.

Results: We identified sixty-six proteins that were upregulated on the corona of PAAF-incubated exos. Many of these proteins were also found in PAAF, suggesting that PAAF-derived proteins became attached to the exos corona after incubation. Gene-ontology enrichment analysis of these proteins revealed their involvement in the regulation of molecular and biological functions. Additionally, the HLA-I peptide repertoire of exos was compared with that of the originating cells. Although there was not complete overlap, most sequences exhibited a length consistent with HLA-I peptidomes and displayed high theoretical affinity.

Conclusion: Our findings provide valuable insights into the dynamics of the protein corona on exos under inflammatory conditions. The attachment of microenvironment-related proteins may influence their biological function, potentially extending inflammation to distant target cells. Furthermore, the immunopeptidome presented by exos offers a new source of peptides that could trigger an immune response complementary to their cell of origin. This information could be instrumental in developing novel therapeutic strategies for cancer and inflammatory-based pathologies, for which the role of exos has been widely proposed.

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P1.12 CELLULAR MECHANISMS IN INNATE IMMUNOLOGY

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365 – P1.12.01

Cytotoxic units of innate lymphocytes and innate-like T cells as regulators of mammary gland immunity.

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Innate lymphoid cells (ILCs) are pivotal for maintaining tissue homeostasis under steady-state conditions and in inflammation. These cells function as a fundamental bridge between innate and adaptive immunity, alongside innate-like T cells (ILTCs), the unconventional T lymphocytes that rapidly respond to non-pepitde antigens. Recently, a cytotoxic subset of ILTCs has been identified in the context of tumor surveillance. Despite originating from αβ- TCR and γδ-TCR lineages, cytotoxic ILTCs exhibit a myriad of characteristics akin to ILCs and bear a striking resemblance to a subset of tissue-resident ILC1s originally described in the liver and in the salivary gland with cytotoxic properties. In this study, we discovered large numbers of cytotoxic ILC1s and ILTCs in breast tissue emerging during the phases of pregnancy and lactation. Using high dimensional flow cytometry and single-cell transcriptomics, we found that cytotoxic ILC1s and ILTCs share a wide array of phenotypic and functional features including Natural Killer (NK) cell-related receptors, residency markers and high levels of Granzymes B and C, implying a high cytotoxic potential. Furthermore, cytotoxic ILC1s and ILTCs are dependent on the transcription factor Hobit for their development and acquisition of the cytotoxicity program. Given their phenotypic similarity, we hypothesize that cytotoxic ILC1s and ILTCs share their location in the mammary niche and cooperate in regulating physiological processes of pregnancy and lactation, as well as in providing protection against threats such as viral infection. Our study will offer valuable insights into the pathophysiological properties of cytotoxic ILC1s and ILTCs, providing a foundation for their potential application in immunotherapeutic strategies.

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417 – P1.12.02

Calcium Macrophage differentiation and maintenance depends on oxidative phosphorylation and fatty acid consumption

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The differentiation of monocytes into calcium-macrophages is initiated by increased extracellular calcium and phosphate concentrations, the formation of calciprotein particles (CPPs), and the uptake of CPPs via macropinocytosis in the absence of additional growth factors. Calcium macrophages are characterised by their needle-like shape, high osteopontin production, and a pro-inflammatory cytokine response to lipopolysaccharide (LPS).

The aim of this study was to characterize the metabolic state of resting and LPS-activated calcium-macrophages and to identify the nutrients necessary for macrophage differentiation.

Monocytes were isolated from the blood of healthy human donors and subsequently differentiated into calcium-macrophages, GM-CSF-macrophages, or M-CSF-macrophages for 7 days. The metabolic state was analysed using the Seahorse analyser.

Resting calcium-macrophages use oxidative phosphorylation over glycolysis as their main source of energy. However, activation of calcium-macrophages with LPS induced a metabolic shift towards increased glycolysis and decreased oxidative phosphorylation. Blockade of glucose, fatty acid, or glutamine consumption revealed a strong dependency of calcium-macrophages on fatty acid consumption. Accordingly, the glucose concentration in cultures of calcium-macrophages was unchanged after 7 days of differentiation, whereas the glucose concentration in cultures of GM-CSF-macrophages and M-CSF-macrophages was decreased. The differentiation of monocytes into calcium-macrophages in culture media with and without glucose, amino acids, or fatty acids revealed a decreased elongation factor (EF) when fatty acids were not available. When two nutrients were removed simultaneously, monocytes differentiated to calcium-macrophages only when fatty acids were present. Glucose or amino acids alone were not sufficient to sustain calcium-macrophage differentiation. Proteomic analysis revealed a strong upregulation of FABP3-5 in comparison to GM-CSF-macrophages and M-CSF-macrophages.

In conclusion, calcium-macrophages strongly depend on fatty acid consumption and oxidative phosphorylation, both during the differentiation process and after maturation.

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547 – P1.12.03

The involvement of Neutrophil Extracellular Traps in Necrotizing Enterocolitis: Exploring the role of Neutrophils and their interaction with other immune cells in different organoid models

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Purpose: Necrotizing enterocolitis (NEC) is a leading cause of mortality in premature infants, but its exact cause remains unclear. Impaired immune responses, particularly neutrophil recruitment and neutrophil extracellular traps (NETs) formation, play a central role. Macrophages exhibit heightened pro-inflammatory responses and can degrade NETs. Various organoid models, including Transwell models and advanced 3D-printed microfluidic models, are used to study NETs' role in NEC and their interaction with epithelial cells and macrophages.

Methods: Intestinal organoids were generated from healthy and NEC tissue, co-cultured with immune cells and stimulated with LPS. Various assays such as MPO-SytoxGreen staining, NETs immunofluorescence, ELISA and Western blot were used to evaluate NETs formation and immune responses. FACS staining panels were used to profile inflammation. Coated Transwell inserts facilitated the establishment of intestinal monolayer models and enabled the investigation of neutrophil recruitment, inflammatory profiles and apoptosis/necrosis of epithelial cells. In addition, a microfluidic model with a vasculature enabled live cell imaging to study the interactions between immune cells during NEC development.

Results: LPS stimulation led to a significant increase in neutrophil recruitment, which was particularly pronounced in NEC organoids compared to healthy organoids. NEC cultures showed a clear upregulation of TLR4 expression after LPS stimulation. The immune profiles differed between the groups, with the healthy organoids showing strong cytokine secretion, while the NEC organoids showed reduced cytokine levels, suggesting immune dysregulation. Healthy tissue exhibited increased epithelial cell wall necrosis after LPS stimulation, with NEC organoids showing the highest levels, especially after co-cultivation. In addition, increased NET formation was observed in LPS-stimulated organoid cultures. Collagen-coated Transwells facilitated the formation of an organoid monolayer and increased experimental versatility by allowing access to both the apical and basal sides of the intestine.

Conclusion: Our organoid models mimic conditions in the intestine and show increased neutrophil recruitment and NET formation in NEC with LPS stimulation, as well as increased TLR-4 expression. LPS induces inflammatory responses and NEC-like lesions, emphasizing the complexity of the disease. Altered cytokine profiles and increased epithelial cell necrosis highlight the dysregulation of the immune system in NEC.

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612 – P1.12.04

The metabolic profile of plasmacytoid dendritic cells from patients with systemic sclerosis

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Purpose: Plasmacytoid dendritic cells (pDC) are innate immune cells specialised in rapidly producing large amounts of type I interferon (IFN), playing an important role in anti-viral immune responses, and in different autoimmune diseases, such as systemic sclerosis (SSc). In fact, pDC are key to the onset and development of fibrosis in SSc, and a recent paper suggested that dysregulation of the tricarboxylic acid (TCA) cycle contributes to the type I IFN signature observed in these patients. However, the metabolic profile of pDCs from SSc patients remains to be elucidated. Thus, our study aimed to apply advanced techniques to characterise the metabolic profile of these cells.
Methods: Peripheral blood mononuclear cells (PBMCs) were isolated from blood of healthy donors and SSc patients. SCENITH, a single-cell flow cytometry-based method, was applied to infer the metabolic profile of circulating pDCs, which were identified as $CD304^+$ Lin^-, and analysed at steady-state and upon stimulation with CpG A. Toll-like receptor (TLR)9 activation by CpG A was confirmed by staining for S6 phosphorylation.

Results: Circulating pDCs were analysed in samples from 10 healthy donors and 14 SSc patients. pDCs from anti-centromere antibody-positive (ACA^+) patients displayed higher mitochondrial dependence and lower glycolytic capacity than those from anti-topoisomerase I antibody-positive (ATA^+) patients. Furthermore, pDCs from both ACA^+ patients and those with limited cutaneous SSc (lcSSc) showed a stronger response upon CpG A stimulation than cells from ATA^+, anti-RNA polymerase III antibody-positive (ARA^+) or diffuse cutaneous SSc (dcSSc) patients.

Conclusions: Our results suggest that pDCs from ACA^+ patients rely more on oxidative phosphorylation (OXPHOS) and are more responsive to external stimuli. These findings point to the possible contribution of the metabolic profile of pDCs for the SSc clinical course, unveiling new possible targets for therapeutic approaches.

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688 – P1.12.05

Monocytes drive myofibroblast contraction in a 3D skin model used to understand fibrosis in systemic sclerosis

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Purpose: Systemic sclerosis (SSc) is an autoimmune disease characterized by vasculopathy, fibrosis, and immune dysregulation. In SSc pathogenesis, circulating monocytes can be recruited to the skin and differentiate into macrophages, activating resident fibroblasts. However, how these cells exert their functions is not fully understood. Here, we investigated the role of monocytes in mediating (myo)fibroblast contraction and activation using an innovative 3D collagen hydrogel model.

Methods: For our 3D skin model we cocultured human primary dermal fibroblasts with either peripheral blood mononuclear cells (PBMCs) or CD14+ monocytes in a 3D collagen type 1 hydrogel. Subsequently, monocyte-driven tissue contraction was measured over time. We performed immunostainings for CD68, fibroblast activation protein (FAP), and alpha-smooth muscle actin (α-SMA) to evaluate cells activation. To investigate the signaling pathways involved, we measured transcription factor-driven luciferase production using reporter constructs in the same dermal fibroblasts. The activity of the following reporter constructs was determined after 24 hours: Sis-Inducible Element (SIE); SMAD-Binding Element (SBE); Nuclear Factor of Activated T-cells 5 Response Element (NFAT-5), and NFκB Response Element (NFκB).

Results: After 60 hours of co-culture, hydrogel plugs containing fibroblasts + monocytes displayed a strong contraction, seen by a (approx. 80-90%) decrease in the area of the plugs. Hydrogels containing only fibroblasts did not contract. Hydrogels with fibroblasts + monocytes contracted as fast as fibroblasts + PBMCs, but depletion of CD14+ cells from PBMCs slowed down contraction, showing that monocytes might strongly activate fibroblasts. The expression of FAP and α-SMA by fibroblasts also increased in monocyte-containing hydrogels, as well as CD68 expression, indicating enhanced monocyte differentiation/activation into macrophages by fibroblasts. Evaluating which intracellular pathway leads to fibroblast activation, we observed that SIE and NFκB reporter fibroblast constructs were strongly elevated in the presence of monocytes. Enhanced TGF-β activity using the SBE reporter was not observed. Together, our results suggest that inflammatory mediators may contribute to the monocytes-driving myofibroblast contraction.

Conclusion: This study highlights the importance of monocytes and the SIE and NFκB intracellular signaling pathways in (myo)fibroblast contraction in a 3D skin model, contributing to understanding the basic mechanisms of these cells in SSc skin fibrosis and thickness.

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707 – P1.12.06

Systemic immune response to cold exposure is linked to γδ T cells dynamics

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Purpose: This study aims to investigate the systemic immune response during cold acclimation in a model subjected to short-term or long-term mild cold exposure, with a focus on understanding the interplay between cold exposure, neural signaling, immune response, and thermogenic regulation.

Methods: A rat model was utilized to examine the effects of short-term or long-term mild cold exposure. Additionally, a group of regular cold swimmers was included to provide human relevance. Various parameters were measured, including activation of brown adipose tissue, adipose tissue cytokine production, systemic immune response (including leukocyte proportions, activation status, and cytokine production), and the involvement of γδ T cells using flow cytometry, ELISA, and qPCR. RNA-seq was employed to elucidate the mechanisms by which γδ T cells participate in the response to cold. Furthermore, rats exposed to cold were challenged with a Pam2CSK4 (Toll-like receptor 2 agonist) to assess modulation of the immune response.

Results: One-day cold exposure triggered a stress response characterized by cytokine production in white adipose tissue, subsequently activating brown adipose tissue and inducing thermogenesis. Systemic immune response analysis revealed the pivotal role of γδ T cells in the broader response to cold adaptation. RNA-seq analysis provided further insights into the mechanisms underlying the involvement of γδ T cells. Additionally, the challenge with a Toll-like receptor 2 agonist showed significant modulation of the immune response in cold-exposed rats. Moreover, experiments performed on human volunteers confirmed the role of γδ T lymphocytes also in the human organism exposed to cold.

Conclusion: This study sheds light on the complex relationship between cold exposure, neural signaling, immune response, and thermogenic regulation. The findings significantly contribute to our understanding of the physiological acclimation that occurs in response to cold exposure, emphasizing the importance of γδ T cells in orchestrating the immune response during cold adaptation.

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791 – P1.12.07

BNT162b2 COVID-19 vaccination elicits the expansion of CD16+CD8+ T cells endowed with NK cell features

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Despite the success of the BNT162b2 mRNA vaccine, molecular mechanisms by which the BNT162b2 vaccine stimulates immune responses still remain not completely elucidated. In the course of analysis of CD8+T cells in individuals undergoing BNT162b2 coronavirus disease 2019 (COVID-19) vaccination, we observed in peripheral blood a sizable fraction of the CD8+ T cell pool expressing CD16. Expression of CD45RA in the absence of CCR7, CD28 and CD27 was the dominant pattern among the increased CD16+CD8+ T cells, consistent with TEMRA phenotype and thus a terminally differentiated status. This T cell subset expressed a wide array of classical Natural Killer cell-associated activating and inhibitory receptors as well as intracellular effector molecules, thus showing a distinct phenotype different from other effector CD8+ T cells and more similar to NK cells. CD16+CD8+ T cells were also characterized by interferon gamma (IFN-γ) response gene transcripts and stimulation through CD16 and other classical NK cell innate receptors could elicit a vigorous functional response. Remarkably, both CD16 and NKp30 were specifically upregulated by IL-15 stimulation on NKp80+ CD8+ T cells and engagement of NKp80 in combination with CD16 resulted in a synergic effect for their effector functions. On the other hand, CD16+ CD8+ T cells show a high expression of the inhibitory receptor GPR56, capable to limit their activation via CD16. These data indicate that terminally differentiated CD8+ effector T cells, upon BNT162b2 COVID-19 vaccination, could acquire NK cell characteristics, including Antibody-dependent cellular cytotoxicity (ADCC) and innate receptor-associated cytotoxicity. Thus, BNT162b2 COVID-19 vaccination provides an additional large fraction of ADCC-capable effector cell pool, endowed with innate functions and therefore able to potentially counteract a much wider array of diseases.

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1160 – P1.12.08

Development of the first primary mucosal mast cell model

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Purpose: Active players in the innate immune system, mast cells monitor tissues by responding to various signals from their environment. Their cytoplasm is filled with granules containing pre-formed inflammatory mediators, ready for rapid release through degranulation. There are two main subtypes of mast cells, associated with distinct anatomical niches: connective-tissue mast cells (CTMC) and mucosal mast cells (MMC). These cells differ in origin, location, phenotype, granule composition and functionality. However, the study of MMCs is hampered by the lack of pertinent experimental model.

Methods: We generated primary murine MMC from bone marrow precursors sequentially cultured in two media enriched in key cytokines found in successive sites of MMC ontogeny.

Results: This model summarizes *in vitro* the key features of tissue MMCs, mimicking original phenotype, granular composition and functions.

Conclusion: This model will provide a convenient tool to better investigate MMC responses to various signals involved in pathophysiological processes.

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1164 – P1.12.09

Ankylosing Spondylitis-Specific Isotype Immunoglobulin Glycan Profile Reveal Unique Inflammatory Responses of PMA-Differentiated THP-1 Macrophages

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Purpose: Ankylosing spondylitis (AS) poses a multifaceted challenge owing to its intricate autoinflammatory mechanism, involving both innate immune cells and innate-like immune cells in the inflammatory process. Additionally, although various inflammatory mediators have been implicated in this disease, the mechanisms underlying their expression remain ambiguous. The primary objective of this study is to analyze the inflammatory responses of PMA-differentiated THP-1 macrophages when exposed to AS or healthy control (HC) SIIg, elucidating their unique expressions.

Methods: SIIg were isolated from the serum of individuals with AS and healthy controls (HC) using affinity and gel filtration chromatography. The N-linked glycans of SIIg were enzymatically cleaved using PNGase F to remove glycosylation. The molecular weights of SIIg and deglycosylated SIIg were determined using Western Blot analysis. PMA-differentiated THP-1 macrophages were then stimulated with AS or HC SIIg, either treated or untreated with PNGase F, for 24 hours. The supernatants collected from these cultures were analyzed for the expression of TNF-α and IL-10 using ELISA, and a comprehensive cytokine analysis was performed using the Human XL cytokine array.

Results: In the ELISA analysis, it was observed that the AS group displayed a higher IL-10/TNF-α ratio following 24-hour stimulation compared to the HC group. The comprehensive cytokine analysis revealed increased expressions of many inflammatory mediators in the AS group at the 24-hour mark. Molecular weight estimation indicated larger sizes of N-glycan SIIg in both AS and HC samples compared to deglycosylated SIIg. Moreover, lacking N-glycan SIIg stimulation of THP-1 macrophages resulted in decreased TNF-α and IL-10 expressions compared to stimulated by SIIg N-glycan.

Conclusion: The findings from this study support the clinical hypothesis, emphasizing the significance of N-linked glycosylation on SIIg from both AS and HC individuals in driving inflammation in THP-1 macrophages. Particularly noteworthy is the cytokine comprehensive analysis, which identified several pivotal inflammation-related molecules linked to AS. These results provide a platform for exploring the intricate interplay between glycosylated immunoglobulin and macrophage cytokine expression, thereby establishing a groundwork for future investigations into AS pathogenesis and the potential development of therapeutics.

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1196 – P1.12.10

Dissecting monocyte metabolic reprogramming to SARS-CoV-2 vaccination in people living with diabetes

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Respiratory viral infections have been clinically linked with higher susceptibility to infection and more severe pathogenesis in people with type 1 diabetes (T1D), however it is not well understood what mechanisms are responsible for this. Hyperglycaemia, a hallmark characteristic for people with diabetes, has been associated with an impaired innate immune response to infection. Additionally, it is poorly understood how chronically elevated levels of a major fuel source, such as glucose, affects the tightly regulated relationship between cellular metabolism and the functional capacity of the immune system during respiratory viral infections. As such, people with diabetes are placed in high-risk categories for vaccination prioritisation strategies despite conflicting evidence regarding the longevity and magnitude of responses for these patients. Thus, we have deeply dissected the metabolic reprogramming observed in monocytes of the innate immune system in people with diabetes, in the context of mRNA SARS-CoV-2 vaccination.

Using clinical PBMC samples from the COVAC-DM study, we compared the metabolic rewiring that occurs upon vaccination in $CD14^+$ monocytes in 4 cohorts of patients: people with type 1 or 2 diabetes and glucose levels either above or on target, as defined by HbA1c values. Samples were taken from participants before vaccination, 7-14 days before their 3rd vaccination and 14 days after their 3rd vaccination. Using Met-flow for single-cell metabolic analysis, our results reveal distinct differences between the global metabolic phenotype of monocytes between healthy and diabetic groups as well as between groups with differing levels of glucose control. Metabolic flux through the glycolysis pathway and TCA cycle is elevated in individuals with T1D compared to healthy individuals before vaccination; maintained throughout the primary vaccination schedule and in response to vaccination. Additionally, individuals with poorly controlled glucose levels had significantly higher metabolic flux through the TCA cycle which is heavily involved in anabolic metabolism and energy production. Decreased metabolic flux through the anaerobic glycolysis pathway was also observed in individuals with T1D in response to vaccination. Determining the vaccine response in these diabetic cohorts will aid understanding into predictors of vaccine response and help ascertain the contribution of glucose control for effective immune responses.

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1202 – P1.12.11

Rheumatoid Arthritis monocytes and monocyte-derived macrophages are more inflammatory, less endocytic but display distinct TET expression and differentiation potential compared to Psoriatic Arthritis

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Purpose: RA and PsA share various pathogenic features, while also displaying significant differences at the clinical, cellular, and molecular levels. We investigate the inflammatory capacity of RA and PsA monocytes and monocyte-derived macrophages (Mo-MACs), in addition to other effector functions.

Methods: $CD14^+$ monocytes from peripheral blood mononuclear cells PBMCs) were isolated, then assessed following *ex vivo* LPS stimulation. Mo-MACs were generated via 7-day stimulation with M-CSF (50ng/mL) and polarised to M1 and M2. Inflammatory responses (IL-6, IL-1b, TNF-a, CXCL9-11, SLAMF1-7) were assessed by real-time PCR (RT-PCR). Frequency of monocyte subsets and expression of activation (CD40) and macrophage signature markers (CD64, CD163, CD206, SLAMF7) were assessed by flow cytometry. Endocytosis assays were performed on monocytes and Mo-MACs. Demethylation genes TET1-3 were assessed by RT-PCR. Finally, $CD14^+$ monocytes were cultured with a methylation activator (budesonide), and pro-inflammatory responses assessed.

Results: Significant increases in LPS-induced IL-6, IL-1b and CXCL9-11 expression were observed in RA vs PsA monocytes (all $p<0.05$). Expression of SLAMF1, 2 and 7 were significantly increased in LPS-induced RA and PsA monocytes vs HC, with SLAMF4 significantly decreased (all $p<0.05$). The increased pro-inflammatory response of LPS-stimulated monocytes was paralleled by significantly decreased endocytic capacity, especially for RA ($p<0.01$). RA and PsA Mo-MACs retained the hyper-inflammatory phenotype of their precursor cell. Expression of IL-6, CXCL9 and CXCL11 were significantly higher in RA Mo-M1 (all $p<0.05$), while IL-1b was higher in PsA ($p<0.05$). Heightened SLAMF7 expression was observed for RA Mo-M1 ($p<0.05$), with SLAMF2 significantly increased in PsA ($p<0.05$). Endocytic capacity was reduced in RA vs PsA Mo-M0. RA and PsA PBMCs exhibited decreased classical but increased intermediate monocyte frequencies vs HC. Expression of CD40, CD64, CD163, CD206 and SLAMF7 were higher in PsA classical and intermediate monocytes vs RA. Increased TET2 expression in RA monocytes ($p<0.05$) and TET3 in PsA ($p<0.05$) were observed. Budesonide decreased IL-6 and TNF-a expression in *ex vivo* LPS-stimulated monocytes.

Conclusion: RA $CD14^+$ monocytes and Mo-MACs are inherently more pro-inflammatory than PsA, though PsA monocytes appear more activated and primed for Mo-MAC differentiation. The distinct inflammatory pre-programming of monocytes may also involve altered epigenetic mechanisms.

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Abstracts

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1221 – P1.12.12

Polyherbal formulation NOQ19 Modulates Human Macrophage Polarization and Function

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Purpose: *NOQ19* is a new medicinal polyherbal formulation containing 19 ingredients derived from 13 different medicinal plants. While there is limited research on *NOQ19*, both pre-clinical and clinical investigations have shown that the herbs in *NOQ19* separately have many beneficial effects such as antiviral, antibacterial, antifungal, antioxidant, antidiabetic, and cough suppressant. *NOQ19* has been licensed by the Indian state ministry of Ayush for managing mild to moderate COVID-19 due to its promising outcomes in clinical studies. However, the impact of *NOQ19* on the immune system is not known. Here, we report a detailed analysis of the impact of *NOQ19* on polarization and function of primary human macrophages.

Methods: Human peripheral blood monocyte-derived macrophages were either left untreated or pre-treated with 500 μg/mL *NOQ19* extract for 2 h. Then they were polarized into M1(LPS, IFNγ), M2a(IL-4), or M2c(IL-10) macrophages for 22 h. M1 and M2 polarization markers were analyzed by RNA-seq, qPCR, flow cytometry and ELISA. Moreover, phagocytosis capacity of macrophages was analyzed using pHRodo conjugated heat-killed *Staphylococcus aureus* bioparticle.

Results: Our data showed that *NOQ19* increased the expression of the M1 markers IL-1β, CCL5, CXCL5, NOTCH3, and C3, while decreasing the expression of the M2 markers CD163, IL-10, MS4A6A, SELENOP and F13A1 at mRNA level. Furthermore, the surface expression of the M1 marker CD64 and the M2 markers CD200R, CD209 and CD163 was decreased in M0, M2a and M2c macrophages. While *NOQ*19 did not induce the production of TNF, IL-12(p70) or IL-1β, it decreased the levels of secreted IL-10 and TGF-β in polarized and unpolarized macrophages. Lastly, *NOQ19* treatment decreased the phagocytic capacity of both polarized and unpolarized macrophages.

Conclusion: *NOQ19* modulates human macrophage polarization and phagocytic function with a downregulation of receptors which are known to have distinct roles in phagocytosis. Recent studies indicate that CD209 may serve as a potential new entry receptor for SARS-CoV2 by facilitating endocytosis. Thus, *NOQ19* could be a promising new supplement to modulate macrophage polarization and function for the treatment of viral infections, such as SARS-CoV-2.

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1247 – P1.12.13

Impact of exogenous and endogenous CD40L on macrophage responses to IL-4

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The impacts of CD40-CD40L interactions on type 2 immunity are incompletely understood. While exogenous CD40 agonists bias responses towards the type 1 arm, the induction of most Th2 responses requires CD40-CD40L, suggesting that exogenous agonist delivery does not imitate endogenous CD40L signals. DC function is impacted by endogenous CD40L both expressed by antigen-specific $CD4^+$ T cells and delivered/sensed in the absence of specific interaction. We are examining these issues with a focus on macrophages, which respond to the type 2 cytokine IL-4 by expressing M(IL-4) markers, including RELM-α and Ym1/Chil3, and proliferating. *In vitro*, exogenous soluble CD40L (sCD40L) inhibited expression of RELM-α but had weak or nil inhibitory effects on expression of Ym1/Chil3 by resident or recruited peritoneal cavity or bone marrow-derived macrophages. The known inability of macrophages to proliferate in response to IL-4 *in vitro* was not rescued by sCD40L. Endogenous CD40L was detected under basal conditions in the peritoneal cavity only in $CD4^+$ T cells, mostly intracellularly but also at the cell surface. *In vivo*, interactions with CD40-expressing cells limit cell-surface CD40L expression by bulk $CD4^+$ cells. Such CD40-CD40L interactions may also generate tonic signaling on CD40-expressing cells including macrophages. Endogenous CD40L in the absence of antigen presentation did not influence RELM-α expression by peritoneal macrophages or allow their IL-4-driven proliferation *in vitro*, even in a "forced" system in which CD40 KO splenocytes were used as a source of $CD4^+$ cells expressing abundant cell-surface CD40L. Blocking endogenous CD40L *in vivo* in the context of IL-4 injection did not alter up-regulation of RELM-α or Ym1 by resident peritoneal macrophages, but caused a modest decrease in macrophage proliferation. In preliminary experiments, CD40 KO peritoneal macrophages transferred into IL-4-injected WT mice up-regulated RELM-α and Ym1 and proliferated similarly to transferred WT macrophages, suggesting no impact on these responses of basal endogenous CD40L detected directly by macrophages. In sum, exogenous CD40L antagonizes some macrophage responses to IL-4, whereas endogenous CD40L appears not to influence macrophage responses to IL-4 in the absence of antigen presentation. We are now extending this analysis to the antigen-specific interaction context. Funding: ICGEB; CSIC, CAP and ANII (Uruguay); Wellcome Trust.

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1325 – P1.12.14

Truncating mutations in NFKB1 lead to decreased expression of CCDC22 and COMMD proteins thereby enhancing inflammatory response

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Copper-metabolism Murr1 domain proteins (COMMD1–10) and coiled-coil domain-containing proteins (CCDC22, CCDC93) form a CCC complex that participates in endosomal recycling of diverse transmembrane proteins. Of these, CCDC22 is highly conserved, and it has been shown to act as a scaffold for COMMD proteins. CCDC22 together with COMMD1 are also known to regulate the NF-κB signaling. CCDC22 acts as an NF-κB activator by degrading IκB, and COMMD1 suppresses NF-κB by regulating its ubiquitination. Despite their known role in regulation of NF-κB, a key inflammatory transcription factor, understanding of CCDC22 and COMMD protein's role in immune signaling is limited. We recently described patients with loss-of-function variants of *NFKB1*. These patients suffer from severe sterile soft tissue inflammations, and their macrophages show overactivation of the NLRP3 inflammasome and type I interferon response caused by defective autophagic degradation of proteins in these signaling cascades. We employed transcriptome and proteome analysis to study the impact of truncating *NFKB1* variant (p.R157X) in THP-1 cell line. In transcriptomics p.R157X variant carrying THP-1 cells express decreased levels of *CCDC22*, and proteomics data shows downregulation of multiple COMMD proteins. To study the effect of CCDC22 alone, we silenced CCDC22 in wild type THP-1 cells and activated them with LPS, which led to increased secretion of IL-1β. Silencing of CCDC22 increased the expression of *IL1B* and *IFIT2*, and reduced expression of *NFKBIA* as analyzed by qPCR. Overall, CCDC22 silencing led to similar RNA expression pattern as observed in p.R157X variant carrying cells. To confirm the transcriptomic findings from THP-1 cell line, we analyzed *CCDC22* expression in *NFKB1* variant-carrying patients and found that expression of CCDC22 is reduced also in patient cells. Truncating mutations in NFKB1 cause decreased expression of CCDC22 and COMMD proteins which promotes proinflammatory state. Our research investigates a potential new role of CCC complex in immune dysregulation.

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1355 – P1.12.15

Macrophages activation upon phagocytosis of structurally different antigens

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Macrophages phagocytose various pathogens and are activated during this process. Macrophage response to viral antigens is insufficiently explored, especially inflammation-related signalling pathways. One of inflammatory pathways is inflammasome activation. NLRP3 inflammasome can be activated by many factors, including lysosomal damage. Previously, we showed that VP1-derived virus-like particles (VLPs) of human KI and MC polyomaviruses (PyVs) induce NLRP3 inflammasome activation in macrophages and this might define phagocytosis profile. Moreover, KIPyV and MCPyV VLP-induced cell activation was of different intensity. We aimed to study the relation of phagocytosis to cell activation.

We investigated phagocytosis of VLPs in comparison to that of *E. coli* and amyloid-beta oligomers (Aβ). VLPs and *E. coli* were labelled with pHrodo dye being fluorescent in lysosomes, while Aβ were labelled with FAM fluorophore. To detect intracellular Aβ-FAM signal, cell surface signal was quenched with Trypan blue. Human macrophages derived from THP-1 cell line were treated with these particles for 3 and 24 h, then stained for inflammation marker CD86 and CD83, and analysed by flow cytometer.

We observed different phagocytosis pattern of investigated particles. MCPyV VLPs were phagocytosed at the highest intensity. We identified cell populations engulfing low to high levels of particles. The distribution of these populations were different for each particle type, indicating variant phagocytosis process of investigated particles. Comparing the uptake of VLPs, we detected co-stimulatory molecules CD86 and CD83 on cell surface only in the case of MCPyV VLPs showing the differences in cell activation profile. MCPyV VLPs are large homogeneous particles while KIPyV VLPs are of heterogeneous size. The differences in sequence of MCPyV and KIPyV VP1 could also influence cell activation.

In conclusion, we demonstrated that the structurally distinct particles are engulfed differently and this is related to macrophage inflammatory response.

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1368 – P1.12.16

Modulation of macrophage inflammatory response by Polyherbal formulation Kabasura Kudineer choornam

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Purpose: *Kabasura kudineer choornam (KKC)* is a new polyherbal formulation containing 15 different ingredients. These ingredients have been separately shown to have anti-inflammatory, anti-pyretic and anti-bacterial properties. *KKC* has high binding affinity and interactions with SARS-CoV-2 spike protein shown in *in-silico* studies and exhibits good anti-viral properties against SARS- CoV-2 shown in a few clinical studies. However, the effect of *KKC* on macrophage polarization is not known. Here, we report a detailed analysis of the impact of *KKC* on the polarization of primary human monocyte-derived macrophages.

Methods: Human peripheral blood monocyte-derived macrophages (M0 macrophages) were either left untreated or pre-treated with 500 μg/mL *KKC* extract for 2 h. Then they were polarized into M1 (LPS, IFNγ), M2a (IL-4), or M2c (IL-10) macrophages for 22 h. M1 and M2 polarization markers were analyzed at mRNA level by qPCR and at protein level by flow cytometry and ELISA. Lastly, phagocytosis capacity of macrophages was analyzed using pHRodo conjugated heat-killed *Staphylococcus aureus* bioparticle.

Results: *KKC* extract treatment decreased the surface expression of the M1 marker CD64 (FcγRI) and increased the surface expression of the M2 markers CD200R, CD209, and CD163 in both polarized and unpolorized macrophages. Although, the mRNA expression of TNF, IL-12(p70), IDO1, IL-1β and TGF-β was increased; only the secretion of TNF was induced after KKC treatment.

Interestingly, the M2 cytokine IL-10 showed a unique pattern of response; a decrease in mRNA expression but increase in secretion in M0 and M2a macrophages, while the opposite response was observed in M1 and M2c macrophages. Lastly, *KKC* extract treatment decreased the phagocytic capacity of both polarized and unpolarized macrophages.

Conclusion: In conclusion, KKC extract modulates macrophage inflammatory response and could be a potential supplement for the treatment of infectious and inflammatory diseases.

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1377 – P1.12.17

Long live the macrophages – memantine as cellular youth elixir?

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Memantine is an uncompetitive NMDA antagonist, blocking the ionic channels of NMDA receptors, influencing the Ca^{2+} metabolism. This drug is clinically used for treating Alzheimer's disease, and there have been studies on animal models covering a variety of disease models and tissues, including the heart, liver and brain. Results presented here are from a peculiar observation made in the following study: female Dark Agouti rats of two ages (young – 3 months, and old – 24 months) were fed a memantine solution (60 mg/kg body weight/day for 7 consecutive days) through oral gavage from the first day after experimental autoimmune encephalomyelitis was induced, and were sacrificed on day 8 (inductive phase of disease). We confirmed the effects of systemic memantine application through histological analysis – livers of young control group rats showed signs of steatosis, which was ameliorated in the treated group; rats from both old and young control groups showed signs of liver mononuclear infiltration, which was absent in all rats from treated groups, regardless of age. However, the curious unexpected result was of a technical nature – peritoneal macrophages isolated from the rats showed substantially greater viability even 11 days after the experiment. Memantine had a stronger effect on peritoneal cells of young rats – the peritoneal cell yield was two times lower in the memantine treated group, the percentage of $CD45RA^+$ peritoneal B-cells was substantially higher than in the control group, and 11 days post-experiment there were 3 times more live macrophages ($CD11b^+CD163^+$). Macrophages are usually short living cells upon extraction from tissues, and these results may point in a direction of an intrinsic mechanism for longevity of macrophages and potentially other cells expressing the NMDAR. Since memantine is known to block extravasation of blood mononuclear cells into peripheral tissues, it is possible that the dynamic of peritoneal cells replenishment has been disturbed. The implications of our observations are unclear and further research will be need to establish the full scope of possibilities it unlocks, however we feel confident that memantine could be the proverbial shamrock for macrophages, bringing them long and happy lives.

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Abstracts

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1430 – P1.12.18

Mouse and human macrophages can be activated to fight cancer

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Macrophages are pivotal in tissue homeostasis and inflammatory responses, and they may reversibly alter their function in response to environmental cues. Macrophages are abundant in solid tumors, but it remains controversial whether they promote or inhibit tumor growth. The aim of the present study is to elucidate the potential of macrophages for cancer immunotherapy. For this purpose, we have used non-activated or activated mouse bone marrow-derived macrophages (BMDM) and human macrophages differentiated from monocytes. The macrophages have been tested in mouse models of cancer and in co-cultures with cancer cells. Mice injected subcutaneously with colon carcinoma cells alone, or non-activated macrophages in combination with cancer cells developed tumor. In contrast, injection of the activated macrophages together with cancer cells completely inhibited tumor growth. Upon rechallenge with cancer cells, the mice exhibited delayed tumor growth compared to naïve mice, suggesting that the initial co-injection of activated macrophages with cancer cells induced prolonged immunity. In vitro, mouse or human cancer cells in co-culture with non-activated macrophages proliferate to the same extent as the cancer cells grown alone. In contrast, activated macrophages efficiently killed cancer cells. Imaging by holotomography revealed that cancer cells (human breast cancer and melanoma) show typical morphological features of apoptosis when co-cultured with activated macrophages. Finally, we have observed that in a tumor-on-chip, T cells migrate into a 3D environment consisting of cancer cells and activated macrophages embedded in collagen. These results suggest a new strategy for cancer immunotherapy, based on the activation of tumor-associated macrophages.

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1436 – P1.12.19

Staphylococcal enterotoxins influence the monocyte transcriptional profile and shape subsequent macrophage differentiation

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Staphylococcal enterotoxins (SE) are superantigens produced by *Staphylococcus (S.) aureus*. SE crosslink the MHC-II on antigen presenting cells (APCs) with the T cell receptor, triggering a polyclonal T cell response. Although APCs are the first cells to encounter SE and direct the subsequent T cell response, very little is known regarding the effects SE have on APC activation. Our aim is to investigate how the enterotoxin SEA is affecting monocytes in the presence and absence of other cell types, and the effects that encountering SEA will have in the resulting differentiated macrophages.

We have stimulated human peripheral blood mononuclear cells (PBMC) or isolated monocytes with SEA. Upon stimulation, we collected the monocytes or differentiated them into macrophages and exposed them to polarizing conditions. The cells were collected, and we analyzed their gene expression, surface markers and protein secretion using RNAseq, flow cytometry and ELISA respectively.

Our results show that upon treatment with SEA, monocytes within the PBMC population differentiate into an HLA-DR^{high} and an HLA-DR^{low} population between 8 and 16 hours after stimulation. Furthermore, in the absence of T cells, SEA-treated monocytes show a similar transcriptional pattern as unstimulated cells, but they upregulate T cell-attracting chemokines such as CXCL-9, -10 and -11.

We also observed that macrophages derived from SEA-primed monocytes are phenotypically different to those that have not encountered the toxin, with decreased HLA-DR and activation markers CD80 and CD86, as well as diminished expression of the M2 markers CD163 and CD206 in both M1 and M2 polarizing settings. Altogether, our results suggest that interaction with SEA may influence the monocytic antigen presenting capacity and subsequent macrophage function. This could potentially lead not only to disrupted T cell activation to other antigens and the breaking of T cell tolerance, but also affect macrophage functions such as wound healing and phagocytosis.

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1517 – P1.12.20

Quantitative proteomic analysis of natural dendritic cell subsets

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Background: Dendritic Cells are the primary antigen presenting cells of the immune system and thus bridge the gap between innate and adaptive immunity. DC are a family of ontologically related subsets that exist across a range of maturation states. However, the molecular mechanisms that underpin the division of labour between these subsets and maturation states are not fully characterised. In recent years, cellular metabolism has been shown to be intricately linked to DC fate and function. We hypothesis that metabolism is a key determinant of DC subset specific functions. Furthermore, nutrients are the fuels of metabolism and are thus key mediators of DC function. However, how nutrients effect cDC is unexplored poorly understood.

Methods: We isolated splenic conventional DC1 and DC2 from mice that were either treated with PBS or ODN-CpG for 18h *in vivo*. Total protein was extracted from whole cell pellets and subjected to quantitative proteomic analysis. Statistical analysis was performed to investigate the metabolic proteome and nutrient sensing machinery of immature (PBS) and mature cDC (CpG) subsets.

Results: Herein, we reveal that primary mouse spleen cDC1 and cDC2 have distinct metabolic proteome features that correlate with function. We show that upon activation cDC metabolic proteins do not significantly change after 18h of stimulation. We find that cDC1 and cDC2 differentially express amino acid transporters. In particular, cDC1 have elevated expression of the system-L amino acid transporter, SLC7A5 whose activity was found to regulate anabolic signalling in cDC1s by controlling the activity of the kinase mTORC1. Additionally, pharmacological targeting of SLC7A5 transport was shown to modulate cDC1 cross-presentation.

Conclusion: This study quantifies the absolute protein expression of cDC1 and cDC2. cDC have subset specific metabolic proteome features that underpin their functions. Amino acid transport through SLC7A5 was found to be a cDC1 specific regulator of function. We propose that this is an evolutionary mechanism to support enhanced T cell activation. This data paves the way for future work to investigate the subsets specific nutrient requirements of DC and to identify molecular mechanisms to target these features therapeutically.

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1551 – P1.12.21

Mitochondrial fission-inducible lipid droplets in macrophage antimicrobial responses.

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Mitochondria and lipid droplets (LDs) are recognised for their important roles in innate immunity, with both organelles contributing to inflammatory signalling and bacterial killing by macrophages. Mitochondria are highly dynamic organelles, changing their state from a fused network (fusion) to undergoing fragmentation (fission), with this process affecting cellular metabolism. LDs are vital in cellular metabolism and have a role in inflammatory signalling and direct antimicrobial responses. Mitochondria and LDs interact during cell metabolism, demonstrating that these organelles have an intimate relationship. This study aimed to investigate the relationship between mitochondrial fission and LDs in macrophage antimicrobial responses. Here we have demonstrated that the Toll-like receptor 4 (TLR4) agonist lipopolysaccharide (LPS) induced mitochondrial fission and LD synthesis in both murine and human macrophages. To determine whether there is a functional link between mitochondrial fission and LD formation in murine macrophages, LPS-inducible mitochondrial fission was genetically or pharmacologically inhibited by targeting the fission-promoting GTPase DRP1. Both regimes abrogated LPS-induced LD formation, suggesting that LPS-inducible mitochondrial fission drives LD formation. Signalling mechanisms driving LPS-inducible LDs are poorly understood. One potential candidate is the lysine deacetylase HDAC7 which is required for TLR-inducible mitochondrial fission. LPS-inducible LD formation was abrogated by either genetic or pharmacological targeting of HDAC7. Whereas overexpression of HDAC7 in primary macrophages was sufficient to induce both mitochondrial fission and LD formation in a basal state. In bacterial infection studies, challenge with *Escherichia coli* (*E. coli*) increased both mitochondrial fission and LD formation. Interestingly, infection with *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) didn't induce fission but induced LD formation, indicating a potential form of pathogen manipulation. Finally, whereas LPS-inducible fission and LD formation required HDAC7, these responses occurred independently of HDAC7 in macrophages responding to bacterial challenge. These data further support a connection between mitochondrial fission and LD formation and suggest that distinct mechanisms can initiate inducible fission and LD formation, depending on the nature of the stimulus. Collectively, my data suggest that the antimicrobial effects of mitochondrial fission in macrophages may be mediated by inducible production of antibacterial LDs.

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1581 – P1.12.22

MTADV 5-MER peptide suppresses lung fibrosis of a mouse model and inhibits human fibroblasts biological functions by targeting of SAA, which fuels fibrosis

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Focusing on therapy of chronic inflammations, we have reported in our previous communication (J Autoimmune. 2021 Nov; 124:102713) that a 5-MER peptide (5-MP; Methionine, Threonine, Alanine Aspartic Acid, Valine (MTADV) attenuates the pathology of animal models of Rheumatoid Arthritis, Crohn's Disease/Ulcerative Colitis and Multiple Sclerosis. In this report we described the ability of 5-MP to alleviate the lung inflammation and fibrosis of Bleomycin-induced fibrosis (BIF), a mouse model of human Idiopathic Pulmonary Fibrosis (IPF). All these maladies share Serum Amyloid A, that fuels chronic inflammation and fibrosis. In our previous communication we presented evidence that 5-MP targets SAA and consequently interferes with formation of SAA oligomers and aggregated fibrils, which are involved in chronic inflammations and fibrosis *in vivo* and in release of pro-inflammatory cytokines *in vitro*. In this report we focus, as indicated above, on the suppressive effect of 5-MP on fibrosis *in vivo*, and on fibroblast biological functions *in vitro*. Inflammation-induced quantitatively uncontrolled biological activities of fibroblasts and monocytes generate fibrosis, stressing the linkage between inflammation and fibrosis. The last is much less responsive than inflammation to medical intervention. Indeed, we found that 5-MP inhibits mRNA expression of pro-inflammatory cytokines (IL-6 IL-1β, TNFα) in fibroblasts and monocytes and their release from SAA-activated corresponding cells. Furthermore, the *in vitro* growth potential of fibroblasts was suppressed following their incubation with 5-MP, which explains the ability of the peptide to suppress the fibroblast proliferation-dependent fibrosis *in vivo*. In conclusion 5-MP displays therapeutic potential in lung fibrosis-associated diseases, which face unmet remedy at present.

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1676 – P1.12.24

IFN-γ induced plasticity in human alveolar macrophages can be resolved with IL-10: Implications for macrophage plasticity as a therapeutic target for tuberculosis

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Background: Alveolar macrophages (AM) are the most prevalent immune cells in the lung and the first line of defence against invading pathogens. However, AM are also the primary host cell of *Mycobacterium tuberculosis* (Mtb) in the lung. Interferon-γ induced Th1 mediated immunity is crucial to control pulmonary infections. IFN-γ induces the human AM to become more inflammatory and metabolically active which is thought to improve pathogen clearance. There is a paucity of data on the whether human AM can revert to a homeostatic state after activation by Th1 cytokines such as IFN-γ.

We wanted to investigate if the human AM can revert to a homeostatic regulatory phenotype post treatment with IFN-γ. Furthermore, we wanted to examine the ability of AM that have resolved Th1 inflammation, to respond to Mtb.

Methods: To model plasticity and resolution of inflammation *in vitro*, human AM and monocyte derived macrophages (MDM) were treated with IFN-γ for 24 h, to mimic an inflammatory lung environment. Macrophages were subsequently treated with IL-10 for 48 h. Activation marker expression was determined using flow cytometry. Metabolic profiles were assessed by Seahorse metabolic-flux analysis and PCR. To examine functional responses, AM were stimulated with Lipopolysaccharide (LPS), irradiated H37Rv (iH37Rv) or Mtb lysate and cytokine production was quantified by ELISA. Alternatively, macrophages were infected with Mtb, lysed and colony forming units were quantified.

Results: IFN-γ increased expression of CD40 and HLA-DR by human AM, subsequent IL-10 stimulation only reduced HLA-DR surface expression. IFN-γ treated AM stimulated with LPS, iH37Rv or Mtb lysate had increased TNF and IL-1β production which was attenuated by IL-10 treatment. IFN-γ primed IL-10 treated AM had increased glycolysis and oxidative phosphorylation compared to controls, suggesting metabolic control of homeostasis in human AM. Additionally, IFN-γ primed IL-10 treated AM have a better ability to control intracellular Mtb growth. Similar results were observed in MDM.

Conclusion: IL-10 can revert AM to a homeostatic state after IFN-γ which may mechanistically require increased metabolic activity. The data also suggests that the resolution of inflammation in the macrophage may also play a critical role in killing Mtb from the lung.

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1686 – P1.12.25

Limosilactobacillus reuteri regulate the monocyte transcriptome and functional macrophage polarization

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Background: Species from the bacterial genera *Lactobacillus (L.)* are among the first gut colonizers that play a vital role to built-up healthy immune system. In the neonatal gut, they are known to have beneficial health effects, and their presence has been associated with protection against allergy. We have previously demonstrated how lactobacilli-derived factors (secretome) suppress pro-inflammatory T cell responses and that this activity was mediated via monocytes/DCs.

Aim: To investigate the effect of lactobacilli-derived secretome on the human primary monocyte transcriptome, differentiation to M1 (proinflammatory-like) and M2 (anti-inflammatory-like) macrophages and their functional responses.

Methods: Human monocytes purified from peripheral blood were exposed to the secretome from *Limosilactobacillus (L.) reuteri* for 24 hours. mRNA was isolated and subjected to RNA sequencing. Also, macrophages were generated under different growth conditions *in vitro*, and further exposed to the secretome from *L. reuteri* for 48 hours. The cells were analyzed for cell surface expression of M1/M2 characteristics, i.e. HLA-DR, CD80, CD86, CD163, CD206, and PDL-1 by flow cytometry and cell-culture supernatant were analyzed for the detection of TNF, sCD14, sCD163, IL-6, IL-10, IL-19, IL-1RA, and G-CSF by ELISA. Functional analysis was conducted by coculturing macrophages with the autologous T cells.

Results: Preliminary results from transcriptome analyses of *L. reuteri*-treated monocytes indicate a strong effect on many protein-coding genes. Among others, there was a strong down-regulation of several genes in the MHC class II antigen processing pathway, but also the induction of several non-coding RNAs, some with described regulatory functions. For macrophage differentiation, there was a general dampening of both M1 and M2 phenotypic characteristics but a tendency to upregulate PDL-1 and CD14 on M2 macrophages. Furthermore, LR secretome preferentially elicited immunomodulatory cytokines such as IL-10, sCD163 and G-CSF on M2 conditioned macrophages. Moreover, LR treated macrophages suppressed the autologous T cell responses.

Conclusion: *L. reuteri* induce a broad transcriptional gene activity in monocytes with a clear reduction of the antigen presentation pathway in both monocytes and differentiated macrophages, which could have implications for their capacity to modulate lymphocyte functions and towards therapeutic approaches.

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1806 – P1.12.26

Platelets as possible biomarker candidates to differentiate between neurodegenerative disorders. Platelets as possible biomarker candidates to differentiate between neurodegenerative disorders.Platelets as possible biomarker candidates to differentiate between neurodegenerative disorders.Platelets as possible biomarker candidates to differentiate between neurodegenerative disorders.

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Platelets (PLTs) have recently been recognized as immunoregulatory elements that modulate immune responses with an increasing evidence of their role in the pathogenesis of neurodegenerative disorders. Alzheimer's disease (AD), Parkinson's disease (PD) and Lewy body dementia (DLB) are complex diseases that usually overlap in their neuropathological manifestations preventing a correct clinical diagnosis and management of the disease.

The aim of this project was to explore the interaction of PLTs with other immune cells in these different neurodegenerative disorders and determine the usefulness of PLTs as possible biomarkers. Sixty-six individuals from Germans Trias i Pujol Hospital were analyzed in this pilot study. The percentage of PLTs (CD41+CD61+) attached to T lymphocytes (CD3+CD4+, CD3+CD8+), B lymphocytes (CD19+), monocytes (CD14+), and theiractivation grade (CD25+/ CD68+) were measured in peripheral blood by flow cytometry.

Results revealed an increase in the percentage of PLTs attached to CD4+ and CD4+ CD25+ T lymphocytes in PD compared to DLB(p=0,0186) (p=0,0317). A similar result was obtained with CD19+ B lymphocytes attached to PLTs in CTRL(p=0,0233), PD (p=0,0038) and AD (p=0,0386) compared to DLB. In the same tendency an increase was also observed in the percentage of PLTs attached to CD8+ T lymphocytes in PD compared to DLB (p=0,0132).

Moreover, the percentage of PLTs attached to CD14+ monocytes was higher in AD (p=0.0164) and CTRL(p=0,0384) compared to DLB as well. However, the expression of the activator marker CD68 in monocytes was higher in DLB compared to AD (p=0,0044).

The percentage of PLTs attached to B lymphocytes, CD4+T lymphocytes and monocytes could be a promising biomarker to discriminate between PD, DLB and AD. Moreover, it seems that there is a tendency of a decrease percentage of immune cells attaches to PLT in DLB. This difference should be further explored as a tool for the differential diagnosis between DLB and PD at early disease stages, as it is still very difficult to differentiate them. A correct diagnosis and treatment is crucial to improvethe quality of life of these patients. Further studies with higher number of participants are needed to validate these results.

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1860 – P1.12.27

High production of IL-12p70 by human dendritic cells stimulated with combinations of pattern-recognition receptor agonists

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The cytokine IL-12p70 is crucial for T helper 1 (Th1) polarization and the generation of type 1 immunity required to fight cancer and pathogens. Therefore, strategies to optimize the production of IL-12p70 by human dendritic cells (DCs) may significantly improve the efficacy of vaccines and immunotherapies. However, the rules governing the production of IL-12p70 remain obscure. Here, we stimulated pattern recognition receptors (PRRs) representing five families of PRRs, to evaluate their ability to elicit high production of IL-12p70 by monocyte-derived DCs. We used ten well-characterized agonists and stimulated DCs *in vitro* with either single agonists or 27 different combinations. We found that poly(I:C), which engages the RNA-sensing PRRs TLR3 and MDA5, and LPS which stimulates TLR4, were the only agonists that could elicit notable IL-12p70 production when used as single ligands. We identified six different combinations of PRR agonists, all containing either the TLR3/MDA5 agonist poly(I:C) or the TLR7/8 agonist R848, that could synergize to elicit high production of IL-12p70 by human DCs. Five of the six combinations also triggered high production of the antiviral and antitumor cytokine IFNβ. Overall, the tested PRR ligands could be divided into three groups depending on whether they triggered production of both IL-12p70 and IFNβ, only one of the two, or neither. Thus, combinations of PRR agonists were found to increase the production of IL-12p70 by human DCs in a synergistic manner, and we identified six PRR agonist combinations that may represent strong adjuvant candidates, in particular for therapeutic cancer vaccines.

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1889 – P1.12.28

NFAT is important for NK cell maturation and function

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Purpose: With the key aim to block false T-cell activity, patients receive cyclosporine A (CsA) or tacrolimus (FK506). However, other cell types like natural killer (NK) cells will be affected, too. Both drugs inhibit the enzymatic activity of the phosphatase calcineurin (CN), which primarily but not exclusively dephosphorylates and activates transcription factors of the *nuclear factor of activated T-cells* (NFAT) family. In T cells, CN is activated upon engagement of the T-cell receptor with a subsequent increase in intracellular Ca^{2+} and NFAT nuclear translocation. Here, NFAT proteins directly translate antigen recognition signals into altered gene expression. NK cells possess several receptors, known or predicted to increase intracellular Ca^{2+} as well. In addition, NK cells – like T lymphocytes – express NFATc1, c2 and c3.

Methods: Initially, mice harboring the *Nfatc1eGFP* reporter mice were exploited to verify NFATc1 expression in NK cells of different organs. *Nkp46iCre.YFP*$^{fl/fl}$ were utilized to ascertain the efficacy and specificity of the Cre recombinase system specifically within NK cells. Subsequently, mice with the *Nkp46iCre.Nfatc1*$^{fl/fl}$ and/or *Nfatc3*$^{fl/fl}$ genotype as well as *Nfatc2*$^{-/-}$were employed. Flow cytometry analysis was conducted to assess NK cell maturation and function.

Results: The *Nfatc1eGFP* reporter mice testified that at least NFATc1 is highly expressed and activated in NK cells of the bone marrow, secondary lymphoid organs as well as lung and liver. The *Nkp46iCre.YFP*$^{fl/fl}$ mouse model confirmed the precise targeting by NKp46 iCre solely to NK cells, i.e. without affecting other immune cell populations including $CD4^+$ and $CD8^+$ T cells. Mice with NFAT-deficient NK cells exhibited an increased proportion of NK cells in more mature stages compared to wild-type mice. Furthermore, these NFAT-deficient mice demonstrated reduced levels of inflammatory cytokines such as IFN-γ, but heightened expression of cytotoxic molecules such as granzyme B alongside with their killing capacity measured by CD107a expression.

Conclusion: These findings suggest that NK-specific deletion or inhibition of NFAT supports the anti-inflammatory effect it has on T cells but preserves late NK-differentiation stages with their cytotoxic activity.

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1931 – P1.12.29

Immune excitation of hematopoietic stem cells

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Hematopoietic Stem Cells (HSCs) are the source of blood and immune cells. During everyday healthy life, HSCs are mostly quiescent in the bone marrow. Following inflammation, HSCs can gain activation, presumably accelerating the generation of the needed blood and immune cells. Surprisingly, however, our knowledge regarding the ability of various pathogens' to perturb HSCs is minimal. Moreover, the early- and late-effects of such activation are only beginning to reveal.

We had first revealed surface markers of immune-activated HSCs. Utilizing improved identification of activated HSCs provide us with molecular insights, and better ability to follow the process dynamically. We recently found that HSC's activation is much faster and broader than previously thought -- evident as early as 2 hours for both Stem- and progenitor-cells. The response is systemic, highly sensitive, and dose-dependent down to a surprisingly low amounts of stimulant. Interestingly, other recent studies challenge the concepts of HSCs' contribution to emergency hematopoiesis, highlighting further interests.

Chronic activation may deplete HSCs' potency and increase the risk of malignancies. We find extraordinary impact following prolonged bacterial infection in mice, and even more exciting ability for recovery following clearance of the pathogen. Single-cell analysis let us novel insights into molecular states of naïve, potent-less, and recovered HSCs.

Changing concepts of hematopoiesis are fundamental for the generation of new immune cells through health and disease. New data will allow for preserving the potency for a longer and healthier life.

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1955 – P1.12.30

Evaluation of NK cell anti-tumor activity by detection of subcellular nitric oxide in response to BCG treatment

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Purpose: BCG therapy induces a local inflammatory response that is associated with an immune response capable of eradicating residual tumour cells. NK cells play a central role; however, not all patients achieve a response. NKG2A ligands, HLA-B leader peptides presented in HLA-E with dimorphism at position -21 (threonine -T- vs. methionine -M-), impact the survival of bladder cancer patients. Preliminary results from our series show that "M" ligands are associated with poor survival in patients undergoing chemotherapy but with very good survival in patients under BCG, particularly in patients with concurrent KIR2DL5.

Methods: We evaluate whether the M/T ligands could have an impact on nitric oxide production of NK cells from healthy donors and bladder cancer patients in response to BCG. The method used was described in PM 34821904, with the antibody panel: CFSE-FITC, CD45-APC-Cy7, CD3-BV786, CD4-APC, CD16-V450, CD56-PeCy7, CD8-BV605.

Results: In the presence of KIR2DL5, MT patients performed better clinically in the first 3 years of BCG treatment than TT patients. The proportion of patients in whom strong nitric oxide production is induced in NK cells after BCG stimulation is much higher in KIR2DL5+ genotypes. BCG stimulation in both KIR2DL5+ genotypes (TT and Mx) induces a much stronger nitric oxide response in NK-bright cells and CD8+ and CD4+ T cells ($p<0.05$) than in TT genotypes.

Conclusion: Preliminary results from in vitro assays have given us a glimpse that NK cell activation with BCG is associated with higher rates of nitric oxide secretion in M/KIR2DL5+ genotypes, which would explain their better response to BCG therapy. In contrast, the genotypes with the best response to BCG therapy show the worst survival curves in patients with infiltrating tumours who do not receive BCG therapy, and who may have benefited from BCG therapy.

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1999 – P1.12.31

Dengue virus exploits secretory autophagy mechanisms to promote viral dissemination by human dendritic cells

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Dengue virus (DENV), transmitted by infected mosquitoes, is a major public health concern, with half the world's population at risk for infection. Recent decades have increasing incidence of dengue-associated disease alongside growing frequency of outbreaks. Although promising progress has been made in anti-DENV immunizations, post-infection treatment remains limited. Dendritic cells (DCs) are amongst the first human cells to encounter DENV upon injection into the skin mucosa, and thereafter promote systemic viral dissemination. Autophagy is a vesicle trafficking pathway involving the formation of cytosolic autophagosomes, which engulf the cargo for subsequent lysosomal degradation. Recent reports have highlighted the extensive manipulation of autophagy by DENV for viral replication. However, the temporal profiling of autophagy activity and function of autophagy vesicles in DENV infection of human primary DCs remains elusive. Herein, we demonstrate that mechanisms of autophagosome formation and extracellular vesicles (EVs) release have a pro-viral role in DENV dissemination by DCs. We show that DENV exploits early-stage ULK1/ATG16L1-dependent autophagy mechanisms to establish infection in primary human DCs. DENV replication enhanced autophagosome formation in DCs, and intrinsically-heightened autophagosome biogenesis correlated with relatively higher rates of DC susceptibility to DENV. Furthermore, our data demonstrates targeting of viral replication intermediates to autophagosomes, while productive DENV infection institutes a block at the late degradative stages of autophagy in infected DCs but not in uninfected bystander cells. We identify for the first time that approximately one-fourth of DC-derived CD9/CD81/CD63+ EVs co-express canonical autophagy marker LC3, underscoring the relevance of secretory autophagy in DC-mediated intercellular communication. Notably, we demonstrate that DENV hijacks host exocytosis pathways leading up to the release of viral RNA-laden EVs, including extracellular LC3+ autophagy vesicles, by human DCs for viral dissemination to other recipient target cells. Taken together, our study highlights intersections between autophagy and secretory pathways during viral infection, and puts forward cytosolic autophagosome accumulation and release of infectious EVs populations as host determinants of DC-mediated DENV infection in humans. Host-directed therapeutics targeting autophagy mechanisms and EV biogenesis thus have potential to enhance DC-driven resistance to DENV acquisition and thereby limit viral dissemination by initial human target cells following mosquito-to-human transmission of DENV.

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2009 – P1.12.32

IL-2 drives glucose flux through glycolysis and the PPP

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Purpose: NK cells are important lymphocyte effector cells that kill cancer cells and virally infected cells. NK cells are activated by cytokines, including IL2, which cause a metabolic reprogramming of the cells towards increases in both glycolysis and oxidative phosphorylation. An alternative TCA-like cycle has previously been shown to be important in *in vitro* expanded murine NK cells. The goal of this work was to undertake an unbiased approach in terms of tracing glucose metabolism in human primary NK cells that were activated for 18 hours with IL2.

Methods: For this study isolated NK cells were left untreated or stimulated with IL-2 (500IU/ml) and cultured with universally labelled glucose to map fate of that glucose in these cells and to measure that relative abundance of metaboilites that are derived from imported glucose. The overall metabolic profile of the NK cells was carried out using metabolomics, while extracellular and intracellular pyruvate and lactate concentrations were determined using commercially available assays.

Results: NK cells utilised glucose for glycolysis and IL2 increased this modestly. There was little evidence of TCA cycle use under these conditions; however, there was evidence to support glucose shuttling into the pentose phosphate pathway (PPP). It was interesting to note that NK cells secreted pyruvate, that has been metabolised from labelled glucose, both at rest and stimulateed with IL-2, in addtion to lactate. This was confirmed by blocking the MCT 2 transporter, via the selective inhibitor VB-124, which reduced the amount of pyruvate in the supernatant.

Conclusion: NK cells metabolised glucose through a variety of pathways including glycolysis and the PPP. The evidence for active secretion of pyruvate by NK cells is of note, as it contrasts reported increased rates of OxPhos in IL-2 stimulated NK cells. Our current research is investing the reason or potential role of pyruvate secretion in NK cells.

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2171 – P1.12.33

miRNA content in subpopulations of NK cell-derived extracellular vesicles connects to different functional activity

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NK cells release extracellular vesicles (EVs) in response to cytokine stimulation or receptor engagement. These vesicles contain cytolytic proteins, and can induce apoptosis of a wide range of cancer cells. The EV secretome is heterogeneous, and we previously showed that NK cells release distinct vesicles originating from the plasma membrane or from intracellular sources. Only the intracellularly-derived EVs mediate apoptosis of cancer cells. To further understand how these vesicles target and kill cancer cells, we profiled the miRNA expression in EV subpopulations derived from both primary human NK cells and the NK-92 cell line via the NanoString hybridization platform and quantitative PCR. EVs were harvested from NK cells cultured in IL-15 or a combination of IL-12/IL-15/IL-18 for 48 hrs in serum-free culture. We found that miR-22-3p, miR-129-5p, miR-382-3p, and miR-3140-5p appeared enriched in EVs derived from the plasma membrane, while there was an enrichment of miR-16-5p, miR-19b-3p, miR-20a/20b-5p, miR-23a-5p, miR-29b-3p, miR-142-3p, miR-181a-5p, and miR-630 in internally-derived cytotoxic EVs. No major differences were observed when comparing the different cytokine treatments. Bulk, unfractionated EVs were also harvested from primary NK cells activated through the CD16 receptor, and these EVs showed an overlapping profile with the fractionated, internally derived cytotoxic EVs. Target prediction analysis suggested that miRNAs enriched in cytotoxic EVs converge on genes related to apoptosis and TGF-β receptor signaling, and we further show that the cytotoxic EV subpopulation specifically reduced expression levels of *BCL2*, *RICTOR* and *TGFBR1* in the HCT-116 colon cancer cell line. Subjecting HCT-116 cells to miR-16-5p and miR-19b-3p led to increased apoptosis compared to scrambled controls, as well as reduction of *RICTOR*. To conclude, our data indicate that cytotoxic NK-EVs can target cancer cells through miRNA-mediated modulation of anti-apoptotic pathways.

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P1.13 CHEMOKINES AND THEIR RECEPTORS

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2001 – P1.13.01

Dissection of neutrophil heterogeneity in glioblastoma

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Diffuse gliomas are severe brain tumors accounting for 30% of central neural system malignancies. Glioblastoma multiforme (GBM) is one of the most severe form with a very poor prognosis. Its heterogeneity and immunosuppressive nature are the principal causes of failure of different therapeutic approaches, including immunotherapy. Despite the common occurrence of neutrophilia in GBM patients, our understanding of the interplay between neutrophils and cancer remains limited.

The aim of the study is to delineate the phenotype and maturation state of circulating neutrophils, clarifying their role in the tumor microenvironment (TME).

From the analysis of 70 sera of HGG patients we observed no differences in the levels of IL-17 and CCL2, while CXCL8 levels were increased in GBM patients compared to healthy controls. According to these evidence, we choose to focus on the CXCL8-CXCR2 axis to deeper elucidate the role of neutrophils in HGG tumor and find new potential therapeutic approaches.

Our observations revealed high levels of circulating neutrophils in patients with poorer prognosis. However, the neutrophil maturation and activation states emerged as critical factors, indicating that patients with a higher number of immature neutrophils exhibited longer survival. Experiments are ongoing to understand the interplay of immature neutrophils with GBM.

To better investigate the role of neutrophils in this context, we set up an in vivo model of glioma, by the orthotopic injection of the glioma cell line GL261 in WT and neutropenic (CSF3R KO) mice. Our results showed a better survival of CSF3R KO mice compared to WT, suggesting a pro-tumoral role of neutrophils. To further elucidate these findings, we are performing in vivo experiments using the SB28 glioma cell line, known for better recapitulating the immune profile found in human patients.

Further studies are necessary to deepen our understanding of the role of neutrophils in the biology of brain cancer, paving the way for novel therapeutic approaches.

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P1.14 CONTROL OF INFLAMMATION AND TISSUE REPAIR

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53 – P1.14.02

Role of PTX3 in Staphylococcus aureus-induced sepsis

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Background. Sepsis is associated with dysregulated coagulation and impaired fibrinolysis resulting in disseminated intravascular coagulation (DIC), which contributes to multi-organ damage and mortality. PTX3 is a humoral pattern recognition molecule of the innate immune system, which interacts with defense fibrinogens amplifying effector functions of the innate immunity. By interacting with fibrin and plasminogen (Plg), PTX3 regulates injury-induced thrombotic responses and promotes fibrinolysis. In human sepsis, PTX3 is a marker of severity and prognosis, and is associated with altered coagulation parameters.

Objective. To define the role of PTX3 in fibrinolysis in the thrombotic response associated with *Staphylococcus aureus* (SA)-induced sepsis.

Methods. Models of SA-induced sepsis in *Ptx3* targeted mice, 2P-IVM and confocal microscopy, multi-plexed histology, bioinformatic analysis.

Results. In mouse SA-induced sepsis, PTX3-deficiency was associated with increased susceptibility to infection, bacterial dissemination, early abscess formation in major organs with increased fibrin deposits and thrombosed vessels, and death. Circulating parameters of coagulation and multi-organ dysfunction indicators were increased in PTX3-deficient mice, whereas levels of D-dimer were decreased. PTX3 expression was restricted to aorta, lung and heart, associated with PDGFRa^+ mesenchymal cells. PTX3 interacted with Plg in blood and was associated with fibrin deposits in damaged organ parenchyma, SA abscesses and vascular thrombi. In bone marrow chimeric mice, PTX3 resistance to SA was totally dependent on non-hematopoietic cells. The phenotype was recapitulated in *Cre*-inducible mice with selective *Ptx3* deletion in PDGFRa^+ cells. Use of agents targeting coagulation molecules or boosting fibrinolysis rescued the phenotypes.

Conclusion. PTX3 is involved in SA-induced hemostatic response, possibly through regulation of the thrombotic response by promotion of fibrinolysis. These results will be instrumental for the development of PTX3 as a prognostic and therapeutic target in sepsis.

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64 – P1.14.03

Optimizing bronchoalveolar lavage yield in infectious disease immunology research: a comprehensive review

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Bronchoalveolar lavage (BAL) is a valuable tool in infectious disease immunology research, providing insights into the local immune response within the respiratory tract. However, optimizing the yield from BAL samples in human participants presents several challenges that must be addressed to maximize the utility of this technique. This abstract offers a comprehensive review of strategies for optimizing BAL yield, focusing on sample collection, processing, and analysis methodologies.

Effective sample collection techniques are crucial for obtaining high-quality BAL specimens. Proper patient preparation, bronchoscope insertion, and lavage fluid recovery are essential steps to minimize contamination and maximize sample yield. Additionally, the choice of lavage fluid and the volume instilled can impact the quantity and quality of recovered cells and soluble mediators.

Optimizing sample processing protocols is equally important for preserving cellular integrity and minimizing artifacts. Standardized procedures for cell isolation, viability assessment, and storage conditions help ensure reproducibility and reliability of experimental results. Furthermore, innovative techniques such as flow cytometry, multiplex cytokine assays, and transcriptomic analysis offer valuable insights into the immune profile of BAL samples.

Infectious disease immunology research often requires specialized analyses to elucidate host-pathogen interactions and immune responses. Integration of BAL data with clinical and microbiological parameters allows for a comprehensive understanding of disease pathogenesis and progression. Moreover, longitudinal studies and comparative analyses across different patient populations enhance the generalizability and translational relevance of findings.

Despite its utility, BAL sample collection in human participants poses ethical and logistical considerations that must be carefully addressed. Informed consent, patient safety, and adherence to ethical guidelines are paramount in conducting BAL procedures. Collaboration between clinicians, researchers, and regulatory bodies is essential for navigating these complexities and ensuring ethical conduct of research involving human participants.

In conclusion, optimizing the yield from BAL in infectious disease immunology research requires a multifaceted approach encompassing sample collection, processing, and analysis. By employing standardized protocols, leveraging advanced methodologies, and adhering to ethical principles, researchers can maximize the utility of BAL samples and advance our understanding of respiratory immune responses in health and disease.

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77 – P1.14.04

Loss of TLR2 signalling slows retinal degeneration in mouse models of disease

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Purpose: Age–related macular degeneration (AMD) is the leading cause of blindness in the developed world, estimated to effect 180 million people worldwide. Despite this staggering figure there are few therapeutic options available to patients. Our aim is to use newly characterised mouse models that recapitulate features of AMD and examine the role of TLR2 in this disease progression.

Methods: Claudin 5 (Cldn5) is a tight junction protein that is highly expressed at the inner blood retina barrier. We have shown that suppression of Cldn5 in mice provided a high cholesterol diet can induce dry AMD pathology. Here, we induce Cldn5 deficiency through the use of Cldn5 shRNAs and the Cre-LoxP system. Using these models we investigate the effect of inhibiting TLR2 signalling. TLR2 plays a key role in the innate immune response, however, overactivation of TLR2 can contribute to disease pathology by causing chronic inflammation. Previously we have shown that inhibiting TLR2 is protective against oxidative – damage induced retinal degeneration in an acute model of retinal degeneration. Here we investigate the effect of TLR2 or MyD88 deficiency in this AMD mouse model.

Results: Cldn5 deficient mice show evidence of degeneration in the light absorbing layer of the retina, in addition to mononuclear phagocyte infiltration. The complement membrane attack complex (MAC) is a key effector of the immune system and MAC formation is increased in the choroid of AMD patients. Our findings show increased MAC formation in the retinas of Cldn5 deficient mice. TLR2 deficient mice present with a less severe phenotype, with the light absorbing layer of the retina more intact, and significantly less MAC deposited. Loss of TLR2 signalling also altered the dynamics of mononuclear phagocyte infiltration. Migration assays and chemokine receptor expression suggests TLR2 activation increases immune cell infiltration in this model.

Conclusion: TLR mediated signalling will trigger a robust inflammatory response in the eye. Our findings support the idea that overactivation of the innate immune response is a significant contributor to AMD pathogenesis. Inhibiting TLR2 signalling pathway may represent a powerful target for prevention of RPE degeneration.

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121 – P1.14.06

Dietary fibre and its metabolites regulate alveolar macrophage transcriptional landscape and alter bone marrow progenitor phenotype

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Inflammatory cells play a major role in the pathogenesis of pulmonary disease. Metabolites from dietary-fibre fermentation, mainly short chain fatty acids (SCFA), can alter alveolar macrophage phenotype and reduce inflammatory cell infiltration through modulation of gene expression. However, they can also affect macrophage progenitor cells from the bone marrow altering the phenotype of bone marrow derived alveolar macrophage that are often present in the lung during inflammation.

Aim: Upon treatment of mice with high-fibre diet (HFiD) and low-fibre diet (LFiD), we aimed to sequence broncho-alveolar (BAL) cell-derived RNA and identify differentially expressed genes *in vivo*. We investigated potential role of SCFA on gene expression in murine alveolar macrophages (MH-S) *in vitro* in homeostasis and inflammation. We also derive bone marrow macrophage (BMDM) from HFiD and LFiD fed mice and investigate effects on polarization.

Method: Cells from mice BALF were processed for RNA sequencing and data mapped to reference genome library by GENEWIZ to obtained differentially expressed genes. MH-S cells was treated with SCFA in homeostasis, LPS and hypoxia induced inflammation and q-PCR to assess gene expression. BMDM from mice fed HFiD and LFiD were polarized, and M1 and M2 markers were assessed using q-PCR.

Result: Alveolar macrophages from mice treated with HFiD and LFiD showed 680 differentially expressed genes, HFiD mice showed upregulation/downregulation of genes (such as NFKBiα, SOCS3) and chemokines that are associated with pathways of ‘resolution of inflammation’ and ‘recruitment and chemotaxis of phagocytes. *In vitro* analysis of SCFA treated MH-S mimic *in vivo* gene expression in homeostasis, while SCFA exhibit differential modulation of genes in LPS, and hypoxia induced inflammation showing condition dependent protective effects. HFiD skewed BMDM towards anti-inflammatory M2 macrophage.

Conclusion: HFiD affects inflammatory cells *in vivo* through modulation of gene expression. We demonstrated that SCFA (acetate, butyrate, and propionate) constitute for this affect through alveolar macrophage as seen *in vitro*. A diet lacking fibre (LFiD) predisposes mice to increased susceptibility to inflammation when challenged with injurious insults.

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174 – P1.14.07

The effect of Th1 and Th2 cells on properties and transcriptomic profile of mare endometrial stromal cells – an in vitro study

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Purpose: Fibrotic diseases cause about 45% of global premature deaths, yet their molecular mechanisms remain incompletely understood, leading to a lack of effective treatments. Results of recent studies indicate, that Th1 and Th2 cells may play an important role in modulating processes important in the development of fibrosis. However, whether and how these cells affect the key cells for the development of fibrosis – fibroblasts – hides many secrets. In our study, we utilize fibroblasts isolated from mare endometria, providing a non-traditional model of spontaneously occurring fibrosis known as endometrosis. Endometrosis involves fibrosis formation around endometrial glands and in stroma, adversely affecting endometrial structure and function. To better comprehend the role of Th1 and Th2 cells at fibrotic sites, we aimed to assess their impact on the viability, proliferation, and transcriptomic profile of mare endometrial fibroblasts.

Methods: To obtain Th1 and Th2 conditioned media (CM), Th naive cells were isolated from mare's (n=5) peripheral blood and differentiated *in vitro* into Th1 and Th2 cells. Then, mare endometrial fibroblasts (n=4) were isolated, cultured and treated with Th1-CM or Th2-CM for 48 hours. The proliferation and viability of fibroblasts were determined using BrdU and MTT, respectively, while the transcriptomic profile was determined using NGS followed by bioinformatic analyses.

Results: The proliferation and viability of fibroblasts increased after treatment with Th1-CM and Th2-CM ($p<0.05$). The treatment of fibroblasts with Th1-CM and Th2-CM resulted in 2140 and 1033 differentially expressed genes (DEGs; $p_{adj}<0.05$, $log2FC\geq1.0/log2FC\leq-1.0$), respectively. Further functional analysis using the KEGG database revealed, that DEGs identified exclusively after Th1-CM treatment enriched cytokine-cytokine receptor interaction, calcium, and PI3K-Akt signaling pathways. DEGs identified after both Th1-CM and Th2-CM enriched, among others, ECM-receptor interaction, cell cycle, protein digestion and absorption, and JAK-STAT signaling pathway, while Th2-CM changed DEGs enriched the AGE−RAGE signaling pathway.

Conclusion: Th1 and Th2 cell secretome enhances proliferation, viability, and alters transcriptome of mare endometrial fibroblasts, impacting processes linked to fibrosis development. This highlights the necessity for more research to fully grasp their mechanisms and potentially modulate them to mitigate fibrosis development.

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216 – P1.14.08

The disease with a thousand faces: what's neutrophils' portrait?

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Purpose: Systemic lupus erythematosus, also referred to as "the disease with a thousand faces", is an autoimmune disease hallmarked by a plethora of autoantibodies, interferon-signature, high levels of immune complexes, dysregulation of soluble factors in circulation, and neutrophils with altered phenotypes. While lupus neutrophils might exhibit inherent detrimental traits, we hypothesized that factors present in the circulation of people with lupus could also be responsible, at least in part, for these alterations. Thus, we investigated the influence of the "lupus environment" on the neutrophils' viability and activation level to understand better what causes the abnormalities observed in lupus neutrophils.

Methods: Blood samples from lupus patients and healthy volunteers were analyzed by flow cytometry for viability and expression of surface markers of interest. Neutrophil-enriched suspensions, freshly isolated from healthy volunteers, were stimulated with serum samples from lupus patients, and apoptosis was monitored in real-time. Plasma from both groups were subjected to multiplex analysis to measure a selection of over thirty analytes. Analytes exhibiting the most significant alterations were identified as lupus-relevant soluble factors. Finally, healthy volunteers' whole blood and isolated neutrophils were stimulated with the lupus-relevant soluble factors or with heat-aggregated IgGs, an antigen-free model of immune complexes, before flow cytometry analysis.

Results: Lupus neutrophils showed significant alterations in the expression of surface markers of the immune complex response, adhesion, complement regulation, and degranulation. These neutrophils also displayed reduced viability and increased apoptosis at 4 hours. Stimulation of normal neutrophils with lupus serum increased apoptosis. Lupus-relevant soluble factors were identified as IL-1β, CXCL12/SDF-1, CCL5/RANTES, CCL2/MCP-1, CXCL10/IP-10, IL-7, and calprotectin. Together, these factors altered neutrophil viability with similar trends to those observed in lupus neutrophils. Stimulation with heat-aggregated IgGs largely reproduced trends in surface marker expression observed in lupus.

Conclusion: This study identifies factors that can alter the viability and the phenotype of lupus neutrophils. Ongoing studies focus on how such alterations can contribute to the implication of neutrophils in the progression of "the disease with a thousand faces".

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225 – P1.14.09

Circulating levels of Low Density Granulocytes and cell-free DNA as predictors of cardiovascular disease and bone deterioration in SLE patients

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Purpose: To evaluate the predictive value of low-density-granulocytes (LDGs) for the development of cardiovascular disease (CVD) and/or bone deterioration (BD) throughout the disease course of Systemic Lupus Erythematosus (SLE). Considering the high SLE-LDG capacity to form Neutrophil Extracellular Traps (NETs), circulating levels of cell-free DNA (cirDNA) were tested as an LDG-associated biomarker to identify SLE patients at risk of CVD and BD.

Methods: The frequency of total blood LDGs, as well as the $CD16^{neg}CD14^{neg}$ (nLDG) and $CD16^{pos}CD14^{low}$ (pLDG) subsets, was quantified by flow cytometry in 33 controls and 144 SLE patients. The serum levels of cytokines and bone metabolites were analysed by immunoassays. Total cirDNA and relative amounts of mitochondrial (mtDNA) and nuclear (nDNA) cell-free DNA were measured by fluorometry or qPCR in plasma from a subgroup of 117 patients and 23 controls at enrolment. All the parameters were analysed in relation to the development of CVD and BD in a six-year prospective study.

Results: Increased blood SLE-nLDGs at enrolment were associated with prospective CVD development (pCVD) and the presence of BD. The levels of cirDNA were also increased in patients, especially those presenting traditional CV-risk factors or subclinical atheromatosis, and were directly correlated with C-reactive protein levels, the erythrocyte sedimentation rate, the neutrophil count and triglyceride levels, thus supporting their use as CVD biomarkers. Similar to nLDGs, the nDNA concentration could predict the development of pCVD in SLE. Finally, patients presenting BD during the follow-up period had the highest levels of sclerostin and RANKL, which correlated with the frequency of circulating LDG subsets, cirDNA and/or nDNA.

Conclusion: Our findings highlight the potential role of immature-LDG expansion as a predictor of CVD and BD in SLE evolution and suggest that altered haematopoiesis could underlie both comorbidities in these patients. The present results also support the quantification of cirDNA levels as a surrogate marker of LDGs in clinical practice.

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251 – P1.14.10

Whole-brain light-sheet and live-cell microscopy to investigate the role of Gαi2 and Gαi3 in cerebral ischemia-reperfusion injury

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Aim: The project aimed to develop workflows to investigate Gαi2 and Gαi3 isoforms in neutrophils in cerebral ischemia-reperfusion injury using live-cell imaging and whole-brain light-sheet microscopy.

Background: Stroke-induced tissue damage results from two main mechanisms, ischemia and reperfusion injury. Reperfusion mediates an inflammatory response, accompanied by neutrophil infiltration. Non-canonical signalling, mediated by Gαi2 and Gαi3 isoforms, ameliorates reperfusion injury in myocardial tissue, but their role in regulating neutrophil recruitment to infarcted brain tissue remains unclear.

Method: Chemotaxis towards fMLP and CXCL2 gradients was monitored in *Gnαi2*-deficient neutrophils using Ibidi chemotaxis chambers and manual cell tracking. To simulate reperfusion injury in brain, we used the transient middle cerebral artery occlusion model (tMCAO). Immediately following reperfusion, brains were harvested, stained with a 5-marker antibody panel, and rendered transparent using a modified Ethyl Cinnamate-based tissue clearing protocol for 3D reconstruction by light-sheet microscopy.

Results: The genetic ablation of *Gnai2* in neutrophils affected their chemotaxis towards fMLP and CXCL2 in 3D collagen matrices. Interestingly, ablation of *Gnai2* had no impact on the total track length or total distance travelled but eliminated the directed migration of neutrophils towards the chemotactic gradient. To test if *Gnai2*-deletion affects neutrophil infiltration into inflamed brain tissue, we established a whole-brain tissue clearing and light-sheet microscopy workflow to quantify neutrophil infiltration following reperfusion injury after tMCAO with single cell resolution. 3D reconstructions of whole brains were analysed for neutrophil infiltration (Ly6G) and PNC formation (Ly6G – CD42b) in relation to vessel and tissue damage detected by Iba-1 (microglia), wheat germ agglutin (WGA, perfused vessels) and CD31 (all vessels). Ischemic and reperfused brain regions could be clearly delineated based on the WGA/CD31 signal, revealing microglia-covered vessels but sparse neutrophil infiltration immediately after reperfusion.

Conclusion: In summary, genetic ablation of *Gnai2* in neutrophils disrupts directed chemotaxis towards fMLP and CXCL2 in 3D collagen matrices without altering overall motility. Additionally, we established a multiplexed antibody panel and tissue clearing workflow to investigate the effects of Gαi2 on neutrophil infiltration into reperfused brain tissue.

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278 – P1.14.11

Zymosan-induced trained innate immunity in mice aggravates myocardial infarction

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Introduction: Zymosan has been used to induce trained innate immunity (TII), which can support the immune response against infectious diseases, but could also exaggerate cardiovascular diseases (CVDs). Here, we investigated the impact of zymosan-induced TII (zTII) on inflammatory and functional consequences after myocardial infarction (MI) in mice.

Methods: C57BL/6 mice were stimulated twice (day -7, -4) with zymosan (or PBS as control) before induction of MI (50 min ischemia/reperfusion). Noninvasive magnetic resonance imaging (MRI) was conducted to assess cardiac function and infarct size (late gadolinium enhancement, LGE). Perfluorocarbon nanoemulsions (PFCs or fluorescently labelled ^{A488}PFCs) were injected intravenously. Immune cell counts and cellular PFC uptake were analyzed by flow cytometry.

Results: Flow cytometric analysis of immune cells in blood, spleen, and bone marrow after zTII revealed increased numbers of neutrophils and monocytes on day 0. Induction of MI (day 0) led to an increased infarct size as well as a reduced ejection fraction (EF) on day 1 in zTII mice. Follow up monitoring of cardiac function by MRI showed a reduced EF and higher end-systolic/-diastolic volume after four weeks. Interestingly, injection of PFCs - which are avidly taken up by phagocytic immune cells that accumulate in inflamed lesions - revealed a significantly reduced ^{19}F signal in the infarcted heart and bone marrow in zTII mice on day 2 post MI. Analysis of immune cell counts showed slightly elevated neutrophil counts in the heart and bone marrow of zTII treated mice. Flow cytometric in vivo and in vitro analyses revealed that PFC uptake by phagocytic immune cells of zTII mice was strongly reduced but could only partly be explained by direct zymosan stimulation.

Conclusion: We show that zTII leads to an increased infarct size and a worse outcome after MI. Reduced ^{19}F signals in the infarcted heart and bone marrow are due to strongly reduced PFC uptake. We conclude that zTII induces functional alterations of phagocytic immune cells, which can be noninvasively detected by ^{1}H/^{19}F MRI, and which may be relevant for the onset, progression and healing after MI or other CVDs.

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335 – P1.14.12

Immune cell activity during anti-TNF treatment in patients with psoriasis and psoriatic arthritis

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Background: Psoriasis is a chronic, inflammatory skin disease characterized by a dysregulated immune response and systemic inflammation. Up to one-third of patients with psoriasis have psoriatic arthritis (PsA). Targeted treatment with antibodies neutralizing tumor necrosis factor (TNF) can ameliorate both diseases. We here explored the impact of long-term infliximab treatment on the composition and activity status of circulating immune cells involved in chronic skin and joint inflammation.

Methods: Immune cells were analyzed by multicolor flow cytometry. We measured markers of immune activation in peripheral blood mononuclear cell (PBMC) populations in 24 infliximab-treated patients with psoriasis/psoriatic arthritis compared to 32 healthy controls.

Results: We observed a significant decrease in the frequency of both peripheral natural killer (NK) cells and their subset $CD56^{dim}CD16^{+}$ NK cells in PsA compared to healthy controls and patients with psoriasis. The latter had a strong positive correlation with psoriasis area and severity index (PASI) in these patients, while $CD56^{bright}CD16^{-}$ NK cells were negatively correlated with PASI. In addition, we observed an upregulation of $CD69^{+}$ intermediate $CD14^{+}CD16^{+}$ and $CD69^{+}$ classical $CD14^{+}CD16^{-}$ monocytes in PsA and increased activity of $CD38^{+}$ intermediate $CD14^{+}CD16^{+}$ monocytes in patients with psoriasis. Compared to healthy controls, psoriasis patients demonstrated shifts of the three B cell subsets with a decrease in transitional $CD27^{-}CD38^{high}$ B cells.

Conclusions: Our exploratory study indicates a preserved pathophysiological process including continuous systemic inflammation despite clinical stability of the patients treated with infliximab.

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346 – P1.14.13

The role of Neutrophil Extracellular Traps in Epidermolysis Bullosa Acquisita

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Purpose: Epidermolysis bullosa acquisita (EBA) is an autoimmune blistering disease (AIBD) clinically characterized by erosions and blisters. EBA is caused by autoantibody against type VII collagen, and its skin lesions feature a marked infiltration of neutrophils into the dermis. Release of reactive oxygen species (ROS) and neutrophil extracellular traps (NET) are among the effector functions of neutrophils which contribute to tissue damage and the resulting inflammation in AIBD. NET formation has been shown to be dependent on ROS generation by NADPH oxidase and chromatin decondensation relying upon the enzymes peptidylarginine deiminase 4 (PAD4), neutrophil elastase (NE), and myeloperoxidase (MPO). This study examines the role of these key players in EBA.

Methods: To provide preclinical proof for the role of neutrophil effector functions during the disease, a mouse model for bullous pemphigoid (BP)-like EBA was utilized. *In vitro,* the study was conducted using bone marrow derived neutrophils stimulated with immune complexes (IC). WT controls were compared to *Rab27a*$^{-/-}$, *Mpo*$^{-/-}$, *NE*$^{-/-}$, and *Padi4*$^{-/-}$ mice.

Results: While WT mice displayed the expected disease progression, and lesional skin stained positive for Ly6G and CitH3, indicating the involvement of NETs, *Mpo*$^{-/-}$ mice were completely protected and did not develop skin lesions or show signs of neutrophil infiltration. Absence of PAD4 or Rab27a did not alter disease severity. *In vitro,* absence of Mpo and Rab27a correlated with a significant reduction in ROS release, NET formation, and leukotriene B_4 (LTB_4) release. These differences were not observed in the absence of NE or PAD4. The specific role of MPO in the inhibition of ROS and formation of NETs could be confirmed by use of PF-1355, a mechanism-based inhibitor of MPO.

Conclusion: Taken together, our results provide evidence that among the various key players of NET formation, MPO plays a crucial role in EBA. The mode of action may be attributed to the inhibition of two disease driving forces, ROS release and NET formation, and release of LTB_4.

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370 – P1.14.14

Investigating the autoimmune response in myocarditis and myocardial infarction

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Purpose: Autoimmunity is becoming a more recognised sequalae following myocardial infarction(MI), where exposure of the adaptive immune system(AIS) to released cardiac antigens exacerbates the inflammatory response. This leads to detrimental remodelling and ultimately heart failure(HF). This phenomenon is more pronounced when an underlying inflammatory disorder is present (ie.myocarditis). We have previously shown in mouse models that specific enhancement of cardiac lymphatics post-MI improves cardiac function, partly due to clearance of innate immune cells (macrophages and dendritic cells(DCs)) from the heart. The interplay between the innate and AIS in this context, however, is poorly understood. We have determined the profiles of adaptive immune cells in mice post-MI that spontaneously develop myocarditis due to T-cells specifically expressing functional receptors to cardiac antigen alpha-myosin heavy chain(αMHC;TCRM) and have begun to manipulate their interactions in draining lymph nodes to prevent T-cell activation and autoantibody production through targeted lymphatic-based clearance from the injured heart.

Methods: Permanent ligation of the left anterior descending artery was performed on anaesthetised wild-type(WT) BALB/c mice or TCRM mice (8-12 weeks). For sham controls, surgical procedure was equivalent minus ligation. The heart and mediastinal lymph nodes(MLN) were harvested 7-days post-MI(7dpi) for processing.

Results: 7dpi, significant enlargement of the heart was observed in MI models versus sham for both mouse groups. A significant inflammatory response was observed in hearts of TCRM mice, especially following MI – with a 4-fold increase in conventional-type 2 DCs(cDC2s) and 5-fold increase in effector CD4+ T-helper cells(eTh) compared to WT. The autoimmune response was elevated in MLNs of TCRM mice – with a 2-fold increase in both cDC2s and eTh, and a trending increase in plasma B-cells compared to WT.

Conclusion: Our studies to-date confirm that activated DCs migrate from the injured heart to MLNs resulting in activation of the AIS and recycling of auto-activated T-cells back to the heart. This is further augmented in the TCRM background sensitised to αMHC. Studies are ongoing to determine how precocious enhancement of cardiac lymphangiogenesis post-MI may traffic immature, non-antigen presenting DCs to blunt crosstalk with T-cells in MLNs, preventing the autoimmune component of chronic MI/HF.

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412 – P1.14.15

Obesity comprises tissue repair by altering type 2 immunity

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Purpose: Obesity represents an evolving global health concern. In addition to well-known comorbidities like cardiovascular diseases and metabolic diseases, obesity is associated with impaired wound healing and drastic changes to the immune cell homeostasis resulting in a constant low-grade type-1/-17-dominated inflammation in both the adipose tissue and the periphery. Therefore, we hypothesize that the impaired wound healing response during obesity is due to a disruption of the type 2 immune response.

Methods: In order to investigate the immune-mediated tissue repair response during obesity, transient lung damage in control-diet fed and high-fat diet-induced obese mice was induced using *Nippostrongylus brasiliensis* infection or papain treatment. Different stages of tissue repair (early damage, acute immune response and late wound healing) were investigated using flow cytometry to characterize infiltrating immune cells, histological analysis for the evaluation of the wound healing progress and ELISAs to evaluate immune cell functionality.

Results: Characterization of immune cells participating in wound healing revealed changes during early immune responses in the lung comparing control-diet fed and high-fat diet-fed animals in both models. In particular, major changes in T cell and macrophage polarization as well as granulocyte recruitment in obese animals were observed. Notably, the acute immune responses in the lung led to long-lasting changes in the immune cell composition within the abdominal white adipose tissue (WAT). ILC2, dendritic cells, eosinophils as well as Th2 cell numbers were still altered long after the primary immune response against the helminth had subsided and the infection was cleared. Similar long-term effects were detected in the WAT immune cell compartment of papain-treated mice.

Conclusion: In summary, our study highlights the detrimental effects of obesity on immune cell behaviour that contribute to compromised tissue regeneration in mice. Long-term effects in the immune cell composition of WAT might be an interesting basis for investigation of subsequent tissue repair and associated immune responses. In our future investigations, we aim to translate these findings with clinical samples obtained from persons living with and without obesity.

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486 – P1.14.16

Modulating effect of astaxanthin in mouse model of osteoarthritis

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Purpose: As one of the most common arthropathies – osteoarthritis (OA) is age related, slowly progressive, condition, affecting more than 240 million people worldwide. The physical manifestations of OA include pain, stiffness, locomotor restriction, bony enlargement, swelling of affected joints, impaired function. Moreover, wear and tear on the joints resulted in bone remodeling, osteophyte formation, fibrosis hyperplasia of the synovial membrane, and damage or loss of articular cartilage. The currently used therapies include pain relieving medicines, non-steroidal anti-inflammatory drugs, and topical therapies. The aim of the study is to investigate the anti-inflammatory and anti-arthritic effects of Astaxanthin (ASTA), a natural keto-carotenoid classified as a xanthophyll, in the collagenase-induced mouse model of osteoarthritis (CIOA).

Methods: Balb/c mice were injected intra-articularly with Collagenase type IA for induction of osteoarthritis. The animals were treated with 40mg/kg ASTA *per os* daily. The effect of the ASTA therapy was evaluated by FACS analysis of synoviocytes, splenocytes, and macropahages, the differentiation of bone marrow-derived osteoclasts and osteoblasts, and the histological assessment of histopathological alterations.

Results: The daily suplementation of 40mg/kg ASTA leads to the reduction of osteoarthritis-associated joint histological manifestations, and improvement of the knee joint remodeling process, confirmed by the evidence of the formation of multinucleated osteoclast-like cells and osteoblasts. The flow cytometry analysis showed a reduction in pro-inflammatory immune cell subtypes.

Conclusion: The animals fed with ASTA showed improved disease symptoms and positive effects on the control and restriction of the disease progression. The results show the potential of the ASTA as the supplement to the main therapy of patients with osteoarthritis.

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491 – P1.14.17

The impact of miR-21 on epithelial intercellular junctions influencing gut inflammation and barrier repair

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Purpose: Inflammatory bowel disease (IBD) is an autoimmune disease of chronic inflammation along the gastrointestinal tract. In the gut, intestinal epithelial cells (IECs) react to changes in the gut environment impacting the abundance of gut microbes, thus affecting gut permeability via key barrier points like intercellular junctions. These tactics can be regulated by microRNAs, short nucleotides that bind to target mRNA and impact protein synthesis. Of focus to this project is microRNA-21 (miR-21), an oncomiR upregulated in IBD and previously shown to target epithelial tight junctions like occludin and Zo-1, thereby increasing barrier dysfunction. However, the molecular mechanisms underlying this phenotype of barrier permeability and repair via intercellular junctions remain inconclusive.

Methods: To evaluate the impact of colitis on direct and indirect miR-21 targets *in vivo*, WT and constitutive knockout mice (miR-21$^{-/-}$) were subjected to DSS-induced colitis. In addition, to assess the consequence of selective miR-21 modulation, we employed an *in vitro* system using the human adenocarcinoma cell line Caco-2 to model the intestinal epithelium. Tissue samples and cell lysates underwent molecular analyses for differential gene expression (qRT-PCR) and protein abundance (fluorescence microscopy) of intercellular junction components.

Results: We show *in vivo* that both basal and dextran sulfate sodium (DSS)-inflamed colonic tissue from miR-21$^{-/-}$ mice have greater expression levels of several tight and adherens junction genes compared to wild type controls, and these were upregulated in other murine intestinal regions. Interestingly, fixed colonic tissue from miR-21$^{-/-}$ mice showed elevated levels of E-cadherin at the top of intestinal crypts. Further isolating IECs from healthy miR-21$^{-/-}$ mice demonstrated an upregulation of claudin-4. Additionally, we demonstrate *in vitro* that selectively modulating miR-21 levels impact the levels of junction components in a predictable manner.

Conclusion: miR-21 plays a regulatory role over IEC intercellular junctions, which dictate paracellular transport, cell adhesion and cell-cell communication. Modulating the levels and function of E-cadherin and tight junction proteins can reduce susceptibility to inflammation and improve mucosal barrier repair during IBD. Importantly, this work elucidates the negative impact of miR-21 over the stability and protection provided by the gut epithelium.

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502 – P1.14.18

Sertoli cell transplantation alleviates acute inflammation and improves sperm quality

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Purpose: Infertility is considered a major problem of the modern-day life with up to 12% of couples affected worldwide. Inflammation-induced testicular damage plays a substantial role in the rising prevalence of infertility. Disappointingly, conventional therapies, often employed during the inflammatory stage, fail to rescue fertility, highlighting the need for pioneering interventions like cell therapy. We have identified Sertoli cells (SCs) as a promising candidate. SCs are crucial for the nourishment, support, and development of sperm cells within the testicular niche. Recently, we have confirmed that SCs possess stem cell-like properties including their immunomodulatory function.

Methods: In this study, we employed an *in vivo* mouse model of acute lipopolysaccharide-induced inflammation and analysed the potential of intravenously administered SCs to alleviate testicular inflammation. Flow cytometry was used to assess the phenotypes of infiltrating and resident myeloid cells. The presence of pro- and anti-inflammatory cytokines in the serum and testicular homogenate was assessed by ELISA. Furthermore, computer-assisted sperm analysis (CASA) and immunohistochemistry were utilized to assess the germ cell quality along with the morphology of the testes.

Results: Intravenously administered SCs showed anti-inflammatory activity *in vivo*. They showed a distinctive migratory pattern, and preferentially accumulated within the testes and liver. Administration of SC resulted in reduced neutrophil infiltration but maintained macrophage population within the testis. Furthermore, the expression of MerTK on testicular macrophages was upregulated. Most importantly, the administration of exogenous SC into an inflammatory model exhibited protective effects on sperm cells, restoring their motility and preserving the physiological morphology of the testes.

Conclusion: Our research provides compelling evidence for the therapeutic effectiveness of SC transplantation in mitigating testicular damage induced by acute inflammation. Hence, highlighting the potential of cell therapies as a novel targeted intervention for male infertility.

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513 – P1.14.19

Dysregulation of type 2 immune responses and microbiota in patients with obesity

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Purpose: Obesity, as one of the most important medical challenges worldwide, is a well-known risk factor for the development of many comorbidities, such as hypertension, diabetes mellitus, or cancer. Moreover, people with obesity (PWO) are more prone to develop surgical wound infections, which can become chronic due to impaired wound healing responses representing severe postoperative complications. Importantly, obesity is associated with a chronic low-grade local and systemic inflammation as well as alterations in gut microbiome composition, both of which affect proper immune function. We therefore hypothesise that tissue repair and wound healing are -at least in part- caused by changes of the microbiome and the altered type 2 immune response in PWO.

Methods: Blood and stool samples were collected from PWO before and after bariatric surgery and analysed in comparison to lean, healthy individuals. To assess alterations in the immune response, the frequencies of different immune cell populations were analysed by flow cytometry. Macrophages, T cells and eosinophils were isolated and cultured to examine polarization capacity, proliferation status and functionality by using qPCR and ELISA. To detect differences in the gut microbiome, stool samples were examined by 16S rRNA metagenomic analysis. We also analysed microbiome and immune cell composition of visceral adipose tissue from PWO and lean controls undergoing reflux surgery in regard to inflammatory conditions.

Results: We observed important changes in composition, functionality and interaction of immune cells, when comparing blood and stool samples from lean and obese individuals. In particular, T cell and macrophage polarisation as well as eosinophil activity were affected by obesity. We have analysed the effects of bariatric surgery and subsequent weight loss on various cell subsets both systemically and locally in adipose tissue. Additionally, we examined the composition of the gut microbiome. Our findings suggest a direct relationship between the loss of specific gut microbes and low-grade tissue inflammation in PWO.

Conclusion: Our results indicate that both the microbiome and polarization capacity and activation are altered in PWO, which may lead to impaired immunity and wound healing. Thus, our data contribute to a deeper understanding of the microbiome-immune axis to avoid surgical site infections.

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570 – P1.14.20

Orthotopically transplanted organoids closely recapitulate human colonocytes in vivo

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The intestinal epithelium plays a central role in human health and disease, and several chronic inflammatory disorders associate with a weakened epithelial barrier. The organoid model allows the cultivation, expansion and analysis of non-immortalized intestinal epithelial cells and has been instrumental in studying epithelial behavior in homeostasis and disease. Recent advances in human organoid transplantation into mouse and human lay the base for models to study human epithelial cell behavior within the intestinal tissue context, and promise novel therapeutic approaches for diseases such as short bowel syndrome and inflammatory bowel disease. It remained unclear how organoid transplantation into the colon would affect epithelial phenotypes and protein expression, which is key to assess the suitability of this model to study human epithelial cells *in situ* and as a therapeutic approach. To address this, we employed Deep Visual Proteomics, which utilizes AI-guided cell classification on high-resolution images, microdissection, and high-sensitivity proteomics, on human colonic epithelial stem and differentiated cells *in vivo*, upon transplantation *in situ*, and organoids cultured *in vitro*. We find that organoids transplanted into the murine colon closely resemble human intestinal epithelial cells *in vivo* compared to organoids grown *in vitro*, indicating that organoid culture induces a transient shift in epithelial phenotypes, which is reversible upon reintroduction into the mucosa. Phenotypic differences between epithelial cells *in vitro* and *in situ*/*in vivo* were largely driven by hallmarks of high proliferation and lower functional differentiation in organoids due to culture conditions. Taken together, we demonstrate that transplanted epithelial cells *in situ* represent a physiological, relevant model for studying functional aspects of mature colonocytes compared to the organoid model.

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574 – P1.14.21

Lymphatic emigration of pro-tolerogenic human epidermal Langerhans cells after tick feeding and tick-borne pathogen transmission

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Langerhans cells (LCs) are the first antigen presenting cells that respond to environmental stimuli including pathogens and may induce immunity or tolerance at the epidermal skin barrier. Here, we investigate the migration and polarization patterns of human LCs in response to clinical and experimental tick bite as well as skin infection with *B. burgdorferi* (acute Lyme borreliosis) using flow cytometry, functional assays, multi-color imaging and single-cell RNA sequencing.

We observed strong emigration of epidermal LCs after tick feeding on human skin and in an *in vitro* experimental tick bite model using tick saliva (TS) injection. Consequently, LCs were increased in the dermis, especially in proximity to dermal lymph vessels. Upon tick bite, LCs over-expressed the migration marker CXCR4 and the lymph node homing molecule CCR7, indicating their capability for lymph node homing. In line with this, LCs stimulated with TS showed increased potential to emigrate from epidermal sheets and invade towards collagen gels supplemented with the CCR7-ligand CCL19. Similarly, acute Lyme borreliosis skin harbored activated LCs expressing CXCR4 and CCR7. Interestingly, monocyte-derived LCs exposed to TS or infected with *B. burgdorferi* exhibited a tolerogenic phenotype characterized by the upregulation of the transcription factors IDO1 and IRF4, when compared to uninfected cells. In contrast, LCs exposed to *S. aureus* infection induced a strong immunogenic response. This could be verified by single-cell RNA sequencing of patients with Lyme borreliosis, where lesional LCs exhibited increased expression of *IRF4, IDO1* and *IL4I1* compared to LCs from healthy skin of the same individuals. Accordingly, when co-cultured with autologous, naïve CD4+ T cells, moLCs pulsed with TS or *B. burgdorferi*, skewed T cell polarization towards regulatory T cells indicating impaired adaptive immune response.

Collectively, our results indicate that tick-feeding induces emigration of LCs to the lymphatic system and a skewed T cell polarization by tolerogenic LCs, which may result in impaired adaptive immune response to tick-borne pathogens. These findings explain the low immunogenic response and high transmission rates of Lyme disease and other tick-borne infections observed in human skin.

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578 – P1.14.22

Targeting NPM1 epigenetically reprograms macrophage metabolism to facilitate post-infarction cardiac repair

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Background: Reparative macrophages play a crucial role in limiting excessive inflammatory responses and promoting cardiac repair after myocardial infarction (MI). Thus, timely and proper transitions of macrophage phenotypes are essential for effective wound healing post-MI. Nonetheless, the mechanisms that governing this transformation are not well-defined.

Objectives: To explore the function and mechanisms of macrophage NPM1 in orchestrating cardiac repair post-MI.

Methods: Peripheral blood mononuclear cells (PBMCs) were collected from healthy individuals and patients with MI to analyze NPM1 expression and its correlation with prognostic indicators of MI. A mouse strain with macrophage-specific knock-out of NPM1 was developed to observe the effect of NPM1 deficiency. Metabolomic profiling was used to analyze the influence of NPM1 deletion on metabolic patterns. Transcriptomic sequencing combined with Cut&Tag was used to analyze the epigenetic mechanisms by which NPM1 regulates macrophage metabolic remodeling.

Results: Firstly, we found that the expression of NPM1 in the PBMCs of MI patients was higher compared to that in healthy individuals, and it showed a positive correlation with indicators of prognosis. Deleting NPM1 specifically in macrophages significantly lessened cardiac fibrosis, encouraged angiogenesis, enhanced cardiac performance, and prevented ventricular remodeling in mice with MI. Additionally, the elimination of NPM1 promoted the reparative phenotype and function of macrophages by increasing mitochondrial oxidative phosphorylation and reducing glycolytic pathways. Mechanistically, NPM1 was observed to bind directly to the promoter region of *Tsc1*, recruiting KDM5b to diminish H3K4me3 modifications, suppressing TSC1's transcriptional activity, blocking mTOR signaling activation, and leading to metabolic reprogramming in macrophages. Both antisense oligonucleotides targeting NPM1 and inhibitory agents against NPM1 showed significant protective effects on cardiac repair following MI.

Conclusion: Our findings indicate that NPM1 participates in the metabolic reprogramming and phenotypic transition of macrophages after MI through epigenetic mechanisms, impeding the repair of cardiac injury and the recovery of cardiac function.

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594 – P1.14.23

SLAMF7 expression of peripheral monocytes in patients with rheumatoid arthritis

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Purpose: Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease of unknown etiology and a complex pathogenesis. Ongoing inflammation results in phenotypic and functional alterations of various immune cell populations. SLAMF7 (also known as CRACC), a typical nk cell receptor, can induce a stimulatory or inhibitory signal in the cell and thereby regulate immune cell functions. For RA monocytes, SLAMF7 has been shown to support activation and induce inflammation, but the SLAMF7-specific function of monocyte subpopulations, classical (CD14+CD16-), intermediate (CD14+CD16+) and non-classical (CD14-CD16+), are not known so far.

Methods: RA Patients and healthy donors (HD) were recruited and clinical parameters, e.g. serum C-reactive protein (CRP) levels and anti-citrullinated protein antibodies (ACPAs) levels were assessed. Disease activity score 28 was determined to evaluate disease activity. Peripheral blood mononuclear cells (PBMCs) were isolated and CD14/CD16 and SLAMF7 expression was determined by flow cytometry. For functional in-vitro assays, monocytes were isolated and stimulated with and without SLAMF7-specific antibody and analyzed for cytokine production using ELISA.

Results: First results show a general decreased SLAMF7 expression on RA monocytes (n=19) compared to healthy controls (n=22). Within the subpopulations, SLAMF7 is mainly expressed on CD16+ non-classical monocytes in RA as well as in HD (mean: 57,8 % and 53,3 % SLAMF7+), but the SLAMF7 expression level was significant lower on CD16+ RA than CD16+ HD monocytes. Furthermore, The increased frequencies of SLAMF7+CD16+ monocytes are associated with age in HD (n=22; r=0,37), but not in RA. However, in RA, frequencies of SLAMF7+CD14+ monocytes are associated with high disease activity (n=15,r=0.63; p=0.01).

Conclusion: We are the first to show a SLAMF7 expression on peripheral blood monocyte subpopulations. SLAMF7+ is mainly expressed of CD16+ monocytes, which seems to be a feature of a healthy aging process that is disturbed in RA. In contrast, frequency of SLAMF7+CD14+ classical monocytes are associated with high disease activity. Because SLAMF7+ monocytes might play a stimulatory role, blocking of SLAMF7 in RA could avoid inflammatory processes.

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620 – P1.14.24

The Fibrin-derived Peptide Bβ15-42 (FX06) Protects Human Pulmonary Endothelial Cells Against COVID-19-Triggered Cytokines

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Objectives: Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has been a major health emergency since 2019. Endothelial dysfunction is a hallmark of COVID-19, which leads to severe illness, i.e. multi-organ failure, coagulopathy, and death. FX06, a fibrin-derived peptide naturally occurring in the human body, formerly known as $B\beta_{15-42}$, protects the vasculature in myocardial ischemia-reperfusion in animal models and clinical trials. Therefore, it is a promising therapeutic candidate for endothelial complications like capillary leak in COVID-19 and other forms of acute respiratory disorders. This project aims to investigate *in vitro* whether FX06 can help to prevent COVID-19 progression.

Methods: To mimic the inflammatory status of COVID-19, cells from a human pulmonary endothelial cell line (ECs) were treated with a cytokine cocktail comprised of ten different cytokines or chemokines at concentrations found in COVID-19 patients' serum profiles. ECs were cultured under both static and shear stress/laminar flow conditions and treated with the cytokine cocktail for 24h, in the absence or presence of FX06 for 2h.

Results: Under both static and shear stress conditions, the COVID-19-triggered cytokine cocktail increased the adhesion and migration of Peripheral Blood Mononuclear Cells (PBMCs) through the endothelial monolayer. This deleterious effect was significantly reduced by FX06. FX06 was shown to mitigate the cytotoxic activity of CD8+ T cells, which increases upon cytokine treatment. Interestingly, FX06 did not protect ECs from cytokine-triggered apoptosis and necrosis, suggesting that restoration of vascular integrity is caused by cytoskeletal reorganisation rather than cellular rescue. Cytokines promote RhoA expression and actin polymerisation, while FX06 treatment reverses cytokine-induced RhoA overexpression and causes morphological changes in F-actin (stress) fibres. Additionally, FX06 restores continuous VE-cadherin/CD144 distribution on the EC surface after disruption by the cytokine cocktail.

Conclusions: FX06 shows great potential in reducing vascular leakage to protect the endothelium against the proinflammatory effect of COVID-19-triggered cytokines. The investigation of immunological markers in ECs will further advance our understanding of the mechanism of action of FX06 and its potential to counteract systemic endothelial inflammation and disease progression.

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668 – P1.14.25

ICOS/ICOSL/OPN in tissue repair: enhancing wound healing with ICOS-Fc treatment

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Background/Purpose: ICOS is a T cell co-stimulatory receptor that binds ICOSL expressed on various cell types. ICOSL also binds osteopontin (OPN), acting as both extracellular matrix protein and cytokine. ICOSL triggering by ICOS or OPN exert different, often opposite, effects on the cell expressing ICOSL, modulating cell migration, angiogenesis and inflammation. In tumours, activation of ICOSL by ICOS-Fc, a recombinant protein comprising the extracellular portion of ICOS fused with IgG1 Fc domain, inhibits cell migration, metastatization and angiogenesis. OPN-triggered ICOSL activation yields contrasting outcomes. Recently, we found that ICOS-Fc improves wound healing in normal skin, enhancing angiogenesis and modulating inflammatory infiltrate. The aim of this work was to extend the analysis to include mice deficient in either ICOS, ICOSL, or OPN, as well as those dually deficient in ICOS/ICOSL, ICOS/OPN, or ICOSL/OPN.
Methods: Skin wounds were induced in C57BL6/J wild type (WT) and the knockout mice cited above (n=4–7 mice per condition-experiment). Mice were treated locally with either PBS or ICOS-Fc and wound healing was monitored for 10 days. Some mice were sacrificed from day 1 to 4 for histological and molecular analyses.
Results: In comparison to WT mice, ICOS-, ICOSL-, OPN- and ICOS/ICOSL -/- dual deficient mice exhibited impaired wound healing, whereas wound healing appeared normal in mice dually deficient in ICOS/OPN or ICOSL/OPN. Treatment with ICOS-Fc ameliorated healing in all mice strains except for those lacking ICOSL (i.e. ICOSL-, ICOS/ICOSL-, ICOSL/OPN -/-). More specifically, the treatment enhanced fibroblast migration, angiogenesis, and immune cell migration on day 3 and day 4 in WT mice and all single knock out mice, without increasing collagen deposition.
Conclusion: These data show that the ICOS/ICOSL/OPN network is involved in wound healing. Surprisingly, healing is defective in the mice carrying an unbalanced mechanism, but not in those whose system is completely unfunctional (i.e. ICOS/OPN and ICOSL/OPN deficient mice). Overstimulation of ICOSL by ICOS-Fc improves healing in all mice expressing ICOSL by exerting effects different from those exerted in tumours.

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703 – P1.14.26

Characterising potential tissue-protective roles of the BTNL-γδ T cell axis in relation to dietary tissue stress

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The intestinal Butyrophilin-like (BTNL)-γδ T cell axis appears highly conserved: human BTNL3+BTNL8 expressed by colonocytes regulates Vγ4+ intraepithelial lymphocytes (IEL), while Btnl1+Btnl6 expressed by mouse enterocytes selects and maintains Vγ7+ IEL. Consistent with IEL being tissue-protective, BTNL3/8 hypomorphism is a risk factor for human inflammatory bowel disease (IBD) severity. Given that high fat intake also disposes to IBD, we hypothesise that dietary stress is a major stimulus triggering the tissue-protective response of the BTNL-gd axis so as to limit progression to severe inflammatory disease. This hypothesis is further supported by evidence from mice that γδ T cells regulate dietary adaptation. Based on this, we are developing a new mouse model of IBD based upon the potential synergy of high-fat diet (HFD) and hypomorphism at the BTNL-γδ T cell axis. Currently, we are examining the impact of HFD on the epithelial expression of BTNL (and other) molecules and on the immunophenotypic and transcriptomic status of Vγ7+ IEL. Thereafter, the physiologic impact of combined defects in the BTNL-γδ T cell axis and HFD, respectively, will be assessed.

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705 – P1.14.27

Deregulated pIgR contributes to steatotic liver disease via systemic IgA accumulation

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Purpose: While IgA is the predominant antibody in the gut where it plays a crucial role in mucosal immunity, increased systemic IgA titers have been shown to propagate steatotic liver diseases (SLD). Yet, the mechanism that contributes to increased circulatory IgA during SLD is currently unknown. Given that IgA levels are regulated by the polymeric immunoglobulin receptor (pIgR), transporting it across intestinal epithelium and hepatic canaliculi, our aim was to elucidate the function of pIgR-dependent IgA secretion in the context of steatohepatitis.

Methods: IgA and *pIgR* levels were determined in different segments of the gut-liver axis of wild-type mice fed chow or methionine- and choline-deficient (MCD) diet for 4 weeks to induce murine SLD. Further, IgA was assessed in serum and stool of biopsy-proven metabolically-associated SLD (MASLD) patients. Next, wild-type (*Wt*) and *pIgR*-deficient (*pIgR*$^{-/-}$) mice, as well as *Wt* and *pIgR*$^{-/-}$ mice transplanted with IgA-deficient bone marrow, were fed MCD diet to investigate the causal role of IgA accumulation during *pIgR*-deficiency in steatohepatitis.

Results: Increased systemic IgA in murine steatohepatitis correlated with reduced *pIgR* expression in the liver, suggesting dysfunctional hepatic IgA secretion. In contrast, increased *pIgR* in colonic epithelium reflected higher fecal IgA, indicating site-specific IgA regulation by pIgR during SLD. In line, MASLD patients displayed increased serum and stool IgA compared to controls, which positively correlated with fibrosis. Furthermore, MCD-fed *pIgR*$^{-/-}$ mice, having higher circulatory IgA compared to their *Wt* littermates, developed more liver inflammation and injury accompanied with increased infiltration of neutrophils and macrophages Elevated neutrophil elastase in liver, as well as higher plasma LPS levels were present in *pIgR*-deficient mice, indicating increased bacterial translocation and NETosis. Importantly, transplantation of bone marrow lacking IgA ameliorated hepatic neutrophil infiltration and injury in *pIgR*$^{-/-}$ mice.

Conclusion: We identified a dysfunctional pIgR-mediated IgA secretion as novel mechanism contributing to steatohepatitis.

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714 – P1.14.28

Development of a novel pathotype-specific therapeutic strategy for osteoarthritis by targeting synovial macrophage subpopulations

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Osteoarthritis (OA) is the most common rheumatic disease affecting over 500 million of people worldwide. Incurable and debilitating joint disease, OA leads to cartilage degradation, bone remodelling with the formation of osteophytes, meniscal alterations, and synovitis, with only symptomatic treatments available. Synovitis has been shown to play an important role in OA initiation and progression. Our group recently identified that OA synovium could be stratified into three different pathotypes, based on a precise histopathological analysis of the synovial cellular infiltration, namely pauci-immune (PI), diffuse-myeloid and lympho-myeloid (LM). Importantly, macrophages represent the most abundant synovial immune population observed in the three pathotypes (Boutet et al., OAC, 2023).

We hypothesized that the fine characterisation of macrophage subpopulations in each pathotype could lead to the development of pathotype-specific macrophage targeting strategies and provide novel personalized therapeutic approaches for OA patients, contributing to giving the “right drug to the right patient”.

Therefore, we collected OA synovial tissues from patients who underwent total knee replacement surgery and selected samples from PI, DM and LM pathotypes (n=3/pathotype), as well as 2 non-OA controls. We performed single-cell RNA sequencing of the $CD45^+$ cells dissociated from these tissues to characterise macrophage subpopulations and we developed a lipoplex-based targeting strategies of these cells.

The single-cell RNA sequencing data revealed 13 distinct macrophage populations. Those macrophage subpopulations were differently represented in the OA synovium compared to control non-OA synovial tissues, as well as across OA pathotypes. We identified *SPP1*$^+$, *LYVE1*$^+$ and *TREM2*^{low} macrophages as respectively enriched in the PI, DM and LM pathotypes. To individually target those pathotype-specific macrophage populations, we selected relevant genes in each population, based on bioinformatic approaches, and developed specific lipoplexes containing a mix of siRNA and mRNA to either reduce or promote their expression. We confirmed the biodistribution of lipoplexes *in vitro* and *ex vivo* on synovial explants, as well as their effective modulation of gene expression.

Overall, this strategy will help better characterise OA pathophysiology and, applied to OA murine models, could ultimately bring the proof of concept that the pathotype-specific modulation of synovial macrophage populations would contribute to prevent OA development.

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726 – P1.14.29

Modulation of lung tissue remodelling induced by Mycobacterium tuberculosis infection by anti-fibrotic drugs

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Purpose: Up to 52% Tuberculosis (TB) patients exhibit tissue remodelling after recovery from *Mycobacterium tuberculosis* (*Mtb*) infection, which is characterised by expression of pro-fibrotic transforming growth factor-β1 (TGFβ1) and collagen deposition by lung fibroblasts. Tissue remodelling is mediated by matrix metalloproteinase-1 (MMP-1), a collagenase that is significantly increased in TB patient plasma. Mechanisms driving fibrosis in TB are ill-defined and the efficacy of anti-fibrotic drugs in reversing TB-driven tissue remodelling are unknown.

Methods: Human monocytes were infected with *Mtb* at a multiplicity of infection of 1, for 24 h. Filter-sterilised supernatant from *Mtb*-infected (CoMtb) or un-infected monocytes (CoMCont) were used to stimulate primary human lung fibroblasts (PHLF) to investigate intercellular networks driving tissue remodelling. Collagen expression was quantified by immunofluorescence microscopy and secreted TGFβ1 and MMP-1 measured by ELISA. To investigate the effect of anti-fibrotic drugs on TB-induced tissue remodelling, PHLF were pre-treated with Nintedanib (a tyrosine protein kinase inhibitor) or Pirfenidone (a pyridinone derivative) for 30 min prior to CoMtb stimulation.

Results: At 72 h, CoMtb stimulation of PHLF significantly increases TGFβ1 secretion compared to CoMCont (149.2±5.7 vs. 106.7.0±2.0 pg/ml, $p<0.05$). Pre-treatment of PHLF with Nintedanib significantly decreases TGFβ1 secretion in a dose-dependent manner, with maximal inhibition observed with 1 μM Nintedanib ($p<0.05$). Pre-treatment of PHLF with Pirfenidone has no effect on TGFβ1 secretion at any tested concentrations (1-100 μM). Nintedanib and Pirfenidone significantly suppress TGFβ1-induced collagen expression from PHLF by 14.0% and 24.4% respectively. However, Nintedanib or Pirfenidone pre-treatment of PHLF has no significant effect on CoMtb-induced collagen expression. However, both Nintedanib and Pirfenidone pre-treated PHLF secrete significantly decreased MMP-1 in response to CoMtb compared to CoMCont stimulation (both $p<0.0001$).

Conclusions: These data demonstrate that primary human lung fibroblasts adopt a tissue remodelling phenotype in response to *Mtb*-induced immune activation secreting TGFβ1 and MMP-1. Nintedanib and Pirfenidone significantly reduced MMP-1 secretion and tyrosine kinase inhibition also decreases TGFβ1 secretion, consistent with blockade of tissue remodelling. Such host-directed therapies may potentially improve outcomes in TB patients.

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793 – P1.14.30

Mesenchymal stem cell extracellular vesicles attenuate dendritic cell maturation.

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Purpose: In inflammatory states, dendritic cells infiltrate bone tissue and activate T cells, increase osteoclast differentiation, and subsequently increase bone resorption and destruction. Therefore, the control of dendritic cell maturation is imperative to potentiate repair. The concept of mediating tissue regeneration using transplanted mesenchymal stem cells was primarily based on the early hypothesis that the cells would engraft and differentiate, to replace damaged tissue. However, low engraftment rates and poor survival remain a barrier for exploitation of the multi-potency of mesenchymal stem cells. Despite this, tissue regeneration is still potentiated. These observations gave rise to the developed hypothesis that mesenchymal stem cells faciliate tissue regeneration via a paracrine method, through secretion of soluble factors and extracellular vesicles. Extracellular vesicles are secreted membrane bound nano-vesicles that contain proteins, lipids, ribonucleic acid (RNA) and deoxyribonucleic acid (DNA). Extracellular vesicles are found in most biological tissues and are involved in cargo transfer between cells. It is hypothesized here that mesenchymal stem cell extracellular vesicles are phagocytosed by dendritic cells, and this subsequently attenuates dendritic cell maturation.

Methods: Extracellular vesicles were isolated from the conditioned media of the D1 ORL UVA mesenchymal stem cell line. Immature dendritic cells were differentiated from C57BL/6 bone marrow monocytes in the presence of granulocyte-macrophage colony-stimulating factor (GM-CSF). Dendritic cell maturation was induced via lipopolysaccharide (LPS) and CpG oligonucleotide stimulation. Mature dendritic cells were subsequently treated with mesenchymal stem cell extracellular vesicles and maturation was assessed via flow cytometry and cytokine analysis.

Results: Mesenchymal stem cell extracellular vesicles are characterised by size, protein content, morphology and flow cytometry. Mesenchymal stem cell extracellular vesicles are phagocytosed by dendritic cells, and this is potentiated by LPS CpG oligonucleotide stimulation. Mesenchymal stem cell extracellular vesicles reduce the surface marker expression of CD80, CD83, CD86, CD40, MHC II and CCR7 on LPS CpG oligonucleotide-stimulated dendritic cells. Secretion of IL-12 and TNF-α from these dendritic cells is also reduced post-extracellular vesicle treatment.

Conclusion: Mesenchymal stem cell extracellular vesicles attenuate maturation in LPS CpG oligonucleotide-stimulated dendritic cells.

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810 – P1.14.31

IL-10 and Gal-3 expression is linked to reduced ulcerative colitis severity in patients with metabolic syndrome

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Purpose: Ulcerative colitis (UC) is defined as a chronic inflammatory condition affecting the gastrointestinal tract, marked by alternating phases of remission and flare-ups. UC is linked with a variety of coexisting conditions, with metabolic syndrome (MetS) being notably prevalent among them. Despite this association, the connection between UC and MetS remains poorly understood. Consequently, our study aims to investigate the influence of MetS on the severity and the immunopathology of UC, both locally and systemically.

Methods: Eighty-nine patients with newly diagnosed and histologically confirmed UC were divided into two groups based on ATP III criteria for MetS: those with UC only and those with UC and MetS. The severity of UC was assessed for each participant using clinical, endoscopic, and histological scoring systems. Concentrations of inflammation mediators were measured in serum and fecal supernatants of UC patients using commercially available ELISA kits. Additionally, the phenotypic and functional characteristics of immune cells in the colon tissue were determined using flow cytometry.

Results: Patients with both UC and MetS exhibited a milder form of the disease, as confirmed by significantly lower endoscopic, clinical, and histological scores, characterized by higher levels of the immunosuppressive cytokine IL-10 in serum and Gal-3 in fecal content, compared to patients with UC only. Moreover, serum ratios of TNF-α/IL-10, IL-6/IL-10, and IL-17/IL-10 were significantly lower, whereas fecal ratios of Gal-3/TNF-α and Gal-3/IL-17 were significantly higher in patients with both UC and MetS. Additionally, a higher percentage of CD56+ NK cells and CD4+ Th cells producing IL-10, as well as a higher percentage of CD3+CD56+ NKT cells and CD8+Foxp3+ Tregs expressing Gal-3, were found in the lamina propria of patients with both conditions compared to those with UC only.

Conclusion: MetS might mitigate colon inflammation by altering the local immune response to enhance the activity of immunosuppressive cells and molecules. The increase in systemic IL-10 and local Gal-3 production could serve as a mechanism to limit inflammation and the resulting tissue damage in UC.

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838 – P1.14.32

Investigating innate immune mechanisms in dental pulp repair

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Introduction: The tooth is composed of several tissues, enamel, dentin, and pulp which is considered the innermost layer. It is a highly vascularised tissue that is responsible for the tooth vitality, sensation, and defence. Whenever it is inflamed either due to bacteria (e.g., in caries) or due to trauma, this inflammation is referred to as pulpitis. Irreversible pulpitis is linked to NLRP3 inflammasome-mediated inflammation. Inflammasomes mediate inflammation and may also play a role in tissue repair and regeneration, a function which is currently not well understood.

We recently demonstrated using a co-culture model that when odontoblasts, the cells responsible for dentin formation, die, they induce differentiation and proliferation of dental pulp cells (DPCs) in an NLRP3 inflammasome-mediated manner. This induces mineralization required for new dentin production suggesting repair of the damaged tooth.

Aim/Purpose: This study aims to determine the underlying mechanisms of inflammasome-mediated dentin regeneration.

In addition, we aim to determine the cell types responsible for inflammasome activation, as well as determining the response of the other cells to inflammasome activation.

Methods: Healthy dental pulp from immature third molars was exposed to bacterial PAMPs and extracellular danger signals to mimic inflammation. The inflammatory response was determined by ELISA and qPCR, inflammasome-positive cells were identified by fluorescent immunocytochemistry and pyroptosis was measured by LDH release. Alternatively, DPCs were cultured in a co-culture model with macrophages.

Results: IL-1β release and cytotoxicity were negligible in DPCs when exposed to NLRP3 activators, suggesting that DPCs do not form active NLRP3 inflammasomes directly. In preliminary results we found that NLRP1 and AIM2 were upregulated in DPCs following inflammatory stimuli in vitro.

Conclusion: Macrophages are the key immune cells in pulpitis, and we have previously shown that DPCs respond to IL-1β in vitro. Using an in vitro pulpitis co-culture model, we will next investigate whether macrophage-derived IL-1β mediates dental pulp cell differentiation.

Future work will identify the role of NLRP1 and AIM2 inflammasomes in pulpitis with the aim to find novel targets that could be used to modulate the destructive inflammation in pulpitis towards regenerative outcome.

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839 – P1.14.33

Immunomodulatory capacity of large and small apoptotic bodies derived from human bone marrow mesenchymal stromal cells: An in-vitro investigation

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Background: Mesenchymal stromal cell (MSC) apoptosis was reported required for their therapeutic function including immunodulation. In our previous study, MSC-derived apoptotic bodies (ApoBDs) also exhibit immunomodulatory potency. However, compared with small extracellular vesicles, there is a dearth of literature to elaborate on the preparation, characterization and biological properties of ApoBDs.

Purpose: This project aims to prepare highly efficient and safe ApoBDs and evaluate their immunomodulatory potential in vitro.

Methods: ApoBDs were collected from the conditioned medium (fetal bovine serum-free) staurosporine-induced apoptotic human MSCs. The 3,000 g and 16,000 g centrifuged speed was performed to isolate large and small ApoBDs, respectively. ApoBDs were collected and characterized comprehensively. In their surface marker characterization, we characterized the surface proteins which were verified expressed or not in their parental MSCs. Then, the ApoBDs were incubated with peripheral blood mononuclear cells and macrophages respectively to investigate their in-vitro immunomodulatory capacity.

Results: Two sets of ApoBDs were harvested which differed in size. The large ApoBDs were about 700 nm and small ApoBDs were about 500 nm. Both ApoBDs expressed CD90, CD44 and CD73 which were verified positive in their parent MSCs. Similarly, PD-L1, CD11b and HLA-DR were expressed lowly in both ApoBDs and their deriving MSCs. Then, ApoBDs were incubated with peripheral blood mononuclear cells. We found that both ApoBDs inhibited allogeneic T-cell proliferation but the large ApoBDs were more effective. Next, in ApoBDs/macrophage incubation assays, both ApoBDs can polarize M1 macrophages towards M2-like phenotype with large ApoBDs being more effective in upregulating the expression of CD163. Besides, large ApoBDs were easier to be phagocytosed by macrophages as detected through flow cytometry analysis and immunocytochemistry images. Finally, the in-vitro safety of both ApoBDs was verified well as no toxicity was observed using staurosporine.

Conclusion: The staurosporine-induced ApoBDs are safe and exhibit immunomodulatory potential in vitro. Compared with small ApoBDs, large ApoBDs are more effective in inhibiting T-cell proliferation and M2 polarization. ApoBDs could be useful in future studies replacing cell therapies with MSCs.

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907 – P1.14.34

Intravenous immunoglobulins: A new therapeutic option for cryptogenic organizing pneumonia?

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Purpose: Cryptogenic organizing pneumonia (COP) is a rare form of idiopathic interstitial pneumonia in which granulation tissue obstructs the ducts and alveolar spaces with chronic inflammation occurring in the adjacent alveoli for which no etiologic agent is found.

The standard treatment for COP is high-dose corticosteroids to reduce the inflammatory process. Approximately 50% of patients with NOC treated with corticosteroids experience disease recurrence.

For recurrent cases and severe infiltrative COP, different immunosuppressive treatments have been tried, such as Rituximab, aziatropine, mycophenolate, cyclophosphamide, all of them with low effectiveness.

The purpose of this work is to present a new therapeutic option for these patients that in our experience demonstrates promising results.

Methods and results: The first is a 60-year-old man with a history of chronic lymphocytic leukemia treated with Venetoclax-Obinutuzumab, who completed the 6 cycles of obinutuzumab. The patient required several admissions due to exacerbations of his cryptogenic pneumonia, with increasing worsening of respiratory function, even requiring home oxygen therapy for the most part. that you are requested to join the waiting list for a lung transplant.

The second is a 53-year-old man with clonal lymphocytosis B being monitored by hematology without active treatment. It begins with symptoms of fever and weight loss with CT images where lung lesions suggestive of NOC and bronchial lymphadenopathy are observed

The third is a 65-year-old patient with a history of non-Hodgkin's lymphoma treated with autologous hematopoietic stem cell transplantation and rituximab who 4 years later has multiple admissions due to exacerbations of cryptogenic organizing pneumonia (COP). With poor response to treatment, it is proposed to join the waiting list for a lung transplant.

The 3 patients received treatment with intravenous immunoglobulins at a dose of 600 mg/kg every 3 weeks, presenting both clinical and radiological improvement with disappearance of the lung lesions, so they were removed from the transplant list.

Conclusion: There are only 10 cases reported in the medical literature of NOC successfully treated with immunoglobulins, so this should be considered as another option for patients who present NOC that relapses to immunosuppressive treatment

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917 – P1.14.35

Immune cell interaction in Pulmonary Arterial Hypertension

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Pulmonary arterial hypertension (PAH) is a cardiovascular disease characterised by a high mean pulmonary arterial pressure of >20 mmHg at rest and a low survival rate. It is associated with chronic inflammation, lung vascular remodelling and vasoconstriction that ultimately leads to heart failure and death. Immune cell infiltration is a hallmark of PAH, and emerging evidence suggests that gamma-delta T (gdT) and plasmacytoid dendritic cells (pDC), with pro-inflammatory and cytotoxic effects, could play a crucial role in the transition to a chronic inflammatory environment.

Therefore, we aim to investigate how gdT and pDC cell populations interact with each other and with the structural cells from all layers of the pulmonary arteries (PAs) and ultimately their contribution to vascular remodelling in PAH.

The methodology consists of immune cell isolations from human healthy buffy coats with magnetic beads, direct co-culture system of gdT and pDC with human primary pulmonary arterial smooth muscle cells (hPASMC) isolated from healthy and PAH lungs, multi-colour flow cytometry, fluorescent microscopy and qPCR.

Our results have shown significant downregulation in activation (CD25) and cytotoxic markers (CD8 and CD56) in gdT and L-selectins (CD62L) in pDC cells, as well as downregulation in extracellular matrix components (Col4A1), cell adhesion (periostin) and upregulation of chemoattractants (CCL4) in hPASMC. These results indicate a potentially unknown homeostatic role of these immune cells on hPASMC.

Even though these results are promising, cellular behaviour in the vascular wall is controlled by the interaction of multiple cell types, which is not possible to replicate in 2D cell culture. Therefore, future experiments will include a 3D human immune cell-blood vessel organoid with induced pluripotent stem cells (iPS) together with gdT and pDC cells to ultimately understand their role in vascular remodelling in PAH.

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926 – P1.14.36

The tricarboxylic acid cycle regulates fibroblast-driven tissue remodelling via hypoxia-inducible factor 1α during Mycobacterium tuberculosis infection

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Background: *Mycobacterium tuberculosis* (*Mtb*) causes extensive lung tissue remodelling, driven by matrix metalloproteinases (MMPs). Fibroblasts are a major source of MMPs in a monocyte-dependent network during *Mtb* infection. Metabolic pathways including the tricarboxylic acid (TCA) cycle, which produces many intermediate compounds, may modulate innate immune responses. Itaconate is a key TCA cycle derivative, which regulates the transcription factor, hypoxia-inducible factor 1α (HIF1α), that drives pro-inflammatory responses in tuberculosis (TB). We investigated the role of itaconate in regulating fibroblast-dependent tissue remodelling and inflammation during *Mtb* infection.

Methods: Primary human lung fibroblasts (PHLF) were treated with 4-octyl itaconate (4OI, a cell permeable derivative of itaconate) for 1 h prior to stimulation with conditioned media from *Mtb*-infected monocytes (CoMTb). MMP-1 (a collagenase), MMP-3 (an MMP-1 activator) and IL-1β secretion, and gene expression were measured by ELISA and qRT-PCR respectively. Functional MMP activity was quantified using the EnzChek™ Collagenase Assay Kit which fluoresces upon collagen degradation. HIF1α protein expression in PHLF was measured at 4-48 h by western blot.

Results: PHLF pre-treated with 4OI upregulated CoMTb-induced MMP-1 and MMP-3 secretion in a dose-dependent manner at 24, 48 and 72 h. The maximal effect was observed with 100 μM 4OI at 72 h when PHLF MMP-1 secretion increased from 156.9 to 232.6 ng/ml (p=0.0002) and MMP-3 concentrations increased from 425.9 to 529.3 ng/ml (p<0.0001). IL-1β secretion was also upregulated with 4OI pre-treatment from CoMTb-stimulated PHLF (286.9 vs. 592.8 ng/ml, p=0.0002). Furthermore, *mmp1* gene expression in PHLF stimulated with CoMTb was increased in response to 4OI treatment ($p=0.034$). Increased collagen degradation was observed from CoMTb-stimulated PHLF pre-treated with 4OI when compared to CoMTb stimulation alone ($p=0.0006$). Lastly, CoMTb stimulation induced HIF1α protein expression in PHLF from 8 h onwards, which was increased with 4OI pre-treatment.

Conclusion: Itaconate, which modulates the TCA cycle, increases *Mtb*-dependent MMP-1, MMP-3 and IL-1β secretion and gene expression in human monocyte-fibroblast networks in TB, as well as upregulation of HIF1α expression in fibroblasts. Specific TCA cycle metabolites may represent potential targets for host-directed therapies to limit inflammation and tissue destruction in TB.

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936 – P1.14.37

Investigating the impact of ECM-based biomaterials in macrophage-mediated tissue regeneration.

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Purpose: Osteoarthritis (OA), is a degenerative disease, stemming from initial injuries or defects of the joint. The immune response, largely mediated by innate immune cells macrophages, greatly dictates disease progression and patient outcome following joint replacement. As a result, modulation of macrophage response has become an attractive therapeutic target to improve tissue regeneration and implant longevity following surgery.

There is now ample evidence which demonstrates how biomaterial design can be used to modulate macrophages in vivo, steering cell phenotype from a destructive pro-inflammatory state to a more favourable pro-regenerative phenotype.

Previous work from the Kelly lab has highlighted that the use of biomaterials derived from mammalian extracellular matrix promotes a hybrid phenotype in primary human macrophages. It is the aim of this study to further characterise this response, and furthermore compare the response of macrophages to ECM derived from distinct tissue sources of the joint including articular cartilage, tendon, and bone.

Methods: Primary human macrophages were treated with ECM derived from porcine bone, tendon, and articular cartilage. Macrophage phenotype was characterised using PCR, ELISA, and western blotting. Metabolic profile of macrophages in response to ECM was also characterised using extracellular flux Seahorse assays. Regenerative potential of ECM-treated macrophages was assessed using indirect co-culture with human MSCs using conditioned media from ECM-treated macrophages.

Results: Results of this study show that musculoskeletal ECM promotes a distinct hybrid phenotype in primary human macrophages, exhibiting markers of both classical and alternative activation. ECM was also shown to limit LPS activation of macrophages, limiting glycolytic capacity of LPS-treated macrophages, as well as pro-inflammatory cytokine production. Finally, Articular-cartilage ECM was shown to drive specific growth factor expression in macrophages, conducive with cartilage regeneration. This result was confirmed with conditioned media experiments, whereby conditioned media from ECM-treated macrophages was shown to promote robust chondrogenesis of human MSCs.

Conclusion: This work builds on previous studies regarding ECM-based scaffolds, further exemplifying the immunomodulatory potential of ECM-based biomaterials, while also highlighting how tissue source can further tune the immune response.

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956 – P1.14.38

Phosphodiesterase 4 regulates monocyte-dependent fibroblast activity in Mycobacterium tuberculosis (Mtb) infection

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Purpose: Tuberculosis (TB) infects around a third of the world's population and even after recovery, up to 94% patients have clinically significant lung fibrosis. Fibrotic lung tissue is characterised by the increased presence of differentiated fibroblasts expressing α-smooth muscle actin (αSMA) and excessive deposition of extracellular matrix proteins. During *Mycobacterium tuberculosis (Mtb)* infection, cyclic adenosine monophosphate (cAMP) is a key regulator of the interface between inflammation and fibrosis and modulated by nucleotide phosphodiesterases (PDE). PDE4 (subtypes A-D) are highly upregulated during TB and so we investigated the hypothesis that PDE4-dependent events drive the development of chronic fibrosis in TB.

Methods: Primary human lung fibroblasts (PHLFs) were stimulated with media, 1 ng/ml TGFβ (positive control), or media from *Mtb*-infected monocytes (CoMTB) or from un-infected monocytes (CoMCon) for 72 h. Expression of αSMA was detected by immunofluorescent microscopy and DAPI nuclear co-staining; quantification of αSMA mean stain area was assessed using ImageJ software. Supernatants were collected at 72 h and profibrotic mediators PDGF-BB and TGFβ quantified by ELISA. RNA was extracted and cDNA reverse transcribed for qRT-PCR analysis of *pde4a-d* expression.

Results: PHLFs treated with CoMTB but not CoMCon enhanced αSMA expression compared to media alone, leading to differentiation into myofibroblasts, a key effector cell in fibrosis ($p=0.011$). CoMTB-stimulated PHLFs secrete significantly more PDGF-BB than fibroblasts treated with CoMCon (73.63pg/ml vs. 35.85 pg/ml, $p<0.01$). CoMTB-stimulated PHLFs secrete increased concentrations of TGFβ1 compared to PHLFs stimulated by CoMCon (748.8 pg/ml vs 552.0 pg/ml, $p<0.05$). Stimulation of PHLF with CoMTB results in upregulation of *pde4a-d*, compared to CoMCon ($p<0.05$ in all subtypes). Pre-treatment of PHLF with PDE4 inhibitor decreases PDE4 mRNA expression and TGFβ secretion.

Conclusion: Fibroblasts activated by Mtb-infected human monocytes secrete pro-fibrotic mediators in a PDE4-dependent manner. PDE4 may be a potential target for host directed therapy in TB treatment, thus reducing the mortality and morbidity associated with TB disease.

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1022 – P1.14.39

Comparative Effects of Vitamin C, Curcumin and Sambucus nigra Extract on Cell Viability & Cytokine Levels of Cigarette Exposed Lung Epithelium Cell Culture

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Introduction: Cigarettes contain harmful chemicals leading to diseases like lung cancer and coronary artery diseases, causing 1 in 10 deaths globally. Smoking depletes antioxidants crucial for immune function. Inflammation, involving cytokines, is a natural defense. Antioxidants like Sambucus nigra, Curcumin, and Vitamin C reduce oxidative stress. However, higher doses of Vitamin C may have a potential pro-inflammatory aspect, revealing a complex relationship between antioxidants and inflammation. Study aims to investigate mutual effects of Vitamin C, Curcumin and Sambucus nigra in lung epithelium cells.

Methods: Human lung epithelium (BEAS-2b) cells were cultured in T25 flasks until 90% confluence at 37°C. Cigarette Smoke Extract (CSE) was prepared for cell interaction. TNF-α and IL-6 cytokine levels from CSE-stimulated cells were measured using ELISA kits. Vitamin C, Sambucus nigra, and curcumin effects were tested through MTT analysis at varying concentrations. CSE-treated cells were supplemented with antioxidants, and cytokine levels were assessed with ELISA kits. Statistical analysis was performed.

Results: After 24 hours of incubation with various Cigarette Smoke Extract (CSE) concentrations, the highest (41% viability) was chosen for subsequent stages. Human lung epithelium cells, stimulated with the CSE for 24 hours, showed high Interleukin 6 levels (28.97) and high but insignificant TNF-alpha levels (6.96). Antioxidant exposure for 24, 48, and 72 hours with 5 different concentrations of each compound yielded varying viability percentages. In the Vitamin C groups, cell viability increased as the dose decreased. Curcumin & S. nigra groups showed overall low viability. Seven groups were selected for cytokine level analysis, and IL-6 or TNF-alpha stimulation was not measured.

Conclusion: Substances' impact on cells depends on dose and exposure duration. Higher vitamin C doses correlated with lower cell viability, indicating potential pro-inflammatory effects. Sambucus nigra and Curcumin consistently showed low viability rates. Adjusting doses in future studies may clarify healing effects. Cigarette smoke uniquely increased TNF-α cytokine levels, revealing its inflammatory effects, while other substances suppressed inflammation, reducing cytokine levels.

Keywords: Cigarette smoke, Cell viability, Cytokine, Antioxidant

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1030 – P1.14.40

Characterization of Fibroblast-like Synoviocytes isolated from the synovial fluid of Juvenile Idiopathic Arthritis patients

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Purpose: The role of Fibroblast-like Synoviocytes (FLS) in Juvenile Idiopathic Arthritis (JIA) is still unclear. FLS have been mostly investigated in the synovial membrane (SM-FLS) or synovial fluid (SF-FLS) of adults rheumatic patients or in murine models of arthritis . In SM, two different subsets of FLS were identified: the lining (LL) and sublining (SL), involved in chartilage degradation or the orchestration of immune responses, respectively. FLS seem to retain multipotent capacity and are able to differentiate into a chondrocyte-like phenotype. The characterization of different FLS subtypes and multipotent properties have never been assessed in JIA patients.

Methods: 10 JIA patients with active disease were enrolled in the study, Cells were isolated from SF by adherence. Skin fibroblasts (sFB) from 4 healthy donors were used as controls. Cytofluorimetric analysis was carried out after staining with anti-CD45, CD34, PDPN, THY antibodies. Gene expression was quantified by RT-PCR under both basal and pro-inflammatory conditions (LPS or TNFα). Chondrogenic differentiation was obtained by culturing pelleted cells in differentiation or normal medium for 28 days, fixed, and stained with Alcian Blue.
Results: Citofluorimetry showed that the majority of SF-FLS cells present a phenotype similar to that of the SM-FLS of the SL region of adult arthritis patients, specifically $CD45^{-}$,CD34-,$CD90^{+}PDPN^{+}$. RT-PCR analysis showed that SF-FLS expressed higher mRNA levels of HAS-1, BMP4, and Aggrecan genes than sFB. LPS stimulation induced high mRNA expression of the pro-inflammatory molecules,IL1β, IL6, IL8, CCL5, respect to metalloproteasis (MMP1, MMP3) supporting the hypothesis of SF-FLS involvement in immune response rather than in tissue degradation. FLS also demonstrated to be able to differentiate into chondrocytes-like tissues when cultured in differentiation medium, as showed by the Alcian-Blue staining Assay.

Conclusion: Results demonstrated that SF-FLS from active JIA patients exhibit a phenotype similar to that of FLS in the sublining SM of adult arthritis patients and might play a role in the inflammation within the joint. Furthermore, SF-FLS ability to differentiate in chondrocytes-like cells suggest the potential of these cells as a model for the study of chondrocytes in JIA and for the predictionof disese severity.

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1137 – P1.14.42

Teucrium montanum extract ameliorates collagen-induced arthritis in rats

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Purpose: Traditional herbal plants attract atention as potential therapeutics in the treatment of arthritis. *Teucrium montanum* L. (TM) is a widely distributed plant in almost all Mediterranean countries. The therapeutic potential of TM extract is insufficiently examined, but its composition implies potential anti-inflammatory effect. We tested amelorating effects of TM extract on rheumatoid arthritis (RA), in rat collagen-induced arthritis (CIA) model.

Methods: Phytochemical analysis of TM methanol extract was performed using LC-DAD-ESI-MS. Female *Dark Agouti* rats were immunized with bovine type II collagen (CII) in incomplete Freund`s adjuvant to induce CIA. CIA rats were treated with TM extract daily *per os* (100 or 200 mg/kg) from day when the first signs of disease started. Clinical evaluation of CIA was performed by scoring and histological analysis. Phenotypic and functional characteristics of splenocytes and draining lymph nodes (dLNs) cells were analyzed by flow cytometry. Cytokine levels in supernatants from hind paw tissue culture, and IgG CII-specific antibodies level in blood were determined by ELISA.

Results: TM extract contained phenylethanoid glycosides, mainly verbascoside, phenolic acids, flavonoid and iridoid glycosides. Applied TM extract doses significantly reduced arthritic score and severity of ankle joint inflammation in CIA rats. This was accompanied by the more pro-oxidant profile in serum, and shifted pro-inflammatory (TNF-α and IL-6) and anti-inflammatory (IL-10) cytokines balance toward later in paws of treated rats. This was in conjunction with less inflammatory phenotype of dLN and spleen CD11b+ cells (monocytes/macrophages) judged by their lower expression of CD86, MHCII, and TLR-4, and lower production of TNF-α and IL-1β. The frequency of TCR+ cells, and IL-17- and IFN-γ- producing CD4+ cells were lower in dLNs and spleen, while frequency of regulatory CD4+CD25+FoxP3+ cells was higher. The lower frequency of CD45RA (B cells) in dLNs and spleen was accompanied with lower levels of serum anti-CII antibodies in treated rats.

Conclusion: These findings suggest, for the first time, that TM extract effectively ameliorated clinical presentation of RA in model of rat CIA, and that this therapeutic effect may be associated with its immunoregulatory/anti-inflammatory action.

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1166 – P1.14.43

Modulating Human Macrophage Metabolism through the Glycolytic Enzyme PKM-2 for the Generation of Bone Regenerative Extracellular Vesicles

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Purpose: The extracellular vesicles (EVs) from pro-inflammatory (M1) and anti-inflammatory (M2) macrophages play crucial roles in terms of bone regeneration. These macrophage phenotypes are dependent on intracellular metabolic pathways such as glycolysis of which the enzyme Pyruvate-kinase M2 (PKM2) is essential. Macrophages switch from M1 to M2 during the bone healing process. Thus, we postulated that we could generate a hybrid M1/M2 macrophage through targeting PKM2, and that their EVs may provide an effective new strategy for bone healing.

Methods: For this aim, primary human macrophages were treated with different concentrations of the PKM2 allosteric activator (DASA-58) before inducing a pro-inflammatory phenotype with LPS (100 ng/mL) + IFN-gamma (20 ng/mL) and compared to LPS + IFN-gamma (M1) or IL4 (20 ng/mL) + IL13 (10 ng/mL) (M2) alone. Metabolic profiling was conducted using Seahorse XF-analyzer, and expression of M1 and M2 markers along with inflammatory and pro-angiogenic markers relevant to bone healing were assessed. EVs were separated and their angiogenic potential was evaluated *in vitro* using tube formation assays with HUVECs.

Results: The activation of PKM-2 in macrophages prior M1 stimulation led to upregulated mRNA levels of *IL10*, *VEGF* and *COX2*, along with downregulation of *CXCL10* and *CD38* expression, as well as reduced IL-6 secretion compared to M1 controls. EVs obtained from PKM-2 re-programmed human macrophages significantly enhanced tube formation in HUVECs compared to M1 controls.

Conclusion: We have shown for the first time that DASA-58 modulates human macrophage metabolism with hybrid features of both pro-inflammatory and anti-inflammatory markers, leading to the production of EVs capable of enhancing tube formation, a critical first step in terms of bone healing. This highlights the link between macrophage metabolism and EV generation, suggesting potential therapeutic strategies for promoting bone healing. Further studies will explore the biological activity of these regenerative EVs in various *in vitro* settings, such as osteogenesis and immune response, prior to incorporating them into a novel biomaterial for localized delivery in an *in vivo* model of large bone defects.

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1191 – P1.14.44

Intermittent actuation decreases the presence of neutrophils in the pericapsular region of implanted biomaterials.

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Background: The Foreign Body Response (FBR) is a complex cascade of inflammatory and fibrotic events that occur in the presence of a foreign object, such as implantable biomaterials. Innate immune cells including neutrophils recognise the foreign material and orchestrate the FBR, leading to the formation of a hypopermeable, fibrous capsule around the device, which is detrimental to its function and long-term durability. We have previously shown that cyclic inflation and deflation (intermittent actuation (IA)) of an implanted device interferes with the progression of the host FBR, resulting in a significant reduction in fibrous capsule thickness. However, the immunological mechanisms by which IA interferes with the FBR are not fully understood.

Methods / Results: Given that neutrophils play a key role in initiating the inflammatory response to implanted biomaterials, we hypothesised that IA affects neutrophil recruitment to the pericapsular region. To test this, devices were implanted subcutaneously in mice and actuated for 5min every 12hrs. Immunofluorescent staining of Ly-6G at the pericapsular region showed that IA significantly reduced the presence of neutrophils 5-days post-implantation, compared to non-actuated control devices. We next established an *in vitro* model to further understand the neutrophil response to IA and investigate if IA alters neutrophil survival and function. Owing to their short lifespan *in vitro*, primary neutrophils are challenging to culture. Therefore, we established a protocol to differentiate neutrophil-like cells from the acute promyelocytic leukaemia cell line, HL-60. HL-60s were cultured with 1.3% DMSO for 7-days and expression of neutrophil markers were monitored by flow cytometry. DMSO did not affect cell viability and significantly increased the expression of CD16, CD11b and CD66b, indicating successful differentiation into neutrophils. Neutrophils will be cultured on devices which will be actuated daily for 5-days. We will examine if IA affects neutrophil survival, cytokine (TNF and IL-10) and Reactive Oxygen Species production.

Conclusions: Results from this project will better our understanding of the mechanisms by which IA interferes with the cellular coordination of fibrosis. Defining this process has critical implications for understanding the host response to implantable biomaterials and how modulation of neutrophils may have important long-term consequences on the FBR.

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1214 – P1.14.45

Butyrate, tributyrin, and high-fiber diet: improving inflammation in acute and chronic psoriasis

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Purpose: Psoriasis is a chronic disease characterized by T cell activation and keratinocyte hyperproliferation. Butyrate is being studied for its potential therapeutic effects on immunological diseases, however, its impact on psoriasis remains unclear. This study aimed to investigate the effects of oral administration of butyrate, trybutirin and a high-fiber diet on experimental psoriasis.

Methods: In a chronic psoriasis model, male C57BL/6 mice were induced with psoriasis by applying an imiquimod-based cream for 6 consecutive days, followed by reapplication from day 21 to day 26. Mice were either given 150 mM butyrate in drinking water or orally administered with 3 g/kg tributyrin (a pro-drug of butyrate) through gavage from day 7 to day 28. In another model, animals were fed a high-fiber diet (25% inulin), starting 7 days before imiquimod application and continuing until the final day of the protocol. In an acute psoriasis model, mice were induced with psoriasis by applying an imiquimod-based cream for 5 consecutive days while simultaneously receiving 150 mM butyrate in drinking water. Skin thickness, body weight, water and food intake, spleen area and weight, and gene expression of resident memory T cell (TRM) markers in the skin were assessed. Statistical analyses were performed using one-way or two-way ANOVA followed by Bonferroni's post-test ($p < 0.05$) (CEUA: 6059-1/2022).

Results: Topical application of imiquimod mimicked psoriatic inflammation (increased skin thickness, redness, and peeling, increase in spleen area and weight) compared to the control group. Treatment with butyrate reduced skin thickness, redness, and scaling in both chronic and acute psoriasis models. The gene expression of CD103, a T_{RM} marker, was reduced at the 28th of the protocol in butyrate-treated animals. Tributyrin or a high-fiber diet also showed some benefits in alleviating psoriatic inflammation, although the effects were not as pronounced as pure butyrate.

Conclusion: Butyrate, tributyrin and high-fiber diet treatment had beneficial effects on psoriatic inflammation. Butyrate led to a reduction in skin thickness and modulated the expression of T_{RM} markers, specifically decreasing CD103 expression. These findings suggest that butyrate may have immunomodulatory properties that can attenuate the activation of T_{RM} cells in psoriasis.

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1270 – P1.14.46

Unravelling stem cell destiny: calcineurin-NFAT axis in early stem cell commitment and immune exclusion

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The Nuclear Factor of Activated T Cells (NFAT) is a family of transcription factors composed of 5 members, four of which (NFATc1-4) are regulated by calcineurin (CN), a calcium-dependent phosphatase. Our group shed light on additional non-inflammatory roles of this transcription factor in dendritic cells. The aim of this work is to unveil how NFAT activation modulates early stem cell commitment and differentiation while exploring new tissue-dependent mechanisms of immune system alertness towards cell proliferation.

We investigated two tissues with distinct regenerative capabilities: the brain, characterized by slow regeneration, and the small intestine/colon, known to be highly proliferative. *In vitro*, we infected neural stem cells with a lentivirus carrying a CN inhibitor (iCN), while *in vivo*, we conducted intracranial injections targeting the ventricular-subventricular zone (V-SVZ) with the same virus. To assess if this mechanism extends to the small intestine/colon system, we utilized mice with inducible inhibition of NFAT in $LGR5^+$ stem cells, predominantly found in intestinal and colon crypts. We also employed 2 models of disease: Parkinson's disease (PD), for the brain system, and dextran sulfate sodium (DSS)-induced colitis.

When NFAT-CN interaction is inhibited, there is an expansion of the neural stem cells in resting condition both *in vitro* and *in vivo*. Furthermore, we showed that this expansion can ameliorate a damage induced by PD. This phenotype is also conserved in the small intestine and colon where there is an expansion of $LGR5^+$ cells leading to tissue elongation. Consistent with our previous findings, this expansion of stem cells can mitigate colitis damage induced by DSS. We also found that NFAT regulates a checkpoint phase mediated by the immune system.

In summary, we propose that NFAT is involved in an early phase of stem cell differentiation, controlling directly or indirectly adult stem cell differentiation and immune system surveillance on activated progenitor cells.

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1332 – P1.14.47

Extracellular vesicles produced by interleukin 4 polarized macrophages suppress atherosclerosis by reprograming Ly6Chi monocytes that improve efferocytosis

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Purpose: Extracellular vesicles (EVs) produced by IL-4-polarized macrophages (Mϕ) suppress inflammation by communicating immunometabolic signaling to recipient immune cells and adipocytes. The purpose of this study was to examine transcriptional responses sensitive to IL-4-polarized Mϕ EVs among circulating Ly6C^{hi} monocytes that could suppress atherosclerosis in hyperlipidemic mice.

Methods: EVs were produced by culturing THP-1 macrophages with IL-4 and purified using cushioned-density gradient ultracentrifugation. Twenty-week old male LDL-receptor deficient mice fed high-fat diet for 16 weeks were treated given intraperitoneal infusions of 10e^{10} THP1-IL4 EVs or sham 3-weekly for 6 weeks. Circulating Ly6C^{hi} monocytes were collected using a cell sorter and extracted RNA was examined using NanoString nCounter Autoimmune and Metabolic gene panels. Plasma cytokines were detected with a V-Plex Meso Scale assay. Atherosclerosis was assessed by detecting myeloid cell subsets in enzymatically digested aortae as well as oil red O positive lesions and Sirius Red collagen staining in the aortic root. Efferocytosis was examined both *in vitro* and *ex vivo* by exposing THP1-IL4 EVs or sham-treated bone marrow-derived macrophages and peritoneal macrophages with CFSE-labeled apoptotic Jurkat cells.

Results: THP1-IL4 EVs caused reduced signaling pathways controlled by MAPK, PI3K, TLR and inflammasomes among Ly6C^{hi} monocytes. We identified reduced levels of Casp-1, Nlrp1a, as well as Aim2, all recognized to drive atherosclerosis including in JAK2^{vf} clonal hematopoiesis. Furthermore, THP1-IL4 EVs upregulated metabolic pathways involved in arginine metabolism, IDH1/2 activity, redox stress control, nucleotide salvage, mitochondrial respiration, and fatty acid oxidation. In contrast, they reduced pathways involved in glucose transport, hypoxia, KEAP-NRF2 pathway and tryptophan metabolism. Control of these pathways in Ly6C^{hi} monocytes contributed to reduced levels of IL-1β, IL-6, IFNγ and TNFα in plasma, as well as CD45^{+} aortic myeloid cells and oil red O positive aortic lesions. This also led to plaque stabilization as noted by increased collagen deposition and reduced necrotic core volumes. *In vitro* and *ex vivo* studies of macrophages treated with THP1-IL4 EVs supported their capacity to enhance efferocytosis.

Conclusions: IL-4-polarized Mϕ EVs communicate profound transcriptional reprogramming to Ly6C^{hi} monocytes, suppressing their propensity to accelerate atherosclerosis while benefitting their ability to contribute to atheroma remodeling and stabilization.

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1335 – P1.14.48

Downregulation of Notch signaling is associated with enhanced activity of human osteoclast progenitors in patients with rheumatoid arthritis

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Purpose: Rheumatoid arthritis (RA) affects approximately 1% of the global population, causing significant morbidity and disability. Despite advances in treatment options, many patients experience suboptimal responses to therapy and decreased quality of life. Inflammation-induced activation of osteoclasts, exclusive bone-resorbing cells of hematopoietic origin, mediates joint destruction and systemic bone loss in RA. We previously characterized highly osteoclastogenic human osteoclast progenitor (OCP) population within the classical monocyte subset (CD45+CD15-CD3-CD19-CD56-CD11b+CD16-CD14+CCR2+), present in the inflamed periarticular tissues as well as among peripheral blood mononuclear cells. As Notch signaling is implicated in hematopoietic cell differentiation and activation, we investigated the effect of Notch modulation on OCP activity in RA.

Methods: Upon Ethical approval, mononuclear cells from peripheral blood and periarticular tissue were collected from RA patients and controls. Expression of Notch pathway components were analyzed in sorted OCPs using flow cytometry and qPCR. In addition, Notch receptors and ligands were assessed in periarticular tissue by immunohistochemistry. Osteoclasts and macrophages were differentiated from common progenitor monocyte subset in vitro using RANKL and/or M-CSF respectively, under stimulation by immobilized Notch ligands, with or without addition of neutralizing anti-Notch 1 antibodies.

Results: Substantial proportion of OCPs within peripheral blood and periarticular mononuclear cells of RA patients express Notch receptors. Moreover, we observed lower gene expression of Notch 1 and 2 receptors in OCPs from RA as well as negative association between Notch 1 and 2 expression with RA activity score DAS28. Differentiating osteoclasts increasingly expressed Notch 3 and DLL1 mRNA. In vitro stimulation of osteoclastogenic cultures by Notch ligands showed that JAG1 and DLL1 inhibited osteoclast formation in a dose dependent manner, whereas neutralizing anti-Notch 1 antibodies partially ameliorated that inhibition. Moreover, macrophages differentiated from the same monocyte progenitor subset were also functionally regulated by Notch signaling, with DLL1 acting to suppress phagocytosis but enhance antigen-presenting potential.

Conclusion: Notch axis is involved in the negative regulation of OCP differentiation and activity, therefore modulation of Notch signaling may serve as an important complementary approach to reduce bone resorption in arthritis.

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1341 – P1.14.49

Dexamethasone and mRNA co-loaded Lipid Nanoparticles (LNPs): A Synergistic Approach for Efficient mRNA Delivery and reduced pro-inflammatory Cytokine Secretion in immunological cells

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Purpose: The objective of this work is to develop two LNP formulations with the ability to efficiently express a reporter mRNA and co-deliver an anti-inflammatory drug (Dexamethasone, DX) in Human peripheral blood mononuclear cells (PBMCs). LNPs-mRNA/DX could reduce the secretion of pro-inflammatory cytokines and attenuate the side effects of repetitive LNPs administration.

Methods: LNPs were optimized by modifying the helper lipids and replacing the 25% of cholesterol with DX, to obtain two different lipid mixes: LM1-DX (ALC-0315/DSPC/Cholesterol/ALC-0159/DX) and LM2-DX (ALC-0315/DOPE/Cholesterol/ALC-0159/DX). LNPs without DX were also prepared (LM1 and LM2). The LNPs were generated using the microfluidic platform and characterized in terms of size, polydispersity and Z potential by Dynamic Light Scattering and mRNA encapsulation efficiency (EE) by fluorescence assay. Transfection efficiencies of LNPs delivering a reporter mRNA (Luciferase) to PBMCs were determined. The release of cytokines induce by different LNP formulation was measured in the supernatant of PBMCs by cytometric bead array. *In vivo* biodistribution in C57BL/6J mice after i.m injection of the LNP formulations was performed.

Results: Stable LNPs-DX were obtained with a mean size around 120 nm, highly homogeneity (PDI less than 0.05) and Zeta potential values close to neutrality. High mRNA EE (95-100%) was achieved. The LNPs efficiently delivered *Luciferase* mRNA to PBMCs with relative luciferase levels higher than $5x10^5$ for LM1-DX and LM2-DX. Cytokine release was evaluated after stimulation of PBMCs with LNPs for 24 h. While LM1 and LM2 LNPs induced an increase in interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and monocyte chemoattractant protein-1 (MCP-1) levels, the respective LNPs-DX strongly reduced 3 to 5 times the cytoquines levels. The biodistribution assay showed that LM1-DX and LM2-DX mostly distributed in the liver, while LM2-DX also exhibited luciferase signal in spleen and limph nodes.

Conclusion: The results demonstrate that LNPs co-loaded with mRNA and anti-inflammatory drug could be considered as effective carriers for transfection of human primary immune cells and reduction of pro-inflammatory effects. Furthermore, LNPs showed differences in biodistribution. These formulations showed more tolerogenic properties with lower pro-inflammatory activity and could be potential candidates for the reduction of adverse effects in the treatment of metabolic diseases.

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Abstracts

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1366 – P1.14.50

Role of macrophage migration inhibitory factor (MIF) on mesenchymal stromal cell (MSC) therapeutic efficacy in acute lung injury

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Purpose: Human mesenchymal stromal cells (MSCs) rely on specific inflammatory disease microenvironments in order to carry out their anti-inflammatory actions *in vivo*. One of the barriers to the success of MSC therapy is the inability to identify potential responders. Macrophage migration inhibitory factor (MIF) has been previously shown to act as a mediator sustaining the pulmonary inflammatory response in acute respiratory distress syndrome (ARDS). This study evaluates the role of MIF in ARDS pathophysiology and how it may be used to predict MSC therapy outcomes.

Methods: To investigate the role of MIF in ARDS humanised MIF mice were subjected to an LPS-induced acute lung injury model. Mice with either the low-expressing MIF promoter polymorphism ($CATT_5$), or the high-expressing MIF promoter polymorphism ($CATT_7$) or wild type (WT) littermate controls were anesthetised by isoflurane, and 2mg/kg LPS (*E.coli* - O111:B4) or PBS control was instilled intratracheally. 4 hours after LPS challenge, human bone marrow-derived MSCs were administered intravenously. Mice were sacrificed 48 hours post LPS challenge and the bronchoalveolar lavage fluid was obtained. Pro-inflammatory cytokines, total protein content and immune cell populations were evaluated.

Results: Mice containing the high-expressing MIF allele ($CATT_7$) exhibit a more severe phenotype with increased clinical score, elevated levels of pro-inflammatory cytokines and greater immune cell infiltration following LPS challenge compared to the low MIF expressing $CATT_5$ and WT groups. Furthermore, $CATT_7$ mice displayed increased lung permeability. Interestingly, BM-MSCs were more efficacious in the $CATT_7$ mice where they significantly reduced inflammatory markers and abrogated immune cell infiltration.

Conclusion: These data demonstrate how disease microenvironments can affect MSC therapeutic efficacy and identifies MIF as a potential biomarker for MSC success.

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1385 – P1.14.51

Time-Dependent Modulation of Peritoneal Adhesion Post-Abdominal Surgery: The Role of PAD2 in Regulating neutrophil extracellular traps and macrophage extracellular traps

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Purpose: This study investigates the inhibition of Peptidylarginine Deiminase 2 (PAD2) as a therapeutic strategy to mitigate peritoneal adhesions resulting from past surgeries, focusing on its effects on Neutrophil Extracellular Traps (NETs) and Macrophage Extracellular Traps (METs) formation inhibition.

Methods: Utilizing a peritoneal adhesion model in C57BL/6 mice, this study assessed the impact of various pharmacological inhibitors on NET and MET at post-operative days 3 and 21, exploring their roles in adhesion formation. Inhibitors tested included Pad2in1 (PAD2), DNase1 and DNase1L3 (extracellular DNA), BMS-P5 (PAD4), Alvestate (Neutrophil elastase), AZD5069 (CXCR2 antagonist), Disulfiram (Gasdermin D), Na11 (NOX-2 for ROS-dependent NETs), and Metformin (HMGB1). Evaluation methods comprised HE staining, immunohistochemistry for NET/MET markers, and immunofluorescence for detailed NET/MET visualization. Macroscopic grading of adhesions was performed using the Leach and Nair grading systems.

Results: PAD2 inhibition significantly reduced adhesion severity, especially in the prolonged post-operative phase (21-day model). The efficacy of PAD2 inhibition in reducing NET and MET formation in both, the early phase (3-day model) and late phase (21-day model), contributed to this reduced adhesion severity.

Conclusion: Targeting PAD2 presents a promising approach to reducing peritoneal adhesions post-abdominal surgery. By modulating both NET and MET pathways, PAD2 inhibition offers a nuanced therapeutic strategy that addresses the complex temporal dynamics of adhesion formation. This underscores the importance of considering the timing of intervention in the development of post-surgical care protocols, suggesting a new avenue for improving patient outcomes following abdominal surgery.

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1406 – P1.14.52

To stress... To resist? The power of stress responses in sepsis

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Restoration of organismal homeostasis after an insult requires a balanced response by two arms of host defence: disease tolerance and resistance. Disease tolerance refers to mechanisms involved in tissue protection from damage caused by inflammation, whereas resistance mechanisms aim to kill and clear out the pathogen that triggered the inflammatory condition.

Inducing stress responses can initiate disease tolerance mechanisms, as our lab previously demonstrated (DOI: 10.1016/j.immuni.2020.09.011). In a severe murine sepsis model, we showed increased survival and less tissue damage upon treatment inducing mild mitochondrial stress. However, the protective mechanisms initiated by stress responses have not been systematically studied. This work focuses on understanding the impact of stress responses on resistance and tolerance mechanisms.

For this, we optimized a mild murine sepsis model to allow monitoring for 5 days. Within this period, pathogen load, immune phenotyping and damage markers were assessed by colony forming unit assays, flow cytometry, histopathology and circulating damage markers quantification, respectively. Mixed effects models, with experiments as random effect, were performed using R software.

So far, we have found a significant pathogen burden decrease of at least 30% in treated animals compared to controls, starting at 72h post-infection and treatment. Culminating in complete pathogen clearance in some organs of treated animals at 120h, with control mice still showing considerable pathogen loads. These observations support the hypothesis that mild mitochondrial stress also triggers pathogen clearance, a hallmark of resistance responses. Furthermore, we found significant differences in adaptive and innate immune cell populations, associated with the treatment of septic animals. Interestingly, the effects of mitochondrial stress appear to be cell-specific: treatment reduced lymphocyte activation whilst, in parallel, increasing innate immune cells. We hypothesize these changes could be underlying protective mechanisms.

Our results show that mild mitochondrial stress can improve resistance mechanisms and tolerance in septic mice. Furthermore, our data suggests that mild mitochondrial stress could be a potential strategy for fine-tuning the type and quality of immune responses. Understanding how mitochondrial stress contributes to homeostasis restoration could inform the development of therapeutic approaches to life-threatening conditions characterized by dysfunctional immune responses.

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1407 – P1.14.53

Neutrophil subsets and their functions in the development of periodontitis

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Periodontitis is a highly prevalent chronic inflammatory disease triggered by the oral dysbiosis, whith *Porphyromonas gingivalis* considered a key stone pathogen. Neutrophils, the most abundant immune cells found in periodontal lesions, play a crucial role in periodontal health and disease. Recent data suggests that neutrophils are differentially distributed in periodontal lesions of experimental periodontitis models, exhibiting differential expression of pro-inflammatory markers, which may indicate distinct subtypes of neutrophils involved in the pathogenesis of periodontitis. This study aims to identify predictive neutrophil phenotypes and determine their functions in periodontitis onset and progression.

Experimental periodontitis was induced on 2 groups of wild-type (WT) mice using ligatures soaked with or without *P. gingivalis.* The ligatures were placed into the palatal sulcus of the first maxillary right molar, while the intact left molars served as control. Alveolar bone resorption was monitored by micro-CT before, during, and at the end of the experiment. All mice were sacrificed for histological and immunostaining analysis. In addition to periodontitis mice model, gingival tissue and saliva samples from periodontitis patients were analyzed using mass imaging cytometry (Hyperion) and spectral flow cytometry (Cytek).

In ligature-induced periodontitis, the presence of *P. gingivalis* modulates the expression of neutrophil serine proteases-expressing cells in connective tissue and along alveolar bone crest, with a differential expression of cathepsin G, neutrophil elastase, and PR3 observed. The multiplex analysis of neutrophils purified from saliva revealed a differential expression of markers related to maturation, activation, survival, and phagocytosis between healthy individuals and those with periodontitis, indicating a pro-inflammatory profile in the latter. We identified the overexpression of neutrophil serine proteases in tissue from periodontitis patients. Deeper analysis of neutrophils distribution and phenotypes showed fewer PMNs in non-inflamed tissue and strong patches of PMN infiltrates, with differential expression of key markers and enzymes (CD16, myeloperoxidase, cathepsin G, neutrophil elastase, etc.) according to their localization, indicating different functions and levels of maturation.

The preliminary data of the present study indicates evidence of different subtypes of neutrophils with distinct functions involved in the development of periodontitis.

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1455 – P1.14.54

Attenuation of apoptosis and inflammation in oral lichen planus patients after low level laser therapy

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Oral lichen planus (OLP) is a T-cell-mediated chronic inflammatory disorder that affects the oral mucosa. The control of symptomatic OLP includes different therapies but a permanent cure is not yet available. Although topical steroids are the first-line treatment, alternative strategies for management are required. Low-level laser therapy (LLLT) is an innovative approach for OLP patients that improves wound healing and decreases inflammation.

The study aimes to determine and compare the expression of pro- and anti-apoptotic markers in patients' biopsies and TNF-α in saliva before and after LLLT treatment.

Material and methods: 20 OLP patients and underwent LLLT with a diode laser (810 nm), 3 times weekly for a month. 20 healthy donors were used as controls. The biopsies were taken before and after therapy and the expression of the markers was examined immunohistochemically. Unstimulated whole saliva was collected and the levels of TNF-α were measured by ELISA.

Results: The results from immunohistochemistry showed that p53 and caspase-3 expression were high in OLP patients and decreased after LLLT. In contrast, p63 and bcl-2 expression was absent or weak in the OLP group and slightly increased after therapy. Salivary TNF-alpha levels were significantly increased before and decreased after therapy in patients with OLP in comparison with healthy subjects.

Discussion: Based on our immunohistochemical results, the reverse correlation between p53-p63 and the lack of keratinocyte expression of bcl-2 observed in OLP patients makes keratinocyte cell death possible through a caspase cascade activation. Decreased caspase 3 and TNF-a expression after LLLT are linked with significant improvement in the symptoms and clinical signs of OLP. We showed decreased inflammation and stimulated cell survival and proliferation, counteracting in this way to the pro-apoptotic assault blamed for the tissue damage. All this determines LLLT as a useful and harmless treatment modality for OLP patients.

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1514 – P1.14.55

Immuno profile of Inflammatory bowel disease patient response to anti- TNFa therapy

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Tumor necrosis factor (TNF) α is a pleotropic cytokine with a potential proinflammatory capacity and has been recognized as one of the triggering cytokines in the induction of colorectal inflammation and involved in the pathogenesis of inflammatory bowel disease (IBD). As a result of this, blocking of TNF signal by anti-TNF antibodies becomes a main therapeutic strategy for severe steroid refractory or dependent IBD patients today. Clinical evidence showed that anti-TNF therapy results in an improved clinical success including a rapid disease remission, a high rate of mucosal healing, and improved quality of life in up to 70% of IBD patients. However, about 30% of IBD patients do not respond to anti-TNFα induction therapy. To provide new insights and to optimize the precision of anti-TNF strategy in patients with IBD, we designed a longitudinal study of patients who received anti-TNFα therapy. We collected biopsies before and after 12 weeks of treatment with anti-TNFα and performed single cell transcriptomic analysis with particular focus on CD4+ T cells. We further integrated our findings with 4 other published data sets to analyze individual cell activity and its interaction to other immune and non-immune cells. Our analysis provides a detailed cellular landscape of inflamed gut tissue compared to healthy or non-inflamed controls. In particular, we found that expanded intestinal T follicular helper (TFH) cells are more associated to inflamed tissue and suggest a potential link to negative response to anti-TNFa therapy. These findings shed light on the possible role of intestinal TFH cells in maintaining gut homeostasis and highlight its pivotal role to intestinal immunity in the pathogenesis of IBD.

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1521 – P1.14.56

Expression of MIR22 Host Gene in different cell populations isolated from Oligoarticular Juvenile Idiopathic Arthritis patients

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Purpose: Oligoarthritis is the most common category of Juvenile Idiopathic Arthritis (JIA). To date, no reliable biomarker of disease activity and response to treatment has been identified for this disease. Recent findings indicate a causal role for MIR22 Host Gene (MIR22HG) in the pathogenesis of adult arthritis and suggest its potential as a biomarker. No information is available on MIR22HG expression/function in Oligoarthritis. We investigated MIR22HG expression in different cell populations from peripheral blood (PB) and synovial fluid (SF) of Oligoarthritis patients at disease onset. MIR22HG putative role in Oligoarthritis was studied by assessing the association of its expression with patient clinical course after 1 year of follow up.

Methods: MIR22HG expression was analyzed by RT-qPCR in the human monocytic cell line, THP1, and in mononuclear cells isolated from both PB (PBMC) and SF (SFMC) of 30 treatment-naïve Oligoarthritis patients at onset and from PB of 10 age/gender matched healthy individuals as controls. Cells were isolated by Ficoll and separated into CD14+ and CD14- subsets using CD14+ magnetic beads. SF-derived fibroblast-like synoviocytes (FLS) were isolated from 8 patients and phenotypically characterized using anti-CD45, CD90, CD34, and Podoplanin (PDPN) antibodies. Results of MIR22HG expression analysis were associated with patient clinical parameters.

Results: We showed increased MIR22HG expression in THP1 cells upon stimulation with the proinflammatory stimuli, LPS, IFNy, and hypoxia. Higher MIR22HG expression was detected in patient-derived CD14+ compared to CD14- cells. Correlation with patient clinical data indicates different MIR22HG mRNA expression levels in CD14+ cells between patients with oligoarticular and polyarticular courses of the disease at follow up. FLS from patients were CD45-/CD90+/PDPN+, consistent with the phenotype of synovial sublining FLS involved in inflammation. MIR22HG was found expressed in FLS. Finally, we demonstrated the chondrogenic differentiation ability of MIR22HG-expressing FLS.

Conclusion: We demonstrated induction of MIR22HG expression in THP1 cells by proinflammatory stimuli in CD14+ and CD14- mononuclear cells and in FLS from Oligoarthritis patients. MIR22HG expression levels differed in CD14+ cells from patients undergoing different disease courses. MIR22HG role in the pathogenesis of Oligoarthritis and as a molecular biomarker/target for therapy will be assessed in the future.

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1547 – P1.14.57

Novel dairy fermentates have differential effects on key immune responses associated with viral immunity and inflammation

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Fermented foods and ingredients, including fermentates derived from lactic acid bacteria (LAB) in dairy products, can modulate the immune system. Here we describe the use of reconstituted skimmed milk powder to generate novel fermentates from *Lactobacillus helveticus* strains SC232, SC234, SC212, and SC210, and from *Lacticaseibacillus casei* strains SC209 and SC229, and demonstrate using in vitro assays that these fermentates can differentially modulate cytokine secretion by bone marrow derived dendritic cells (BMDCs) when activated with either the viral ligand, loxoribine, or an inflammatory stimulus, lipopolysaccharide. Specifically, we demonstrate that SC232 and SC234 increase cytokines IL-6, TNF-α, IL-12p40, IL-23, IL-27 and IL-10, and decreased IL-1β in primary bone marrow derived dendritic cell (BMDCs) stimulated with a viral ligand. In contrast exposure of these cells to SC212 and SC210 resulted in increased IL-10, IL-1β, IL-23, and decreased IL-12p40 following activation of the cells with the inflammatory stimulus LPS. Interestingly SC209 and SC229 had little or no effect on cytokine secretion by BMDCs. Additionally, the metabolite composition of these fermentates differed with SC212 having high relative concentrations of desaminotyrosine, succinate, glutamate, and lactate, while SC234 had higher relative concentrations of succinate, and lactate. Overall, our data demonstrates that these novel fermentates have specific effects and can differentially enhance key immune mechanisms that are critical to viral immune responses, or can suppress responses involved in chronic inflammatory conditions, such as Ulcerative Colitis (UC), and Crohn's disease (CD).

The care, treatment, and experiments involved in this study were approved by the Research Ethics Committee (REC) of Dublin City University (Approval DCUREC/2023/187).

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1566 – P1.14.58

Development of an ex vivo approach preserving natural hair follicles and resident immune cells in order to recapitulate hair loss-associated inflammation

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Purpose: More than 30 years ago, Philpott et al. developed an ex vivo model enabling the survival of hair units inside a petri dish after microdissection. It is now considered as the gold standard human hair in vitro model. However, in biopsies from hair loss patients, the perifollicular region exhibits inflammation related to the infiltration of lymphocytes, macrophages and mast cells. While targeting inflammatory aspects represents a promising therapeutic approach for slowing down hair loss, new translational models with hair follicles and immune cells are lacking. Our objective is to develop an ex vivo inflammatory model maintaining hair follicles in their complex native immune environment.

Methods: We collected face lift samples after plastic surgery with all legal authorizations required from 5 donors. Natural skin biopsies with hairs were embedded in a proprietary solid matrix and maintained in chemically-defined xeno-free medium for 5 days. To reproduce local inflammation, we performed a 24h initial topical treatment with bacteria-derived LPS.

Results: Using multiplex imaging, we showed that skin-resident T cells, macrophages and mast cells were preserved in the hair follicle microenvironment in our models. Histological analysis revealed that 24h of LPS treatment did not have significant impact on tissue integrity. Using a 36-plex pro-inflammatory cytokines panel, we demonstrated that LPS treatment induced an inflammatory response in ex vivo models with increased levels of GM-CSF, IL-8 and MIP-1β. By co-immunostaining of Ki-67 and active caspase 3 post-treatment, we observed that the LPS induced a decrease in proliferation at day 2 and an increase in the percentage of apoptotic cells in the hair follicles around day 5.

Conclusion: Overall, we found that a topical LPS treatment for 24h induces an inflammatory environment associated with high levels of GM-CSF, IL-8 and MIP-1β. Importantly, both IL-8 and MIP-1β were elevated in the serum of patients with alopecia areata. LPS treatment was also associated with decreased proliferation followed by increased apoptosis of hair follicles. This model with an inflammation induced by topically applied LPS could be relevant to evaluate the efficacy of compounds for reducing scalp inflammation in a natural environment.

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1647 – P1.14.59

Prothrombotic activity of adipose tissue in patients with rheumatoid arthritis treated with JAK kinase inhibitors baricitinib or upadacitinib

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Background: Data from clinical trials indicate that patients with rheumatoid arthritis (RA) who were treated with the Janus kinase inhibitor (JAKi) had an increased risk of thromboembolic events. Since adipose tissue (AT) can secrete factors that affect coagulation and fibrinolysis, we examined the prothrombotic activity of AT in RA patients just before and after treatment with baricitinib or upadacitinib.

Materials and methods: Seventeen patients with RA were included in the study. Peripheral blood and subcutaneous AT samples were collected from patients just before the implementation of JAKi treatment and after six months of JAKi therapy. Adipose tissue explants were cultured in the presence of TNF or without TNF for 24 hours. At the end of culture, cells were harvested and supernatants were collected for mRNA and protein analyses of tissue factor (TF), plasminogen activator inhibitor (PAI) and visfatin. (Peripheral blood mononuclear cells served as a control for adipose cell mRNA expression. Concentrations of TF, PAI and NAMPT in blood plasma and AT culture supernatants were quantified by ELISA.

Results and conclusions: We found no significant differences in the levels of TF, PAI and visfatin spontaneously produced ex vivo by AT before implementation of treatment with baricitinib or upadacitinib and after treatment with either of these JAKis in all study participants. However, a significant change in the prothrombolytic activity of AT after TNF- stimulation was detected. In the presence of TNF, AT from RA patients who were treated with JAK**i** produced significantly higher levels of mRNA for TF, PAI and visfatin, as well as significantly higher levels of TF and NAMPT proteins, compared to AT samples explanted before the implementation of JAKi. In addition, a strong correlation was found between plasma levels of visfatin and TF. These results indicate that JAKi therapy may increase the sensitivity of AT to TNF, leading to increased production of prothrombotic proteins by this tissue. This may contribute to an increased risk of thromboembolic events in RA patients treated with JAKi.

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1683 – P1.14.60

A protective function of sclerosing cholangitis on inflammatory bowel disease

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Purpose: There is a strong clinical association between inflammatory bowel disease (IBD) and primary sclerosing cholangitis (PSC), a chronic disease of the liver characterized by biliary inflammation that leads to strictures and fibrosis. Approximately 60-80% of people with PSC will also develop IBD (PSC-IBD). One hypothesis explaining this association would be that PSC drives IBD. Therefore, our aim was to test this hypothesis and to decipher the underlying mechanism.

Methods: Colitis severity was analyzed in experimental mouse models of colitis and sclerosing cholangitis, and people with IBD and PSC-IBD. Foxp3$^+$ Treg infiltration was assessed by qPCR and flow cytometry. Microbiota profiling was carried out from fecal samples of people with IBD, PSC-IBD, and mouse models recapitulating these diseases. Fecal microbiota samples collected from people with IBD and PSC-IBD were transplanted into germ-free mice followed by colitis induction.

Results: We show that sclerosing cholangitis attenuated IBD in mouse models. Mechanistically, sclerosing cholangitis causes an altered intestinal microbiota composition, which promotes Foxp3$^+$ Treg expansion, and thereby protects against IBD. Accordingly, sclerosing cholangitis promotes IBD in the absence of Foxp3$^+$ Treg. Furthermore, people with PSC-IBD have an increased Foxp3$^+$ expression in the colon and an overall milder IBD severity. Finally, by transplanting fecal microbiota into gnotobiotic mice, we showed that the intestinal microbiota of people with PSC protects against colitis.

Conclusion: This study shows that PSC can have protective properties on IBD and provides a comprehensive insight into the mechanisms involved in this effect.

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1703 – P1.14.61

CXCL4, CXCL4-DNA/RNA complexes and anti-CXCL4 antibodies are modulated in Systemic Sclerosis patients under Iloprost therapy

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Background: Systemic sclerosis (SSc) is an autoimmune disease characterized by vascular and immunity alterations, and skin/internal organs fibrosis. Aberrant levels of plasma CXCL4, CXCL4-RNA/DNA complexes, type I IFN (IFN-I) and anti-CXCL4 antibodies characterize SSc. These parameters influence each other: CXCL4-self-DNA/RNA are triggers of IFN-I and pro-inflammatory factors in plasmacytoid dendritic cells (pDCs) and myeloid DC (mDC), respectively, via TLR7/8/9. Anti-CXCL4 antibodies can further amplify these responses.

Methods: Here, we assessed modulation over six months time of plasma CXCL4, CXCL4-DNA/RNA complexes, anti-CXCL4 antibodies, IFN-a and TNF-a expression in a SSc cohort under the synthetic prostaglandin iloprost treatment. Responders down-regulated their disease index (EScSGAI), after six months.

Results: Anti-CXCL4 autoantibodies correlated with both IFN-a and TNF-a levels in SSc plasma. In seven patients with shorter disease duration, who significantly improved disease activity after six months, improvement coincides with decreased plasma concentrations of IFN-a, CXCL4 and TNF-a. Interestingly, iloprost efficiently blocked pDCs IFN-I production i*n vitro.*

Conclusions: This is a longitudinal pilot study that analyzes CXCL4 and related parameters modulation over time in SSc. These findings suggest a possible role of Iloprost as disease modifying drug, which may be mediated by down-regulation of the plasma CXCL4 and IFN-I levels. Since CXCL4, IFN-I and TNF-a down-modulation was more evident in SSc patients with shorter disease duration over iloprost treatment, these results warrant future investigations to address whether the early treatment with iloprost can slow or block SSc progression and complications.

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1715 – P1.14.62

USP43 promotes inflammation-driven colorectal cancer progression through stabilizing HDAC11 protein

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Purpose: Toxic substances or imbalance of intestinal flora homeostasis can easily cause intestinal barrier damage, leading to inflammatory bowel disease (IBD) and colorectal cancer. Here we demonstrated the roles of ubiquitin-specific protease 43 (USP43) in DSS-induced experimental colitis and inflammation-driven colorectal cancer.

Methods: Used DSS treatment for induction of experimental colitis and AOM/DSS treatment to induce colon cancer in Usp43 WT and Usp43 KO mice. qPCR and ELISA assay were used to detect IL-10 expression. HE staining and microscopy were used to assess colon section. Multiproteomics mass spectrometry was used to explore mechanistically relevant candidate proteins. Ubiquitination assay was used to detect proteins ubiquitination modification.

Results: Deficiency of USP43 leads to resistance to experimental colitis and colonic tumorigenesis independent of adaptive immunity, with an alteration of innate immune cytokines that IL-10 increased while IL-6 decreased. Furthermore, we found that USP43 can interacts with and stabilizes histone deacetylase 11 (HDAC11), an epigenetic repressor of IL-10. Knockout or pharmacologic inhibition of HDAC11 shows phenotypes consistent with DSS-induced colitis and AOM/DSS-induced colorectal cancer in USP43 deficient mice. Meanwhile, high levels of USP43 and HDAC11 proteins with a positive correlation were found in human colon cancers.

Conclusion: USP43 deficiency results in increased expression of anti-inflammatory cytokine IL-10 and attenuated experimental colitis and colorectal cancer in murine models. Meanwhile, USP43, as an ubiquitin-specific protease, can deubiquitinate K48-specific polyubiquitin chains of HDAC11 at the site of Lys44, thereby promoting the stability of the HDAC11 protein that represses IL-10 expression. These findings suggest critical roles of USP43-HDAC11-IL-10 axis in IBD and colorectal cancer, which serves as a potential therapeutic target.

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1724 – P1.14.63

High-fructose diet and folate insufficiency exacerbate renal inflammation in adenine-induced murine model of chronic kidney disease

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Purpose: A Western diet and high intake of sweetened beverages could readily result in metabolic syndrome and chronic kidney disease (CKD). Our previous study has demonstrated that folic acid deficiency enhanced inflammation and exacerbated renal fibrosis in high-fat high-fructose diet-fed mice. Therefore, this study aims to examine whether high-fructose diet and folic acid insufficiency might exacerbate renal fibrosis in adenine-induced CKD mice.

Methods: In our experimental design, 7 week-old C57BL/6 mice were divided into four groups: a control diet group (Ctrl), a high-fructose diet group (Hfru), Hfru with adenine (Hfru+ade), Hfru+ade with 2 mg/kg folate (Hfru-f+ade). Then, mice were treated with 0.1% (w/w) adenine on 22-week-old, and all groups were sacrificed on 44-week-old.

Results: The results showed significant increases in blood urea nitrogen, serum creatinine, renal fibrosis area, and immune cell infiltration in the adenine-induced groups compared to those of the control group. Furthermore, the kidney injury molecule-1 (KIM-1) was higher in the Hfru-f+ade group. In addition, TNF-α and IL-6 were significantly elevated in the spleen of the Hfru-f+ade group, and MCP-1 and TGF-β were also significantly increased in the kidneys of the adenine-induced groups.

Conclusion: These findings suggested that folate insufficiency might exacerbate fibrosis, inflammation, and the progression of CKD.

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1764 – P1.14.64

Chronic intestinal inflammation predisposes to the development of acute kidney injury

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Clinical observations suggest that individuals with intestinal inflammation worse kidney function, yet the mechanisms are unexplored. Therefore, the aim of this study is to evaluate the influence of intestinal inflammation in an Acute Kidney Injury model. C57BL/6 mice were subjected to three cycles (5 days each) of 2% dextran sulfate sodium (DSS) in water with an interval of 4 days between DSS cycles. The Disease Activity Index (DAI) was determined based on weight loss, stool consistency, and rectal bleeding. The predisposition to the development of renal injury was evaluated by Ischemia Reperfusion (I/R) Injury model for 15 minutes (in our hands, this time of ischemia does not increase serological urea and creatinine levels). The frequency of immune cells was assessed by flow cytometry. Animals in the DSS group showed a reduction in colon length, greater cumulative DAI and loss of intestinal epithelial cells with inflammatory infiltrate. The DSS-treated group did not exhibit an increase in creatinine or urea in the serum. Interestingly, animals in the DSS group showed 6x higher expression of the KIM-1 gene than the control group, indicating that intestinal inflammation can generate kidney damage in absence of classical hallmarkers of kidney function. In fact, animals subjected to 15 min of I/R that did not receive DSS maintained creatine and urea levels similar to the control group. Notably, animals that previously received DSS and were subjected to 15 min of I/R showed high levels of creatinine and urea, corroborating that chronic intestinal inflammation can lead to susceptibility to the development of kidney disease. Animals that underwent I/R surgery showed an increase in the percentage of neutrophils and a reduction in B lymphocytes, regardless of DSS. Our data indicate that prior intestinal inflammation is capable of increasing susceptibility to I/R, suggesting a potential link between chronic intestinal inflammation and increased risk of AKI.

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1798 – P1.14.65

Gentamicin-induced inner ear injury: modulating immune and inflammatory responses with alpha1-antitrypsin for inner ear function preservation

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Introduction: Aminoglycosides, the most known ototoxic antibiotic agent, lead to vestibular dysfunction and hearing loss. Ototoxic mechanism involves excessive inflammation, tissue damage, reactive oxygen species (ROS) and apoptotic events leading to irreparable cell damage. Currently, no clinical approach exists aside of corticosteroids, carrying controversial effects. Alpha1-antitrypsin (AAT), a circulating immune and inflammation modulating molecule, elevates during inflammation, promotes resolution, reduces ROS, and inhibits apoptosis. Moreover, AAT induces macrophages towards a resting phenotype, reducing inflammation. Present indication is genetic AAT deficiency. Mice carrying transgenic hAAT alleles ($hAAT^{+/+}$) exhibit elevated circulating hAAT, protecting from inflammatory flares. These attributes motion investigation of inner ear preservation under hAAT-rich conditions.

Aim: Characterize immunological, functional, molecular, and structural aspects of drug-induced ototoxicity under in-vivo AAT treatment routes.

Methods: A. $hAAT^{+/+}$ and heterozygote mice received daily gentamicin injections (100mg/kg, daily, 9 days, i.p). Vestibulo-cochlear functions were determined and inner ear organs collected for analysis. **B.** Wild-type mice were injected with gentamicin as described. AAT treatment introduced daily (intratympanic, systemic, simultaneous or preemptive). At day 10, vestibulo-cochlear functions were examined, animals were sacrificed; inner ear samples were analyzed for RT-PCR gene expression, paraffin embedded sections made for histological observation and explants for immunostaining.

Results: Expectedly, gentamicin caused hearing deficiency (60dB versus 20-30dB healthy threshold), and vestibular performance decline (2.6 severity score). **A.** $hAAT^{+/+}$ mice exhibited a hearing threshold 20dB better than heterozygote mice ($p=0.0004$) and a 13-fold lower vestibular score. **B.** Intratympanic AAT exhibited superior vestibular functionality than non-treatment, mean rotarod time-to-fall 101.66 sec versus 56.33 sec ($P=0.04$), alongside favorable auditory threshold. RT-PCR analysis showed lower iNOS expression and 3-fold increased IL-1Ra/IL-1β ratio amongst treated group. Additionally, negative correlation between iNOS and IL-1β in treated groups indicates pro-resolution, less aggressive inflammation. Preemptive treatment exhibited earlier and faster vestibular functions normalization, supporting AAT tissue preconditioning. Histological sections of treated mice depicted superiorly preserved organ of corti. Immunostaining for hair cell specific MYO-7 protein revealed a 4-fold increased hair cell survival within treated ears.

Conclusion: Findings support clinically relevant inner ear protection with AAT treatment. Ongoing experiments explore mechanisms and innate immune cell profile of AAT-treated inner ear compartment.

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1838 – P1.14.66

Soluble immune checkpoints as biomarkers for disease activity in large vessel vasculitis

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Purpose: Giant Cell Arteritis (GCA) and Takayasu arteritis (TAK) are different types of large vessel vasculitis (LVV) that share many pathological and clinical features. Both are characterized by immune cells infiltrating the large arteries leading to vessel remodeling and resulting in ischemic events. The pathogenesis is still largely unknown, and the lack of a standardized approach for disease activity definition is the most clinical need. Literature data support a role for immune checkpoints in LVV pathogenesis.

We aimed to identify soluble immune checkpoints deregulated in LVV and those able to discriminate between LVV patients in the active and remission phases.

Methods: 54 LVV patients and 37 controls were enrolled. LVV inclusion criteria: diagnosis of LVV-GCA or diagnosis of TAK; PET/CT with FDG uptake ≥2 in at least one vascular district and at least one among ESR >40 mm/h (or CRP >10 mg/L). The EULAR consensus definitions for disease activity states in LVV were used [Hellmich B., Ann Rheum Dis. 2020;79:19]. For LVV patients, plasma/serum samples were collected longitudinally during the active and remission phases. Concentrations of soluble CD137L, CD137, CD27, CTLA4, CD80, CD40, CD40L, GITR, GITRL, ICOSL, IDO, LAG3, PD1, PDL1, PDL2, TIM3, and VISTA were evaluated by a multiplex bead-based assay.

Results: The comparison between active LVV patients and controls revealed that plasmatic levels of PD-L2 were lower, while VISTA concentrations were higher in LVV patients. Instead, lower levels of ICOSL were observed in serum samples from active LVV patients. The comparison between LVV patients in different disease phases revealed that serum levels of ICOSL increased at least 1.4-fold in 14/31 patients when they transitioned from active to remission phase.

Conclusions: Soluble ICOSL, PD-L2, and VISTA could be involved in LVV pathogenesis. Monitoring the concentrations of ICOSL in serum might help to discriminate between LVV in the active and remission phase.

Founding: This study was supported by AUSL-IRCCS di Reggio Emilia, Italy and th Italian Ministry of Health, Ricerca Finalizzata (GR-2019-12370628).

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1851 – P1.14.67

A dynamic interplay between angiogenesis and neutrophils in systemic sclerosis patients

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Background: Systemic sclerosis (SSc) is an immune-mediated rheumatic disease characterized by microvasculopathy, immune dysregulation, and skin and visceral organ fibrosis. Although SSc is a rare disease, it presents high morbidity and mortality. Pathophysiology involves early endothelial damage, inflammatory infiltration, and a resulting fibrotic reaction. Innate immune system dysfunctions can be involved in the release of cytokines and chemokines, as well as autoantibodies, which can activate fibroblasts. These events give rise to a dysregulated remodeling of extracellular matrix (ECM) proteins, leading to organ failure. SSc pathophysiology involves the imbalance between pro-angiogenic and anti-angiogenic factors. However, evidence related to the prevalence of pro- or anti-angiogenic factors is controversial. Both pro- and anti-angiogenic factors, can be released by human neutrophils (PMNs) under inflammatory stimuli. Moreover, PMNs release Neutrophil Extracellular Traps (NETs), source of autoantigens and players in the pathogenesis of autoimmune diseases. However, little is known about the potential involvement of neutrophils in SSc. The aim of this study was to investigate the angiogenic profile and the involvement of neutrophil-related mediators and NETs biomarkers in SSc.

Methods: Serum levels of angiogenic (VEGF-A, VEGF-A_{165b}, ANGPT1, AGPT2, TGF-β), and lymphangiogenic factors (VEGF-C, VEGF-D) were measured by ELISA in 16 patients with SSc with videocapillaroscopic alterations and 22 healthy controls (HCs). Circulating levels of neutrophil-related mediators such as matrix metalloproteinase-9 (MMP9), CXCL8/IL-8, and NETs biomarkers (MPO-DNA complexes) were also evaluated by ELISA.

Results: SSc patients display higher circulating levels of angiogenic (VEGF-A, VEGF-A_{165b}, ANGPT1, ANGPT2, TGF-β) and lymphangiogenic (VEGF-D) factors compared to HCs. Interestingly, a directly proportional correlation between VEGF-A, VEGF-A165b, and ANGPT2 serum levels was also found. No differences were found in the circulating levels of VEGF-C. In addition, SSc patients display increased serum levels of neutrophil-related mediators (CXCL8/IL-8, MMP-9, and MPO) and the same levels of MPO-DNA complexes compared to HCs.

Conclusions: These preliminary results suggest that SSc patients display a peculiar profile of angiogenic and lymphangiogenic factors compared to HCs. Moreover, SSc patients show increased circulating levels of neutrophil-related and pro-inflammatory mediators (CXCL8/IL-8, MMP-9, MPO, and TNF-α) compared to HCs. A larger patient cohort is needed to confirm these data.

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1863 – P1.14.68

Genetic variants and serum levels analysis of indoleamine 2,3-dioxygenase in Behcet's disease

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Purpose: Behcet's Disease (BD) is a multisystemic disorder with an unknown etiology, having the highest prevalence rates in Turkey. Genetic factors, including familial aggregation, contribute significantly to its pathogenesis. Indoleamine 2,3-dioxygenase (IDO), a key enzyme in tryptophan metabolism, has been implicated in immune regulation and inflammation. IDO activity is believed to play a role in maintaining immune homeostasis by regulating T cell responses and promoting immune tolerance. Herein, this study aimed to investigate the association between IDO gene polymorphisms, serum IDO levels, and BD susceptibility in ninety BD patients and 52 healthy controls.

Methods: Genomic DNA was extracted from the patient's blood by using DNA isolation kit, and single nucleotide gene polymorphisms for IDO (rs7820268, rs10108662) and IDO-2 (rs4503083) were determined using RT-PCR together with the melting curve analysis method using fluorescence resonance energy transfer probs. Serum samples were collected for IDO and IDO-2 measurement using enzyme-linked immunosorbent assay (ELISA). In addition, clinical manifestations of BD such as oral ulcer, genital ulcer, uveitis, and neurological involvement were compared with serum IDO and IDO-2 levels of patients.

Results: Having been in Hardy-Weinberg equilibrium of all allele and genotype frequencies of patients and controls, statistical analyses revealed no significant differences in gene polymorphisms of IDO and IDO-2. On the other hand, serum IDO and IDO-2 levels were significantly lower in BD patients ($p<0.001$ and $p<0.001$, respectively), and a strong correlation was identified between IDO and IDO-2 levels ($r=0.920$, $p<0.001$). Furthermore, only serum IDO-2 levels were significantly lower in patients with a neuro-Behçet group compared to those without neurological involvement seen groups ($n=7$ and $n=83$ respectively, $p<0.001$).

Conclusion: While no significant differences were found in IDO gene polymorphisms between patients and healthy controls, serum IDO levels were notably lower in BD patients. This suggests a potential dysregulation of IDO activity in BD, possibly contributing to the proinflammatory immune response observed in the disease.

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1866 – P1.14.69

Dysregulated innate immune cell activity in children with periodic fever, aphthous stomatitis, pharyngitis, adenitis

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Purpose: Periodic fever, aphthous stomatitis, pharyngitis, adenitis (PFAPA) is a cyclic autoinflammatory syndrome that commonly manifests in children aged two to five years old. Immune dysregulation, rather than infections, is the leading hypothesis to the aetiology of PFAPA. Cytokine levels and infiltrates of adaptive immune cells in tonsils have previously been demonstrated during PFAPA fever flares. Since the innate immune cell phenotype is less known, our aim is to assess functionality of monocytes, dendritic cells (DCs), and NK cells in children with PFAPA compared to age-matched controls.

Method: We collected blood from children with PFAPA during and between flares (n=13), and age-matched healthy controls (n=10). Whole blood cells were used for flow cytometric characterisation of innate immune cells at baseline and after six hours stimulation with lipopolysaccharide (LPS), the toll-like receptor (TLR)7-agonist Gardiquimod, or interferon (IFN)-γ. We quantified production of the cytokines interleukin (IL)-12/23, TNFα, IFNα, IFNγ, and IP10 upon stimulations. We also performed bulk-RNA sequencing on whole blood. Finally, we isolated serum and measured systemic cytokine levels by multiplex.

Results: In line with previously observed lymphopenia during PFAPA flares, we found decreased frequencies of T cells, B cells, and most innate cells during flares, compared to controls and between flares. Cell stimulations showed increased TNFα production in DCs after stimulation with LPS and Gardiquimod in PFAPA subjects compared to controls. TNFα production was also increased in flare-free PFAPA subjects. CD14+CD16+ monocytes had also higher TNFα and IL12/23 production upon stimulation with LPS and at baseline between PFAPA flares. In serum we found increased IL-1RA, IL-15, IL-6, and IL-10 during flares. Between flares, those cytokines return to baseline but GM-CSF and IL-10 levels were lower compared to controls. Analysis of blood transcriptomics showed upregulated pathways associated with an innate antiviral response during flares, that came back to similar level than controls between flares.

Conclusion: Our initial analysis of the innate response in children with PFAPA shows that their DC and monocyte subsets may have a dysregulated responsiveness to innate stimuli. Further functional and genetic analysis of these cells could reveal pathways involved in this dysregulation and association with clinical symptoms.

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1945 – P1.14.70

Role of CD16 receptor in development of diabetes mellitus type 2

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CD16 is an activating receptor expressed on NK cells, macrophages, monocytes and neutrophils. This receptor was mostly investigated in the context of ADCC while its role in other aspects of immune responses remained poorly investigated. We noticed that mice lacking CD16 receptor have higher body weight in comparison to control animals which lead us to our hypothesis that this receptor might play a role in adipose tissue homeostasis. To investigate how CD16 mice cope with metabolic stress we used a model of obesity induced diabetes mellitus type 2 (DM2). In this model mice were fed *ad libitum* with normal diet (NCD) or high fat diet (HFD) in which 50% of calories were derived from animal fat. After 12 weeks on HFD diet mice develop insulin resistance and glucose intolerance. We noticed that CD16 deficient mice gained more weight after HFD and were more prone to development of DM2, seen from greater glucose intolerance and insulin resistance in comparison to control mice.

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1946 – P1.14.71

Characterisation of T lymphocytes in a rat model of Duchenne muscular dystrophy

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Duchenne Muscular Dystrophy (DMD) arises from a mutation in the *dystrophin* gene on the X chromosome. The resulting absence of functional dystrophin protein results in a disorder marked by recurring and unresolving muscle damage and leads to progressive loss of musculoskeletal function. Numerous extensive studies have highlighted the role of macrophages in the context of muscle damage. However, despite the presence of other immune cell populations such as T lymphocytes in the DMD muscle, its role remains poorly understood. In this study, we aim to understand the T cell responses in the DMD muscle using a rat model of DMD. Via single cell RNA sequencing of the wildtype and DMD rats, we identified increased infiltration in absolute numbers of not only macrophages, but also neutrophils, NK, T, and B cells across the DMD diaphragm, tibialis anterior, and extraocular muscle compared to that of WT. We then confirmed this observation by flow cytometry. To further understand the functional capacity of the T cells in the context of DMD, we stimulated T cells from the spleens and popliteal lymph nodes of wildtype and DMD rats *ex vivo* and analysed their production of inflammatory cytokines by flow cytometry. Taken together, this study highlights the need for understanding the T cell responses to chronic musculoskeletal damage in DMD. This knowledge will help to reveal potential immunotherapy avenues for managing the disease progression in DMD patients.

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1960 – P1.14.72

Upregulation of DNase I in systemic lupus erythematosus: a role of mithocondrial DNA

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Purpose: The inflammatory environment present in SLE and the high capacity of low density granulocytes (LDG) to induce NETosis promotes the increase of circulating cell-free DNA (cirDNA) and contributes to the formation of anti-dsDNA antibodies. The present study aims to analyze the role of DNase I, responsible for cirDNA degradation, in relation to LDG subsets and cirDNA levels in SLE.

Methods: DNase I and anti-DNase I were determined by ELISA, DNase I activity by a fluorescence-based assay, total cirDNA by fluorometry, mitochondrial/nuclear circulating DNA (mtDNA/nDNA) by qPCR and the frequency of LDG and T-cell subsets by flow cytometry in 42 controls and 144 SLE patients.

Results: Serum levels of DNase I were increased in SLE patients (p=0.040), especially in those presenting anti-dsDNA antibodies (p=0.005). However, the DNase I/cirDNA ratio was reduced in patients compared with controls, mainly in those with active disease (p=0.017). Remarkably, DNase I levels in controls correlated with mtDNA (r=0.438, p=0.012), a main component of NETs. Furthermore, SLE patients displayed increased levels of anti-DNase I antibodies (p=0.040), that correlated with the DNase I/cirDNA ratio (r=0.247, p=0.007). Thus, the upregulated levels of DNase I in SLE, probably induced by the elevated cirDNA amounts, could not be fully functional and might promote the generation of anti-DNase I antibodies. No differences were detected in DNase I activity, that was unrelated to DNase I levels. However, the DNase I activity rate (DNase I activity relative to DNase I levels) was reduced in patients, especially in those presenting anti-dsDNA antibodies (p=0.025) and correlated negatively with the amount of mtDNA in controls (r=-0.356, p=0.045) and non-active patients (r=-0.213, p=0.040). Finally, blood levels of LDGs were increased in SLE and associated directly with cirDNA, but only the mtDNA amount was linked to CD16+LDGs. DNase I activity was negatively correlated with LDGs and IFNγ expression on CD4 T-cells, but positively associated with Tang in SLE, especially in non-active patients

Conclusion: Present results highlight the relevance of the "cirDNA-NETosis-DNase I" tandem as a pathogenic mechanism directly involved in the etiopathogenia of SLE.

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2023 – P1.14.73

To BCG or not to BCG – a case against trained immunity?

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Trained immunity (TI) has been a hot button issue in current immunology, and we decided to look at the understudied effect of TI on chemically induced colitis. We used BALB/c male mice, which are more susceptible to TNBS-induced colitis, and immunized them subcutaneously with BCG (TI-TNBS group) or saline (control-TNBS group). Seven days later, we induced colitis by intrarectal injection of TNBS in 50% ethanol. We observed the mice over a period of another week. Most mice in both groups did not survive, but the mice in the control-TNBS group lived significantly longer. We investigated the mice who survived in an additional study. We immunized the mice with another dose of BCG and paired them up with adequate controls (mice who didn't have colitis induced, immunized with BCG or with saline). Prior to the second immunization we isolated the PBMCs from a blood sample and analyzed them on a flow cytometer. Two days later mice were euthanized and their bone marrow, peritoneal, and PBMCs were collected and analyzed on a flow cytometer. The results showed BCG immunization causes an increase in both $CD45R^{+}CD19^{+}$ B cells and $CD4^{+}$ T cells in the circulation, however after colitis induction, the frequency of these cells is reduced in the circulation of the immunized mice compared to controls. Given that the immunized animals had a shorter lifespan after colitis induction, it is possible that the infiltration of these lymphocytes from the peripheral circulation could be a causal factor in aggravated inflammation. In the peritoneal cavity, BCG immunized animals had a greater percentage of $F4/80^{+}CD68^{+}$ macrophages, however after colitis, this ratio was reversed, which could indicate a faster depletion of resident macrophages of the peritoneal cavity, which are known to have a reparative role in visceral tissue damage. Post colitis, a greater percentage of peritoneal macrophages expressed iNOS, and the BCG vaccination did increase it slightly in the two days after immunization, compared to the control group. It was shown that TI could aggravate chemically induced colitis in mice, and our results seem to support this notion, which we will research further.

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Abstracts

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2028 – P1.14.74

Th17-cell derived IL-22 plays a non-redundant protective role in a mouse colitis model

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Purpose: The aim of this study is to investigate the role of Th17-cell derived interleukin 22 (IL-22) in patients with IBD and experimental colitis.

Methods: To understand the functional relevance of Th17-cell derived IL-22 we analysed human IBD samples, induced experimental colitis in a mouse model with Th17 cell specific deletion of IL-22 and used an *in vitro* human intestinal organoid system.

Results: We observed that $CD4^+$ T cells co-expressing IL-17A+IL-22+ were reduced in intestinal biopsies from people with ulcerative colitis (UC) with both active and inactive disease. To assess the functional relevancy of this finding, we used *Il17a*^{Cre} x *Il22*^{flox} mice and dextran sulfate sodium (DSS)-induced colitis. We found that *Il17a*^{Cre} x *Il22*^{flox} mice were more susceptible to DSS colitis compared to littermate control mice. Specifically, *Il17a*^{Cre} x *Il22*^{flox} mice showed a higher disease severity, reduced epithelial proliferation and a delayed tissue regeneration. This was associated with a dysregulation of genes involved in antigen presentation. Therefore, we exposed human-derived intestinal organoids to either IL-22, IL-17A or combination of both cytokines. Analysis on their transcriptome profiles identified cluster of genes specifically regulated by the combination of IL-22 and IL-17A. Gene ontology analysis identified biological processes related to presentation via MHC II to be upregulated in intestinal organoids stimulated by the combination of IL-17A and IL-22. We validated upregulation of MHC class II complex via flow cytometry analysis in this setting. Finally, we showed that colonic organoids promote differentiation of IL17A+IL22+ $CD4^+$ T cells via MHC class II.

Conclusion: Th17-cell derived IL-22 has a non-redundant protective role during intestinal inflammation and mucosal healing. It promotes tissue repair and induction of epithelial MHC class II, thereby further promoting $CD4^+$ T cell producing IL-17A and IL-22. These results indicate that the source of IL-22 is important. Specifically, the loss of Th17-cell derived IL-22 cannot be compensated by IL-22 produced by other cellular sources.

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2064 – P1.14.75

Treatment of experimental autoimmune uveitis and effects of probiotic supplementation

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Uveitis is an intraocular inflammation affecting the iris, ciliary body, and choroid. Non-infection autoimmune uveitis is a heterogeneous disease mediated by T-lymphocytes, without clear pathogenesis. Intraocular inflammation is manifested by infiltration of cells and exudation of inflammatory proteins in various ocular structures. Current treatment is based on systemically administered corticosteroids, often combined with immunomodulators/immunosuppressants and biologics.

Purpose: To analyze which immunosuppressant has the best effects on autoimmune uveitis in mice or which treatment could be improved by prophylactic administration of probiotics. Such probiotic treatment in combination with immunosuppressive drugs could simulate the treatment modalities in human medicine

Methods: To induce experimental autoimmune uveitis (EAU), the interphotoreceptor retinoid-binding protein was subcutaneously applied to C57BL/6 mice. The severity of the EAU was assessed in vivo using an otoscope. Intensity and manifestation of intraocular inflammation were compared in mice treated with various immunosuppressants (mycophenolate mofetil, methotrexate) or corticosteroids (methylprednisolone) and control mice. To test the immunomodulatory properties of *Escherichia coli* Nissle 1917 (EcN), mice received EcN for 2 weeks before the EAU induction. Cervical and inguinal lymph nodes were used for analyses of intracellular cytokine production and immune cell phenotype by flow cytometry as well as the same analyses after the antigen-specific stimulation.

Results: The clinical severity of EAU was evaluated on day 21 and day 27 after the EAU induction. The strongest effect was observed in mice treated with methotrexate, milder but a significant effect was in mice treated with mycophenolate mofetil. The decreased severity of EAU caused by methotrexate was accompanied by a decreased proportion of CD4+ and an increased proportion of CD8+ T cells in both cervical and inguinal lymph nodes together with a decreased proportion of IFNγ+CD4+ in the lymph nodes draining the site of inflammation.

Conclusion: Live EcN protects against EAU when administered before and during its induction, suggesting EcN influences antigen presentation and T cell priming and may have a role in regulating the gut-eye axis.

Grant

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2075 – P1.14.76

A multidisciplinary approach to diagnosis and management of scleromyositis syndrome with positivity of anti-OJ, anti-PL7 and anti-Scl70 antibodies

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Background: Scleromyositis is a rare autoimmune condition that falls between the clinical spectrum of systemic sclerosis and autoimmune myositis that involves more than the overlap syndrome typically characterised by the presence of anti-PM/Scl antibodies.

Purpose: Highlight the clinical manifestations, diagnostic challenges, and therapeutic interventions associated with a rare case of scleromyositis with co-positivity of ASA (anti-OJ, anti-PL7) and anti-Scl70 antibodies.

Methods : A detailed clinical history, physical examination findings and imaging were used for the diagnosis and management of the patient. Sera samples were tested for antinuclear antibodies using both indirect immunofluorescence and immunodot assays to screen for anti-aminoacyl-transfer RNA (tRNA) synthetase and systemic scleroderma antibodies.

Results: We report the case of a 68-year-old female, with a history of dyspnea grade 2 on mMRC scale and joint pain. Physical examination reveals cutaneous sclerosis limited to the fingers not extending beyond the proximal metacarpophalangeal joint. The patient fulfilled the diagnostic criteria for AAS based on both serology, due to positive anti-PL7 and anti-OJ antibodies, and clinical manifestations, with the presence of interstitial lung disease, restrictive pattern on spirometry and arthritis. Systemic scleroderma was diagnosed based on the presence of cutaneous sclerosis along with the detection of anti-Scl 70 antibodies. This phenotype of scleromyositis was distinct for its absence of muscular involvement and severe lung disease that required an intensive therapeutic intervention with high-dose systemic corticosteroids associated to mycophenolate mofetil.

Conclusion: This clinical and immunological presentation offers valuable insights into the spectrum of scleromyositis disease. The entity may vary in severity and therapeutic response reflecting the diversity in clinical presentation and disease progression based on the specific anti-synthetase antibody involved.

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2109 – P1.14.77

Simultaneous hyperbaric oxygen therapy and antioxidant supplementation in the treatment of thermal skin injuries reduce inflammation and alter nociceptive signalling and wound healing

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Purpose: Thermal skin injuries are a prevalent cause of skin damage. Pain is considered to be one of the most striking symptoms of a burn injury, caused by the direct effect of thermal injury, stimulation of nociceptors, and the production of mediators of inflammation. Some adjuvant approaches, such as hyperbaric oxygen therapy (HBO) and antioxidant supplementation, have been found useful in achieving therapeutic goals for primary injuries and their consequences.

Methods: As a thermal skin injury experimental model, we used two-month-old male Wistar albino rats. Thermal injuries were made with a solid aluminium bar at a constant temperature of 75 °C for 15 seconds. Hyperbaric oxygen treatment was performed in a specially constructed hyperbaric chamber for rats (HYB-C 300) for seven consecutive days (100% O2 at 2.5 ATA for 60 minutes). Antioxidant supplementation was performed with *Filipendula ulmaria* extract dissolved in tap water to reach the final concentration of 100 mg/kg b.w., for seven consecutive days. The behavioural testing was performed 24 hours after completing the protocols. The nociception assessment was performed in a hot plate test and tail flick test.

Skin tissue, spinal cords and hippocampi samples were obtained for estimation of inflammation (IL-6, TNF-α, IL-33, IL-10), apoptosis (Bax, Bcl-2), and pain control receptors (μ, δ, and κ opioid receptors; melatonin receptors – MT1 and MT2).

Results: Simultaneous administration of HBO and the antioxidant supplementation ameliorated macroscopic and histopathological characteristics of wound area and healing. Also, this therapeutic approach decreased the levels of IL-6, and TNF-α and increased the expression of the IL-33 and IL-10 as well as μ-opioid receptor, and MT1 and MT2 receptors in the wound area and spinal cord, with the consequent increase in the reaction time in behavioural testing.

Conclusion: In conclusion, the presented results allow evidence for the advantages of the simultaneous employment of HBO and antioxidant supplementation in the treatment of thermal skin injury, with special reference to the attenuation of painful sensations accompanied by this type of trauma.

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2110 – P1.14.78

Premature ageing in long-term homeless adults

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Individuals in lower socioeconomic strata experience an increased burden of disease. People experiencing homelessness (PEH) exhibit extremes of poor health and psychosocial stress. Causation of these extreme disparities is complex and multifactorial, there are known effects of diet, smoking and substance abuse but other factors are also likely to play a role.

We hypothesise that homelessness may accelerate the ageing process, manifesting in poorer health and increased frailty among affected individuals. Furthermore, we believe that lifecourse psychosocial stress plays an important role in mediating accelerated ageing in PEH.

Long-term homeless individuals (n=59, F = 25.42%) aged 20-70 (μ=57) were recruited from six Dublin hostels over eight months.

65% suffered from one or more age-related disease, the most common of which was chronic hypertension. The mean age of participants with an age-related disease was 53.75. The median Clinical Frailty Score (CFS) in this cohort was 4, considered “vulnerable”. While age and CFS were strongly correlated in the housed (R = 0.62, P = 1.3e-11), PEH were as frail as the oldest housed individuals (μ = 4.1) independent of their age (r = 0.053, p = 0.69).

To characterise baseline immune status, banked plasma was analysed using a multi-plex ECLIA. Levels of eight circulating cytokines were significantly increased in homeless individuals, reminiscent of signatures associated with inflammageing and pathological chronic inflammation. Next, we will examine effects on dynamic immune responses to stimulation to investigate the functional consequences of this phenotype.

Additionally, we measured psychosocial adversity in participants, including social isolation and past trauma, which have been linked to accelerated biological ageing. 40% of homeless participants reported feeling lonely either “often” or “very often”. The average number of Adverse Childhood Experiences (ACEs) in our cohort was 4 – just 3-5% of the general housed population report 4 or more ACEs .

We conclude that homelessness is associated with psychosocial stress that may drive physiological dysregulation, including immune dysregulation. Independently, we showed that homeless individuals exhibit age-independent increased frailty and immune dysregulation. Future research will examine how psychosocial stress and immune dysregulation may mediate increases in frailty.

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2116 – P1.14.79

Serum free light chains in systemic lupus erythematosus disease

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Background: Serum free light chains (FLC) are produced by plasma cells and serve as markers of monoclonal proliferation in plasma cell pathologies. They also reflect immune status and renal function. Recently, elevated serum FLC levels have been observed in systemic lupus erythematosus (SLE).

Purpose: This study aims to assess the relationship between serum FLC levels (kappa, lambda, and total FLC) and disease activity in SLE, comparing it with rheumatoid arthritis (RA) as another autoimmune disease model, along with healthy controls.

Methods: Twenty SLE patients and 42 age- and sex-matched rheumatoid arthritis (RA) and 20 healthy controls were enrolled. Disease activity was assessed using the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) definition. Serum FLCs were quantified through a turbidimetric assay using 500 µl of stored thawed serum. Two separate measurements kappa and lambda FLC were conducted. The total FLC is equal to K+L light chains. Nonparametric tests compared FLC levels in SLE versus RA and healthy controls, as well as across different SLE disease activity levels and dsDNA antibody positivity. Correlations between FLC and SLEDAI were examined.

Results: We enrolled 20 SLE and 42 RA patients, with mean ages of 35±10 and 40± 7 years, respectively, and 15.7% and 11% men, respectively. Kappa, lambda, and total FLC levels were significantly higher in SLE compared to RA and healthy controls. FLC elevation did not correlate with anti-DNA antibody positivity or kidney damage but was significantly correlated with SLE disease activity.

Conclusion: Serum FLC levels are notably higher in SLE compared to both RA patients and healthy controls. However, to establish them as reliable markers of disease activity, further investigations with larger cohorts are imperative to ensure the generalizability of these findings.

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2118 – P1.14.80

Uncovering the role of novel protein FKBPL in obesity associated psoriasis

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Psoriasis is a chronic inflammatory disease caused by a complex interplay between the immune system, polygenic inheritance, and environmental factors. Obesity is an important environmental factor that promotes both psoriasis development and severity as well as reducing the therapeutic response to currently available therapies. FK506-binding protein-like (FKBPL) is an immunophilin protein and a deficiency in FKBPL strongly correlates with the development of obesity in both mice and humans. FKBPL is located on chromosome 6p21, a major psoriasis susceptibility locus region (PSORS1). The overall aim of the study is to investigate the role of FKBPL in psoriasis using *in vitro* and *in vivo* models.

Female *Fkbpl*$^{+/-}$ (wild type) and *Fkbpl*$^{+/-}$ (heterozygous) mice were fed a normal chow or high-fat diet (60%) for six months and imiquimod (IMQ) cream was applied to the dorsal skin for six days to model psoriasis. Gene expression changes in the skin were measured *ex vivo* by qPCR. Similarly, *in vivo* experiments were replicated *in vitro* using Human keratinocyte (HaCat) cells. The HaCat cells were transfected with pFKBPL, rs28732176, and siFKBPL to induce overexpression and under-expression of FKBPL. Post-transfection cells were treated with TNF to mimic the psoriasis condition. Pro-inflammatory cytokine expression and DNA repair gene expression was evaluated.

In the *in vivo* IMQ model, obese *Fkbpl*$^{+/-}$ mice had significantly higher IL-1 β ($p=0.0002$, n = 6 *Fkbpl*$^{+/+}$, n = 8 *Fkbpl*$^{+/-}$) and IL-17 ($P= 0.0133$) compared to lean *Fkbpl*$^{+/-}$ mice. In invitro proinflammatory cytokine such as IL-1 β, IL-23A, TNF expression decreased when FKBPL was upregulated ($p=0.002$) similar to this, same trend was observed when FKBPL was knocked down ($p=<0.0001$). Furthermore, we investigated the DNA damage genes. Interestingly, we observed the DNA repair gene (PARP1 and CCND1) expression reduced ($p=0.0002$) when FKBPL was knocked down.

In summary, FKBPL overexpression and knockdown show the same effect on proinflammatory cytokines however the DNA repair gene expression is reduced when FKBPL is reduced. Therefore, FKBPL is a regulator of keratinocyte DNA repair, and novel target in obesity-associated psoriasis. However further experiments are required to narrow down a specific pathway.

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2278 – P1.14.81

Cytotoxicity by micronucleus assay exhibit cellular inflammation in drug-addicted mothers and their newborns

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Purpose: To identify the frequency of micronuclei in drug-addicted mothers and their newborns.

Methods: This cross-sectional descriptive study involved collecting 51 oral mucosa samples from drug-addicted mothers aged 16 to 39 and their neonates. Subsequently, the micronucleus assay was conducted, and the samples were stained using Giemsa-Wright. Bright-field microscopy was employed to examine 1000 cells per individual.

Results: Over 50% of pregnant women exhibited cellular inflammation and a notably high frequency of micronuclei compared to women without cellular inflammation ($P = 0.004$). Preterm newborns show a higher incidence of micronuclei than full-term newborns ($P = 0.050$).

Conclusions: Mothers who consumed drugs and their newborns exhibited a heightened frequency of cytotoxicity. We suggest that micronucleus assay shows cytotoxicity in cellular inflammation and can be considered as an early marker. Further research involving diverse populations is warranted to strengthen these findings.

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2281 – P1.14.82

A switch in macrophage ontogeny leads to increased fibrosis during aging after myocardial infarction

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In tissues, aging is associated with structural and functional alterations that notably affect inflammatory responses to diseases and infections. This is of particular importance when assessing pathological processes at play in elderly, such as myocardial infraction. Although extensive studies have investigated the inflammatory response to myocardial infarction in young mice, much less is known concerning the influence of aging on cardiac healing and immune cell function. We here aimed at investigating the age-related alterations in macrophage populations within the aging heart and how this impacts on cardiac healing following myocardial infarction.

This was achieved by employing a combination of lineage-tracing and pulse-labelling mouse models to examine the immune cell composition at baseline and their dynamic responses to myocardial infarction in both young and aged mice. Additionally, we used single-cell RNA sequencing to delineate the differences in the transcriptomic profiles of cardiac immune cells. While macrophages emerged as the predominant immune cell population in the cardiac tissue even in aged animals, age-dependent differences were observed, including an increased proportion of recruited monocyte-derived macrophages with a proinflammatory phenotype in aged animals. Noticeably, this was associated with a reduced proportion of embryo-derived macrophages, the predominant macrophage population found in the heart of young mice under steady-state conditions. Importantly, such age-related changes in macrophage populations correlated with heightened fibrosis in the aging heart.

In aged mice, monocyte-derived macrophages recruited after myocardial infarction were found beyond the infarcted area and notably in the remote ventricular regions not directly affected by the ischemic event. This is stark contrast to young hearts, where recruited macrophages are restricted to the infarcted zone. Functionally, this basal increase in monocyte-derived macrophages and their extended recruitment in aged animals was associated with increased fibrotic scar size.

Increasing the frequency of monocyte-derived macrophages at the expense of embryo-derived macrophages in young animals before the ischemic insult was sufficient to augment fibrotic scarring post-injury, emphasizing the critical role of the immune cell composition in adverse cardiac outcomes following myocardial infarction in the elderly.

Labex Revive, CNRS, Institut Pasteur

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P1.15 CYTOKINE AND T LYMPHOCYTE-BASED IMMUNOTHERAPY

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357 – P1.15.02

Interleukin-2 dynamically reduces interleukin-2 receptor β expression and function especially in T cells

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Purpose: More than 40 interleukin-2 (IL-2)-based compounds are currently being tested in clinical trials to treat inflammatory diseases or cancer. However, IL-2 therapy can induce systemic inflammation, affecting multiple organs. Similar autoimmune-like symptoms are observed in patients with hypomorphic mutations in the IL-2 receptor β subunit (IL-2Rβ). We hypothesize that high-dose IL-2 (hdIL-2) decreases IL-2Rβ surface expression and reduces IL-2R signaling capacity especially in T cells but not NK cells, similar to patients with defective IL-2Rβ.

Methods: Human peripheral blood mononuclear cells (PBMCs) were continuously stimulated with 1-10,000 IU/mL IL-2 (aldesleukin) for up to 7 days. Receptor surface expression and IL-2R signaling (pSTAT5) of cell subsets were measured using flow cytometry.

Results: While IL-2Rβ surface expression remained high on CD8+ T cells and NK cells after 15 min of hdIL-2 stimulation, receptor expression significantly decreased on CD4+ T cell subsets to levels below 0.4% (vs. 2.5% for unstimulated CD4+ T effector cells [Teffs] and 26.1% for regulatory T cells [Tregs]). Prolonged IL-2 exposure (up to 7 days) reduced IL-2Rβ expression on CD8+ T cells to 0.2%. Expression on NK cells also was reduced but 55.8% of cells remained IL 2Rβ+. CD4+ and CD8+ T cells stimulated with hdIL-2 for 7 days showed lower pSTAT5 signal when re-stimulated with IL-2 than unstimulated cells (reduction in mean fluorescence intensity of 1.9-fold, 1.8-fold, and 1.7-fold for CD4+ Teffs, Tregs, and CD8+ T cells, respectively). In contrast, hdIL-2 did not impact IL-2 signaling in NK cells as pSTAT5 signaling remained unaltered upon re-stimulation with IL-2. Similarly, re-stimulation with IL-15 – also signaling through IL-2Rβ – induced significantly reduced pSTAT5 in T cells after high-dose IL-2 stimulation, while pSTAT5 in NK cells was unaltered.

Conclusion: IL-2Rβ is basically absent on hdIL-2-stimulated T cells, leading to concomitant decreases in IL-2R signaling. NK cells retained relatively high IL-2Rβ expression and IL-2R signaling. Given the resemblance of cellular characteristics of hdIL-2-stimulated cells and cells from patients with defective IL-2Rβ, impact of continuous IL-2 stimulation on IL-2R signaling should be considered in the onset of adverse events during IL-2 therapy.

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450 – P1.15.03

Solving the Mystery of Immunomodulation in Malignant Ascites: an exploration of novel unknown immunosuppressive factors in ovarian cancer ascites, and their impact on modern immunotherapeutics.

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Purpose: Cancer treatment has been revolutionised by the recent discovery of BiTEs (Bispecific T-cell Engagers) and other immunotherapeutics. BiTEs are bi-specific proteins that cross-link CD3 on T-cells to cancer antigens, forcing immunoactivation and cytotoxicity. Despite these advances, ovarian cancer is still predominantly treated with chemotherapy, leading to a poor prognosis. It regularly results in accumulation of peritoneal fluid called malignant ascites. By using this ascites as a model of the tumour microenvironment, we aimed to identify immunosuppressive factors preventing the functionality of modern immunotherapeutics in ovarian cancer.

Methods: Ovarian cancer patients were recruited through NHS ethics agreements. 20,000 OVCAR-3 cells were seeded into 96-well plates. T-cells were isolated from healthy donors by immunomagnetic separation, and were co-cultured with OVCAR-3 cells and EpCAM-BiTE (Epithelial Cell Adherence Molecule) for 72 hours. XTT assay was used to measure cytotoxicity and flow cytometry to measure T-cell activation markers (CD69/CD25/41BB). Chloroform precipitation was used to remove lipids from ascites, and Amicon 3kDa filters were used to remove protein. Cytokine concentrations were measured by ELISA.

Results: Nine ovarian cancer patients had ascites collected. T-cells showed significantly reduced activation and cytotoxicity in response to EpCAM-BiTE or Dynabeads when exposed to ascitic fluid. Ascitic fluid demonstrated similar pH buffering capacity to cell media, but caused heterogenous reduction in EpCAM expression on OVCAR-3 cells. Modification of ascites by removal of lipids or proteins caused a drastic increase in cytotoxicity and T-cell activation beyond normal cell media. However, analysis of previously described ascitic cytokines (IL10/IL6/TGFbeta/IFNg) showed no correlation with immunosuppression.

Conclusion: Ovarian cancer ascites induces immunosuppression of T-cells. This leads to reduced functionality of BiTEs, and likely other immunotherapeutics. This is not due to lack of nutrients or pH buffering in ascites, but rather due to immunosuppressive proteins and lipids. When these are removed, ascites becomes an immunostimulatory environment promoting BiTE-mediated cytotoxicity, representing an exciting therapeutic opportunity. Given the lack of correlation between known ascitic cytokines and immunosuppression, it is likely that this effect is mediated by novel, unknown proteins and lipids. Moving forwards, we will utilise proteolipidomics to identify these factors.

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461 – P1.15.04

A small bispecific antibody induces HIV-1 Env-specific T-cell activation

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Purpose: The introduction of antiretroviral treatment (ART) has been instrumental in controlling the HIV-1 pandemic. However, the virus persists in individuals for a lifetime in the form of a viral reservoir. The development of an HIV-1 cure is a global priority. Bispecific antibodies (bsAbs) are promising HIV-1 cure therapeutic agents to target and eliminate the viral reservoir. Here, we present a next-generation small-sized bsAb that engages T-cells by targeting both the T-cell receptor (TCR)/CD3 complex and the HIV-1 Envelope (Env) on the surface of infected cells. We evaluate the ability of the bsAb "light T-cell engager", called LiTE, to specifically bind to each cognate target and to induce Env-specific T-cell activation.

Methods: We designed LiTEs prototypes and produced them by transfection in Expi-293F cells. The binding capacity and specificity of LiTEs were assessed by ELISA with immobilized CD3 and Env and by flow cytometry in Jurkat T-cells and Env-expressing K562 cells (K562-Env). In addition, on-target, and off-target T-cell activation was studied in Jurkat T-cells and PBMC from healthy donors, using anti-CD3 IgG as a control. Cells were stained for CD4, CD8, CD25 and CD69 markers and evaluated by flow cytometry.

Results: We demonstrated specific binding of the LiTE to CD3 and Env by ELISA. In addition, antigen binding on Jurkat T-cells and K562-Env cells further validated the specificity of LiTEs towards CD3 and Env. Importantly, functional assays demonstrated the ability of the bsAb to promote on-target CD3-mediated activation in Jurkat T-cells, dependent on the presence of Env protein, as determined by a significant increase in the expression of CD69. In PBMCs, the LITE Env-specific activation was determined by a significant increase in CD69 and CD25/CD69 frequency in total $CD4^+$ and $CD8^+$ T-cells.

Conclusion: The LiTE prototype demonstrates specific binding to both targeted antigens and Env-specific T-cell activation in a dose-dependent manner. These data highlight the potential Env-targeted LiTE as immunotherapy in the context of HIV-1 infection. Further functional *in vitro* and *in vivo* experiments are required to understand the full potential of light T-cell engagers as an HIV-1 curative strategy.

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533 – P1.15.05

Development of an in vivo suppression assay to assess Treg function

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Background: Regulatory T cells (Tregs) comprise an important subpopulation of T cells and play a pivotal role in maintaining homeostasis and immune tolerance. Considering their powerful ability to suppress aberrant immune responses, there has been an increasing interest in utilizing Tregs therapeutically for pathologies such as autoimmune diseases and transplant rejection. *In vivo* disease models continue to be used to generate proof of concept and safety data for Treg cell therapy. Chronic inflammation models can be long, with large treatment groups and incomplete incidence. Therefore, murine Treg production processes must be optimised, scalable and robust to minimise the risk of failure in these disease models. Although *in vitro* suppression assays have been routinely used to assess the ability of Tregs to control T cell responses, these fail to mimic complex physiological conditions and can be inconsistent indicators of function *in vivo*. Here, we propose the use of a novel *in vivo* model as a valuable tool to examine the impact of cell production processes on murine Treg function.

Methods: Congenic C57BL/6 mice were used to provide total T cells on a CD45.2 background. These were injected with or without Tregs generated from C57BL/6 mice on a CD45.1 background into irradiated NSG mice. Treg function and phenotype was monitored using tail bleeds and at endpoint blood and spleen was harvested. Analysis involved flow cytometry and cell staining with an antibody panel to quantitate and phenotype the cell populations, ultimately revealing the level of Treg function *in vivo*.

Results: T cells were co-injected with an equal dose of Tregs into NSG mice. T cell responses were significantly abrogated when compared to control animals which did not receive the Tregs, thus demonstrating Treg-mediated immune suppression of both $CD4^+$ and $CD8^+$ T cell populations. Throughout the experiment, the Treg populations were detectable and maintained over 90% FoxP3 expression, demonstrating a stable phenotype under *in vivo* conditions.

Conclusions: We have successfully developed a robust animal model which can be utilized as an *in vivo* suppression assay. The advantages of this model are that it is quick, reproducible and more physiologically relevant than *in vitro* equivalents.

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739 – P1.15.06

Functional regulation of human lymphoid cells by IL-1R8 targeting in cancer

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Purpose: Interleukin-1 Receptor 8 (IL-1R8) is a negative regulator of IL-1 and Toll-like family receptors that controls inflammation and innate and adaptive immunity. Taking advantage of IL-1R8-deficient mice, this receptor was shown to inhibit IL-18-dependent activation of Natural Killer (NK) cells against liver carcinogenesis and metastasis. We recently reported that IL-1R8 tunes CD8+ T cell tumoricidal potential in transplantable mouse models of colon carcinoma and fibrosarcoma. IL-1R8 deficiency promoted cytokine-induced responses of intratumoral lymphocytes, suggesting that IL-1R8 acts as an NK- and T-cell immune checkpoint in mouse. However, its role in human lymphocyte remains largely unexplored, as well as strategies to inhibit its function.

Methods: Using spectral flow cytometry, pharmacological and genetic targeting, and *in vivo* models, we studied the heterogeneity of IL-1R8 expression in human lymphocytes and the therapeutic impact of IL-1R8 blockade in antigen specific and antigen non-specific cell-based immunotherapy.

Results: We found that IL-1R8 is expressed in the NK-92 cell line and in primary lymphocytes from healthy donors, with high expression levels in NK and CD8+ T cells. Higher expression was observed in effector and memory subsets, suggesting a maturation-dependent upregulation of IL-1R8 in human lymphoid cells. Moreover, high levels were observed in intrahepatic NK cells and circulating lymphocytes from colorectal cancer liver metastases and B cell lymphoma patients, respectively. Pharmacological targeting of IL-1R8 increased effector functions (Granzyme B production, IFNγ secretion) in NK-92 cells and intrahepatic NK cells *in vitro*. Increased IFNγ production was observed in polyclonally activated $CD8^+$ T cells treated with an IL-1R8 inhibitor. Taking advantage of OT-I mice, we reported increased antigen-induced proliferation for IL-1R8-deficient OT-I cells *in vitro*. Coherently, the adoptive transfer of IL-1R8-deficient OT-I cells better controlled MC38-OVA tumour growth in mice. Finally, IL-1R8 was expressed by CD19 Chimeric Antigen Receptor (CAR) T cells post-infusion. CRISPR-Cas9-mediated IL-1R8 silencing in CAR-T cells improved their tumoricidal effects against Acute Lymphoblastic Leukemia.

Conclusion: Collectively, we show that IL-1R8 loss-of function using both genetic and pharmacological approaches, effectively boosts clinically relevant immune reactivity against cancer which may be exploited in adoptive cell immunotherapies.

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870 – P1.15.07

IL-10 enhances the antitumor immunity in the orthotopic hepatocellular carcinoma mouse model by increasing CD8+T cell cytotoxicity

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IL-10, known for its immunoregulatory functions, has recently garnered attention for its anti-tumor effects. However, its role in the tumor microenvironment remains controversial due to the diverse cancer types and organ-specific conditions. Hepatocellular carcinoma (HCC) is the primary liver cancer characterized by an immunotolerogenic microenvironment. We sought to understand whether and how IL-10 functions in liver cancer under such tumor-promoting conditions. To address this issue, we utilized an adeno-associated virus to deliver IL-10 (AAV-IL-10) in an orthotopic HCC mouse model. After inoculating Hep55.1c hepatoma cell lines into the liver, mice received intravenous injections of either AAV-IL-10 or controls (AAV-mock). Two weeks post-tumor cell inoculation, we assessed tumor size and tumor-infiltrating leukocytes (TILs). Mice treated with AAV-IL-10 showed dose-dependent reductions in tumor size and significant increases in TILs, particularly $CD8^+$ T cells, accompanied by elevated secretion of effector cytokines such as IFN-γ, perforin, and granzyme B. $CD8^+$ T cells from AAV-IL-10-treated mice exhibited enhanced cytotoxicity against Hep55.1c cells compared to those from AAV-mock-treated mice. Importantly, no significant adverse effects were observed in other adjacent organs despite increased serum IL-10 levels following AAV-IL-10 injection. In addition, serum ALT was in the normal range. These findings suggested the therapeutic potential and safety of IL-10 in HCC by enhancing $CD8^+$ T cell cytotoxicity.

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1149 – P1.15.08

Induction of Resident Memory CD8+ T cell phenotypes to eliminate the HIV reservoir

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Background: The major hurdle to HIV-1 eradication in people with HIV (PWH) is the establishment of viral reservoirs. In tissues, where most of the HIV burden persists, antiviral resident memory CD8+T cells (TRM) may be critical to eliminate cellular reservoirs and transcriptionally-active HIV infected cells. Here we aimed to address the functional capacity of $CD8^+$TRM phenotypes and the control they exert on the viral reservoir.

Methods: In ART-suppressed HIV^+ women, we determined total viral DNA in blood (n=8) and cervix (n=7) and its correlation with the frequency of cervical $CD8^+$ TRM ($CCR7^-$ $CD69^+$ $CD103^+$). A functional assay was established to assess suppression of reactivated $CD4^+$T cells by cervical $CD8^+$ TRM from ART-suppressed HIV^+ women undergoing hysterectomy. Moreover, PBMC obtained from PWH were treated with cytokines to expand TRM-like phenotypes, which were subsequently exposed to Gag to determine degranulation and IFNɣ secretion by flow cytometry (n=7). An additional functional assay was established to evaluate the capacity of expanded $CD8^+$ TRM-like cells to eliminate the autologous ex vivo reactivated HIV reservoir (n=5) and to determine intact viruses after ex vivo reinfection (n=5). Last, single cell RNA sequencing was performed to determine the nature of PBMC-expanded cells after cytokine treatment.

Results: The frequency of cervical $CD8^+$TRM cells inversely correlated with proviral HIV-1 DNA in cervix (n=7; p=0.03). Gag-specific $CD8^+$TRM were rarely detected in biopsies, which was likely limited by sample size. Still, cervical $CD8^+$TRM cells from two patients with a large sample were more efficient at eliminating HIV-reactivated $CD4^+$T cells than circulating effector $CD8^+$T cells. Circulating Gag-specific $CD8^+$ T cells presented higher expression of CD107a and IFNɣ after TRM-like induction treatment with more capacity to eliminate reactivated HIV-infected cells (p=0.031). CD8+ TRM-like cells from PBMC-expanded cells were more effective at decreasing proviral intact HIV-1 DNA than other phenotypes, likely caused by an increase in cytotoxic potential of expanded clusters.

Conclusion: Our results highlight an active role of $CD8^+$ TRM phenotypes in limiting tissue viral persistence. Overall, we provide evidences that $CD8^+$TRM-like phenotypes should be potentiated to enhance control of viral persistence and identify a promising immunotherapeutic strategy to achieve control of reactivated viruses.

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1233 – P1.15.09

Apheresis characteristic impacting response to Chimeric Antigen Receptor T-cell therapy

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Introduction: Chimeric Antigen Receptor T-cell (CAR-T) therapy stands as a transformative advancement in cancer immunotherapy. This therapeutic modality has yielded significant rates of remission in patients afflicted with B-cell Acute Lymphoblastic Leukaemia (B-ALL), Diffuse Large B-cell Lymphoma (DLBCL), Primary Mediastinal B-cell Lymphoma (PMBCL), and Mantle Cell Lymphoma (MCL). However, despite these notable achievements, a subset of patients across these malignancies experiences relapse or recurrence. Hence, the imperative for research persists, particularly in the realm of identifying biomarkers that offer prognostic insights into patient responses.

Materials and methods: We have assembled data from 11 DLBCL patients undergoing treatment at our institution, all of them with an anti-CD19 CAR-T cell. Each patient has undergone a minimum 3-month follow-up regimen, inclusive of PET/CT scans at month +1 and month +3 post-treatment. Employing flow cytometry, we have undertaken an investigation into lymphocytic subpopulations on the day of apheresis.

Results and discussion: As of the study cut-off date on March 25, 2024, 45% of patients exhibited complete remission at the 3-month mark post-CAR-T therapy, as assessed via PET/CT imaging. Leveraging this dataset, we partitioned the cohort in two groups: those in remission (n=5) and those experiencing relapse (n=6). Upon scrutinizing the composition of T lymphocyte subpopulations during apheresis, a clear difference emerged, revealing higher percentages of central memory CD8+ T lymphocytes (CM CD8+T) in remission-afflicted patients ($P < 0.05$). There exist studies correlating the percentage and functionality of CM CD8+T cells in CAR-T cell product. Nevertheless, in our research, we identify this correlation at a significantly earlier phase of treatment. This underscores the potential of CM CD8+T cells in apheresis as a marker of treatment response, merits further research and validation.

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1356 – P1.15.10

Monitoring of immune system in CAR-T infused patients diagnosed of hematological malignancies

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Purpose: CAR-T cell therapy is emerging as an alternative against cancer, especially in hematological malignancies such as diffuse large B cell lymphoma (DLBCL), acute lymphoblastic leukemia (ALL), and multiple myeloma. The objective of this study was to monitor the immune system of patients treated with the commercial CAR-T products Tisagenleucel (Kymriah®) and Axicabtagene-ciloleucel (Yescarta®) at the Reina Sofía University Hospital.

Methods: Circulating level of serum cytokines (IL-6, IL-8, IL-10, MCP-1 and IP-10; CBA, Beckton and Dickinson) and IgG subclasses (Optilite, Binding Site) were measured. The proportion of $CD3^+CAR\text{-}T^+$ cells was identified by flow cytometry (Miltenyi). Additionally, a detailed cellular phenotype of CAR-T and non-CAR-T subpopulations was performed. All determinations were conducted weekly in the first month and monthly thereafter.

Results: A total of 7 patients with DLBCL (85%) or ALL (15%) were included in this study. Of them, 85% were adults (>25 years) and 15% were pediatrics (<25 years). Among all, 57% relapsed and 50% died. The Yescarta® product was applied to 5 adults, while 1 adult and the pediatric patient received the Kymriah® product. The $CD3^+CAR\text{-}T^+$ population expanded between +7d and +14d and disappeared between +2M and +4M. $CAR\text{-}T^+CD4^+$ and $CD8^+$ effector cells were the main subpopulations detected and remained over time, while central memory and naïve subpopulations were the least abundant. Th1 cells were the predominant among both $CAR\text{-}T^+$ and $CAR\text{-}T^-$ T-helper cells. Throughout the follow-up time, an inversion was detected between CAR-T $TCR\alpha\beta^+$ and $TCR\gamma\delta^+$ cells in the first month after the infusion. Cytokines reached their maximum level between +0d and +14d, matching with the expansion of CAR-T cells. Given the small cohort studied, no correlation with clinical factors were performed.

Conclusions: Immune system of patients infused with adoptive cell therapy suffers changes througout the therapy course and its monitoring could be of transcendental relevance to identify possible differences between underlying pathologies, patient categories, implemented therapies and, particularly, prognostic biomarkers.

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1670 – P1.15.11

Changes in immunological and metabolic parameters in patients with spondyloarthropathies treated with biologics

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Background: Psoriatic arthritis (PsA) and ankylosing spondylitis (AS) are chronic inflammatory disorders marked by joint inflammation, with AS additionally affecting the spine, causing stiffness and pain. PsA, associated with psoriasis, impacts joints globally, resulting in pain, swelling, and diminished mobility. Both conditions are linked to an elevated risk of metabolic syndrome, characterized by hypertension, hyperglycemia, central obesity, and dyslipidemia. The therapeutic landscape has been transformed by the advent of biologic therapies, including tumor necrosis factor inhibitors (TNFi) and interleukin-17 inhibitors (IL-17i), which target specific immunologic and metabolic pathways. This study aims to assess the immunological and metabolic effects of TNFi and IL-17i in PsA and AS patients.

Materials and Methods: We enrolled three cohorts: PsA patients receiving TNFi (n=9), PsA patients on IL-17i therapy (n=16), and AS patients treated with TNFi (n=19). Pre-treatment and follow-up blood samples were analyzed to quantify regulatory lymphocytes, Th1, Th2, Th17, and Th1/Th17 populations, along with cytokine IL-17A, Il-23, IL-26, IL-9, IL-10, IL-18, IL-18BP levels. Comprehensive clinical data were also compiled**.**

Results and Conclusion: Contrary to expectations, cytokine profiles remained unchanged across all groups. Nevertheless, a significant reduction in CD4+CD25+ lymphocyte percentages was noted in all cohorts, indicating diminished Th lymphocyte activation. Specifically, the PsA+IL-17i group showed a notable decrease in regulatory lymphocyte percentages. Metabolic effects varied by treatment and disease condition: in the PsA+TNFi group, significant reductions in LDL and increases in HDL were observed alongside DAPSA improvement, without notable changes in ESR or CRP levels. The PsA+IL-17i cohort experienced significant declines in total cholesterol, triglycerides, DAPSA, and CRP. For the AS+TNFi group, notable decreases in LDL, non-HDL, BASDAI, CRP, and ESR were documented. This evidence suggests that TNFi and IL-17i treatments not only reduce systemic inflammation but may also favorably affect metabolic parameters, including lipid profiles. However, effects vary by therapeutic agent and patient, advocating for personalized treatment strategies. These findings underscore the complex relationship between inflammation, immune response, and metabolic health in PsA and AS management, spotlighting the need for ongoing research in this interdisciplinary field.

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1691 – P1.15.12

Exploring TCR and gene expression diversity: Single cell RNA and TCR sequencing on NY-ESO-1-specific HLA-DRB3*02:02-restricted CD4+ T cells from cancer-free individuals

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Purpose: Natural transgenic T-cell receptors (tTCRs) represent an emerging type of cell therapy against cancer, capable of recognizing peptides expressed both intracellularly and extracellularly. This offers a significant advantage over CAR therapy that can only target extracellular surface proteins. These surface proteins are often challenging to define as Tumor Associated Antigens (TAAs), especially in the context of solid tumors. However, it is important to acknowledge that TCR therapies against TAAs could potentially generate off-tumor on-target toxicity. To address this, our focus is on NY-ESO-1, a cancer/testis antigen expressed in various tumors but minimally in healthy tissues besided testicles. While several tTCR clinical trials have targeted NY-ESO-1, they all focused on peptide presentation to class I HLA molecules, risking HLA class I downregulation by tumor cells and loss of therapy efficacy. In contrast, our work is focused on HLA-DRB3*02:02, an HLA class II allele expressed approximately by half of the Caucasian population in a similar manner to HLA-A*02:01, for which many more studies have been performed. Our aim is to obtain specific TCRs against NY-ESO-$1_{119\text{-}143}$ presented on HLA-DRB3*02:02.

Methods: $CD4^+$ $CD25^-$ T cells isolated from 12 healthy donor PBMCs were co-cultured with NY-ESO-$1_{119\text{-}143}$-pulsed monocytes for 14 days to promote specific T cell sensitization, stimulating them at days 0, 7 and 14 with NY-ESO-$1_{119\text{-}143}$ peptide. At day 15, $CD4^+$ $CD154^+$ T cells were sorted. Then, cells underwent a 14-day expansion with feeders followed by a second $CD4^+$ $CD154^+$ T cell sorting before freezing them for further single cell isolation followed by single cell RNAseq and TCRseq.

Results: After 30 days of culture and two sorting rounds on days 15 and 30, specific $CD4^+$ $CD25^-$ T cells targeting NY-ESO-$1_{119\text{-}143}$ on HLA-DRB3*02:02 were obtained. Purity and specificity assessments utilizing CD4 and CD154 markers respectively, revealed a sustained purity of 97%, with specificity significantly enhanced after the second sorting round. Single-cell TCR sequences were successfully obtained.

Conclusion: Our study demonstrates the feasibility of obtaining specific TCRs from $CD4^+$ $CD25^-$ T cells targeting NY-ESO-$1_{119\text{-}143}$ presented on HLA-DRB3*02:02. This was achieved through our specialized protocol involving pre-sensitization and expansion of $CD4^+$ T cells.

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1828 – P1.15.13

Unveiling therapeutic potential of adoptive T-cell products in cancer treatment by integrating full spectrum flow cytometry with image-based assays

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The cancer treatment landscape is rapidly evolving with the emerging field of adoptive T-cell therapy, offering promising avenues for patients across various cancer types. Central to the success are precise assessment tools of T-cell products, particularly in solid tumor diseases entrenched within immunosuppressive microenvironments. Here, we propose a comprehensive approach aimed at evaluating the performance and conducting a thorough analysis of T-cell products, introducing a customized multicolor full spectrum flow cytometry (FSFC) panel combined with image-based assays. Our FSFC panel provides a streamlined platform for assessing T-cell composition, functional attributes, and potency, all of which are critical for the efficacy of therapy. By integrating extracellular surface markers, intracellular cytokines, and immune checkpoint molecules, we delineate the essential features of therapeutic T-cells necessary for targeting solid tumors in a single staining process. Through the integration of FSFC data with image-based assays, we can assess the performance of T-cell products and their correlation with treatment efficacy in a clinical trial focusing on solid tumor diseases. This detailed analysis uncovers disparities in the composition of patient-specific T-cell products, directly influencing their effectiveness against cancer. Ultimately, our refined methodology offers valuable insights to enhance the precision and effectiveness of adoptive T-cell therapy, thereby contributing to the ongoing advancement of cancer treatment strategies.

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2129 – P1.15.14

Isolation of a high avidity TCR targeting a newly identified epitope of a common cancer testis antigen expressed by solid tumors

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Purpose: Developing T cell receptor (TCR)-based immunotherapy holds promise in cancer treatment due to its ability to target a wide range of tumor antigens, including both intracellular and extracellular antigens. Our newly identified cancer-testis antigen (CTA) is an intracellular antigen, characterized by its exclusive expression in cancer and reproductive tissues and thereby presents a promising target for cancer treatment. Our objective is to isolate a TCR specific to an HLA-A*02:01 restricted CTA epitope from the human HLA-A*02:01 negative repertoire for subsequent safety and efficacy evaluation *in vitro* and *in vivo*.

Methods: CTA expression and epitope presentation are assessed in hematological and solid neoplasms using immunohistochemistry, qRT-PCR, and mass spectrometry. TCR isolation involves co-culturing naive HLA-A*02:01 negative CD8+ T cells with antigen-presenting cells. Epitope-specific T cells are identified through multimer staining and sorted via fluorescence-activated cell sorting. Subsequent sequencing of TCR alpha and beta chains enables the identification of mutation specific TCRs. Functional avidity of TCR-transduced CD8+ T cells is evaluated by measuring IFNγ release (ELISA). Cross-reactivity is measured through the Alanine- and Glycine-Scan-Assay, and alloreactivity is assessed by co-culturing immortalized B lymphoblastoid cell lines with different haplotypes with TCR-transduced T cells.

Results: CTA expression was identified in patient samples of colorectal, breast, and head and neck cancer using immunohistochemistry and qRT-PCR. Quantification of epitope presentation was conducted via mass spectrometry in myeloma and leukemia cell lines, and PDX models of colorectal and breast cancer. To date, four potential CTA-specific TCRs with minimally murinized constant regions have been identified and synthesized, pending additional safety assessment and efficacy testing in PDX models.

Conclusion: We have discovered an undisclosed epitope of a CTA not targeted by immunotherapy thus far. From human HLA-A*02:01 negative donors, we have isolated four potential high avidity epitope-specific TCRs. Confirmation of target CTA expression and epitope presentation has been achieved across various common solid neoplasms. These findings suggest a significant therapeutic potential of our identified TCRs, offering promising prospects for a wide patient population with relapsed/refractory solid tumors in a tumor-agnostic manner.

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POSTER SESSION 2

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P2.01 CYTOKINES AND THEIR RECEPTORS

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113 – P2.01.01

Pro-inflammatory cytokine/chemokines pattern in obesity induced experimental psoriasis

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Purpose: Psoriasis (Ps) is a multi-factorial disease with a deep inflammatory outline where genetic and certain extrinsic factors like obesity initiate and sustain psoriatic lesions. A network of cytokines intercedes inflammation and supports epidermal cell proliferation, and cytokines of adipocytes origine can enhance the autoimmune reaction. The key changes in Ps are recorded at the IL-22/IL-23/IL-17 axis level, in addition a wide range of chemokines (e.g., CXCL1, MCP-1, Eotaxin etc.) produced by keratinocytes modulates inflammation in Ps. Thus, the purpose of our work was to depict a multi-analyte cytokine/chemokine pattern in experimental Ps in relation with induced obesity as frequently associated co-morbidity.
Methods: We used an experimental imiquimod mice model of psoriatic dermatitis comprising C57 BL/6 mice (Jackson Laboratory, Bar Harbor, ME, USA), males and females (10–11 weeks). The following experimental conditions were tested: (i) the Ps group with induced psoriatic dermatitis; (ii) mice with induced obesity (Fat diet C1090-45 obesity-inducing diet with w/45% energy form fat, *Altromin International*) for ten weeks; (iii) obesity induced mice with induced Ps. A normal group of mice was runed in parallel with the treatment groups. A panel of 40 analytes were tested in mice plasma with Quantibody® Mouse Inflammation Array 1 (#QAM-INF-1, *RayBiotech*).
Results: There was a clear delineation of analytes profile regarding experimental conditions and gender mice ratio. Some of our results indicate that certain proinflammatory Th1 cytokines level (e.g., IL-15) are differently modulated by fat diet in Ps condition and registered distinct patterns for male and female mice, sustaining an enhanced proinflammatory profile in females. Analyzing the chemokines portrait in the frame of Ps combined with fat diet there it is an individualized panel closely dependent on the mice gender as we found for eotaxin, MIP-1 or MCP-1 levels.
Conclusion: Despite all the current clinical improvements Ps remains an incurable disease where new tools or new visions are to be explored to reconcile the cellular and molecular foundations with the clinical course.
The presented study was financed through grants PN-III-P4-PCE-2021-0549 (PCE9/2022), NASR, [PN 23.16.01.03] and authors acknowledge also COST Action CA21108 - European Network for Skin Engineering and Modeling (NetSkinModels).

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279 – P2.01.02

A sensitive functional cell-based bioassay for the screening of different recombinant variants of soluble mouse LIGHT to be used in in vivo anti-tumor studies

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Purpose: LIGHT binds to two receptors HVEM (TNFSFR14, CD270) and LTβR (TNFSFR3). The bidirectional interaction of HVEM/LIGHT delivers costimulatory and survival signals to T cells, whereas the engagement of LTβR by LTα1β2 and LIGHT has been involved in lymph node development and in the formation of ectopic lymphoid structures at sites of chronic inflammation. The classical approach to produce soluble recombinant proteins bound to the Fc fragment of immunoglobulin G (IgG) turned out to yield a functionally inactive mouse LIGHT protein.
Methods: To overcome this hurdle and gain insight into the preclinical potential of LIGHT as a proinflammatory cytokine secreted by tumor cells, various formats of recombinant soluble mouse LIGHT proteins were expressed in eukaryotic cells (dimeric Ig.LIGHT, trimeric FF (Flag-Foldon)-LIGHT and hexameric Ig.FF-LIGHT). These proteins were assessed for their ability to bind and engage HVEM. The different mouse LIGHT recombinant proteins were quantified in culture supernatants by a newly developed sandwich ELISA using anti-mouse LIGHT specific monoclonal antibodies.
Results: In contrast to the dimeric format of recombinant LIGHT that neither bound nor killed reporter cells, the bioactive trimeric and hexameric recombinant variants mouse LIGHT can kill reporter cells transduced with chimeric constructs containing the extracellular domain of mouse HVEM fused to the intracellular domain of the human death receptor FAS. This LIGHT-mediated cytotoxic effect was abrogated in a dose-dependent manner by antibody-mediated blockade of LIGHT.
Conclusion: In summary, this work brings a novel set of tools for the preclinical evaluation of the therapeutic potential of soluble recombinant mouse LIGHT secreted by tumor cells in the context of immune check-point blockade as an immunotherapeutic intervention to enhance the proinflammatory tumor microenvironment for effective activation of the innate and adaptive response against the tumor cells.

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781 – P2.01.03

Transcriptional and epigenetic regulation of IL23R expression in human CD4+ T cells

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The importance of interleukin-23 (IL-23) and its specific receptor, IL-23R, in the pathogenesis of several chronic inflammatory diseases has been established, but the underlying pathological mechanisms are not fully understood. IL-23 signals via a heterodimeric receptor composed of a specific subunit, IL-23R, and the IL-12Rβ1 subunit shared with the IL-12 receptor. IL-23/IL-23R signaling has been studied, in particular, in the context of Th17 cell differentiation and function, however the mechanism controlling the expression of the *IL-23R* gene in different human lymphocyte populations remains poorly understood. Here we aim to enhance our understanding of IL-23/IL-23R biology by elucidating the transcriptional and epigenetic mechanisms controlling *IL23R* expression in human naïve T cells and thus their responsiveness to IL-23.
To define the early molecular events that lead to *IL23R* expression, we isolated naïve $CD4^+$ T lymphocytes from cord blood and stimulated them under different T helper polarizing conditions. *IL23R* expression was induced in both Th17 and Th1 cells, and among Th17 polarizing cytokines, IL-21 was necessary and sufficient to initiate *IL23R* expression in TCR-stimulated cells.
ATAC-seq profiling of the *IL23R* locus revealed accessible chromatin regions with putative regulatory function. IL-21, in the presence of TCR stimulation, induced chromatin accessibility similar to the one observed in Th17 differentiating conditions, confirming the important role of IL-21/IL-21R signaling in initiating *IL23R* gene expression. In addition, ATAC-seq footprinting highlighted potential transcription factor candidates for regulating *IL23R* expression. The role of these transcription factors was tested by siRNA-mediated downregulation and their binding to their target sequence was confirmed by DNA affinity capture assay.
Together, our data demonstrate that IL-21 plays an important role in early induction of *IL23R* in human CD4+ T cells and suggests cell subset specific regulation of *IL23R* expression.

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828 – P2.01.04

GDF-15-Driven Macrophage Reprogramming: Impact on IL17RB Expression and Immune Tolerance

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GDF-15 is a multifunctional cytokine involved in immune tolerance that is elevated in stress conditions, correlating with disease severity and survival. The role of GDF-15 in macrophages and its effects on the macrophage transcriptional program have been studied to only a limited extent. In this study, we identified the transcriptional program induced by recombinant human GDF-15 (rGDF15) on human macrophages in the presence or absence of lipopolysaccharide (LPS). Human monocytes were isolated from buffy coats by CD14+ positive selection and differentiated into M0 (non-stimulated), M1 (IFN-γ stimulated), and M2 (IL-4 stimulated) macrophages for 6 days. On day 6, macrophages were pre-treated with 50 ng/mL rGDF-15 for 1 hour, followed by a 6-hour challenge with 100 ng/mL LPS. Total RNA Seq revealed that rGDF15 altered the expression of 210 genes in M0 and of 372 in M2. rGDF15 and LPS in M0 and M2 exhibited changes in 230 and 295 genes, respectively. Gene Ontology analysis highlighted enrichment in blood vessel morphogenesis and angiogenesis pathways following rGDF-15 treatment in both M0 and M2. The top-upregulated GO terms in M0 and M2 under rGDF-15 and LPS belonged to TGF-β receptor and cytokine signaling pathways. Validation through RT-PCR and flow cytometry confirmed increased expression of IL17RB across all groups, indicating an anti-inflammatory effect mediated by rGDF-15 in macrophages. These findings highlight the ability of GDF-15 to induce a tolerogenic phenotype in human macrophages, promote angiogenesis, and modulate TGF-β signaling.

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1029 – P2.01.05

IFN-γ response by medullary thymic epithelial cell in the absence of Aire gene

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Purpose: In humans, the deficiency of the autoimmune regulator (AIRE) gene may cause several autoimmune disorders, like the autosomal recessive autoimmune polyendocrine syndrome type 1 (APS-1). Chronic mucocutaneous candidiasis (CMC) is one of the earliest and most frequent clinical manifestations of the disease. To explain the occurrence of CMC in APS-1 patients, It has been hypothesized that AIRE deficiency is associated with excessive STATI-mediated mucosal IFN-γ production by 'pathogenic' T cells, impairing the integrity of oral epithelial cells. We evaluated the IFN-γ response in the absence of Aire gene in medullary thymic epithelial cell .
Methods: The Aire WT mTEC 3.10 and the Aire$^{-/-}$ mTEC 3.10E6 cell lines were cultured as monolayers and seeded in 12 well plates and stimulated or not with IFN-γ (Thermo Fisher Scientific, Waltham, MA), maintained in culture for 3 days, the gene expression of IFN-γ receptors and GTPases that are induced by IFN-γ was evaluated by RTqPCR.
Results: Both cell lines expressed the IFNγ receptors 1 and 2 (Ifngr1 and Ifngr2). The WT presented the same pattern of Ifngr1 along the culture, nonetheless the Aire-/- overexpressed the Ifngr1 after 3 days of stimulus with IFN- γ. Regarding the Ifngr2, we observed for both cells line an increase after IFN-γ stimulation, more accentuated for the WT cells. The GTPases Irgb6, Irgm1 and Irgm3 were expressed in both cell lines. Although GTPases were increased in Aire$^{-/-}$ both cells responded to IFN-γ stimulation.
Conclusion: These findings indicate that, in this model, WT and Aire$^{-/-}$ mTECs responded to IFNγ stimulus; however, the Aire$^{-/-}$ cells presented an imbalance of the heterodimer Ifngr1/Ifngr2 expression.

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1032 – P2.01.06

The gene expression of TGF-β receptor subunits in blood is altered in relapsing-remitting multiple sclerosis and under the influence of dimethyl fumarate

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Purpose: Relapsing-remitting multiple sclerosis (RRMS) is the most common form of multiple sclerosis. Dimethyl fumarate is one of the first-line treatment drugs used in the treatment of RRMS. The aim of the study was to determine the alterations of gene expression of TGF-β receptor subunits in blood cells and in TGF-β1 concentration in serum of patients with RRMS untreated or treated with dimethyl, compared to healthy controls.
Methods: Expression of TGFBR1, TGFBR2 and TGFBR3 genes in blood of patients and healthy donors was determined using digital droplet PCR. Serum concentration of TGF-β1 was determined using ELISA. All patients were in remission.
Results: Untreated patients had significantly lower expression of TGFBR3 gene in blood and TGF-β1 serum concentration than healthy controls. Patients treated with dimethyl fumarate had significantly lower TGFBR1 and TGFBR2 gene expression in blood than untreated patients, significantly lower TGFBR2 and TGFBR3 gene expression in blood than healthy controls and significantly higher serum TGF-β1 concentration than untreated patients.
Conclusion: This study demonstrates, changes in the expression of molecules related to TGF-β signaling under the influence of dimethyl fumarate. Dimethyl fumarate affects the Nrf2-related signaling pathway, which may also interfere with TGF-β-related signaling.

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1694 – P2.01.08

Changes in levels of leptin, adiponectin, chemerin and resistin in response to progressive resistance training in highly trained athletes and non-trained individuals

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Background: Despite the old view that adipose tissue (AT) has only a storage and thermoregulatory role, it is now known to be a highly active and complex endocrine and metabolic gland. Cytokines secreted by adipocytes are important in inflammation and many pathologies, such as cardiovascular, metabolic disorders, cancer, dietary behaviour, and even mental illnesses or microbiota disorders. Adipokines can easily pass from AT into systemic circulation, so they are a valuable and readily available indicator. Individuals who regularly engage in physical activity (PA) have a more favourable adipokine profile. They have elevated levels of anti-inflammatory cytokines and reduced levels of inflammatory cytokines. In addition, PA leads to changes in the gene expression of adipokines, protein ligands, and their binding to receptors. Therefore, regular PA is essential for managing AT and adipokine-related diseases. Previous studies have shown that not all adipokines show predictable patterns in secretion in response to PA.
Purpose: We aim to analyze changes in levels of leptin, adiponectin, chemerin, resistin in response to progressive resistance training in highly trained athletes and non-trained individuals.
Methods: Venous blood samples (volume 500 μl) were collected from study participants at time points (before and after the training). Total RNA isolation and cDNA synthesis were performed to assess gene expression levels by real-time PCR (quantitative PCR, qRT-PCR). All results were standardized to the levels of GAPDH, and statistical analysis was performed.
Conclusion: Due to the varying methodologies of studies on PA, it is difficult to determine its effect on adipokine secretion subjectively. For this reason, there is a need to collect data on how different adipokines interact and to study the entire panel of major adipokines involved in developing pathologies of various systems in the human body. In addition, most studies focus on overweight/obese patients, where the adipokine panel is disrupted by excessive AT accumulation. However, there are limited studies on adipokines in regularly physically active individuals whose AT levels remain within normal limits.

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1925 – P2.01.10

Evaluating the cytokine profile in steroid naive patients with polymyalgia rheumatica

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Purpose. Despite polymyalgia rheumatica (PMR) being the most common inflammatory rheumatic disease in those over the age of 50 years, there remains a significant unmet need for a reliable serum biomarker of disease activity, to aid the diagnosis, and the identification of disease relapse. The aim of this pilot study was to evaluate the levels of 7 serum cytokines in a cohort of newly diagnosed PMR patients.
Methods: 15 consecutive patients, who had active, untreated PMR were recruited from a fast track PMR clinic. The following circulating cytokines were then measured via ELISA using the participants serum: MCP-1(CCL2), interleukin-6 (IL-6), CXCL9 (MIG), MIP-1 alpha (CCL3), Tumour necrosis factor alpha (TNF-α), interleukin-1 beta (IL-1β) and interleukin-17A (IL-17A). The statistical analysis was performed using STATA MP Version 18.
Results: A total of 15 patients with PMR were studied (33% female; 67% male). The mean age was 71.71 (SD 5.97). In reference to defined manufacturer parameters, at diagnosis serum MCP-1 levels were elevated in 14/15 (93%), with a median level of 732.5pg/ml (571, 921). Serum IL-6 levels were elevated in 10/15 (67%) with a median level of 11.85 pg/ml (6.05, 31.8). CXCL9 was elevated in 14/14 (100%), with a median level of 2184pg/ml (1180, 3897.5). Levels of MIP-1 alpha were elevated in 4/14 (28.6%) patients, with a median level of 91.1 pg/ml (76.5, 105). IL-1β was elevated in 2/15 (13.3%) with a median level of 0.445pg/ml (0.27, 0.815). TNF-α was elevated in 1/15 (6.7%) with a median level of 15.35pg/ml (12.85, 16.8). Finally, IL-17A was only available in 6 patients, with 5/6 (83%) demonstrating increased levels, with a median IL-17A level of 3.02 (2.89,3.27).
Conclusion: Our pilot study has demonstrated the potential utility of serum CXCL-9, MCP-1 and IL-17A in the diagnosis of those with pure PMR. Our study offers the advantage of inclusion of a pure PMR cohort, who are naïve to any treatment including steroid. Our findings provide promise for future large scale prospective studies exploring the role of these cytokines in PMR, which may also facilitate a deeper understanding of the inflammatory and immune mechanisms underlying PMR pathophysiology.

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2290 – P2.01.11

Adipokines dysregulation in the dysfunctional body adipose tissue is associated with immunometabolic biomarkers increase, highlighting dyslipidemias with a greater contribution from the TG-VLDLC-APO B metabolic pathway

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Purpose: CCL2, chemerin, and adiponectin are adipokines with crucial roles in immunometabolism. The presence of dyslipidemias is also involved in metabolic control. Remarkably, levels of LDLc have shown controversial results regarding the obesogenic profile (adipose tissue distribution in the immunometabolism process). Our study aimed to evaluate the association of adipokines levels with the immunometabolic biomarkers in the dysfunctional body adipose tissue of individuals with obesogenic profile.
Methods: In this cross-sectional study, 230 males and females aged 20 to 59 were included and split up into three groups: reference (healthy), study (overweight/unhealthy), and comparison (overweight/healthy), based on obesogenic profile. We evaluated body fat storage and distribution using bioelectrical impedance. Immunometabolic biomarkers by routine methods. Meanwhile, insulin, chemerin, CCL2, and adiponectin-oligomers serum levels by the ELISA method.
Results: The magnitude of all adiposity parameters increased in the following order: the study group was greater than the comparison group, and this, in turn, was greater than the reference group. The comparison between the study group and the comparison and reference groups showed differences in elements of the lipid profile, mainly the TG-VLDLc-Apo B metabolic pathway and insulin resistance. C3 and C-reactive protein levels showed an increase in the study and comparison groups versus the reference group. HMW and MMW + LMW adiponectin oligomers showed a decrease in the study group compared to the reference group. The chemerin levels showed a pattern that tends to decrease in the following order: the reference group is greater than the study group, and this, in turn, is greater than the comparison group, while a reverse pattern was observed in the CCL2 levels. The CCL2-Chemerin relationship coincides with the obesogenic profile.
Conclusion: The increase in dysfunctional adipose tissue coincides with the increase in the lipid profile with a greater contribution from triglycerides-VLDLc-Apo B metabolism. We suggest that the imbalance in the adipokine scenario, which is contributed by the inflammatory process (characterized by the increase in biomarkers of subclinical inflammation), agrees with the imbalance in the immunometabolic indices.

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P2.02 DIVERSITY OF ANTIGEN RECOGNITION

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1835 – P2.02.01

Cross-regulation of antibody responses against viral and host proteins by commensal microbiota via molecular mimicry

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The commensal microflora provides a repertoire of antigens that elicit mucosal antibodies. In some cases, these antibodies can cross-react with host proteins, inducing autoimmunity, or with other microbial antigens providing protection against pathogens. We demonstrate that several human commensal bacteria can induce salivary anti-SARS-CoV-2 Spike IgG antibodies via molecular mimicry. In particular, S. salivarius, was recognized by SARS-CoV-2-neutralizing monoclonal antibodies and induced cross-reactive anti-Spike antibodies in mice, facilitating SARS-CoV-2 clearance. A specific S. salivarius protein, RSSL-01370, contains regions with homology to the Spike receptor-binding domain, and immunization of mice with RSSL-01370 elicited anti-Spike IgG antibodies in the serum and oral S. salivarius supplementation enhanced salivary anti-Spike antibodies in vaccinated individuals. In case of autoimmunity, we observed that pathogenic ANCA antibodies against myeloperoxidase can be induced by distinct commensal bacteria.
Altogether, our data show that distinct species of the human microbiota can express molecular mimics of viral and host proteins that contribute to human health.

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P2.03 EPITHELIAL AND STROMAL CELLS

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392 – P2.03.01

Packaged Food Emulsifiers Trigger Pro-Inflammatory Activation and Disruption of the Gut Epithelial Barrier

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The barrier function of the epithelia is crucial for maintaining homeostasis. Environmental exposures may alter the epithelial barrier integrity and influence the development of diseases. Recent studies have shown that certain surfactants and emulsifiers damage the epithelial barriers. We investigated the effects of three commonly used food emulsifiers, sunflower-derived lecithin (SunLec), soy lecithin (SoyLec) and diacetyl tartaric acid ester of mono- and diglycerides (DATEM) on gut organoids and organs-on-a-chip and adult stem cell derived intestine-on-a-chip. All emulsifiers were examined at consumer-relevant doses using transepithelial-electrical resistance (TEER), RNA-seq, and targeted proteomics. SunLec, SoyLec and DATEM elicited a dose- and time-dependent decrease in epithelial barrier function in TEER measurement. RNA-seq analysis indicated that both lecithins upregulated the pathways of response to lipid and cell death at 6.25 mg/ml. Specifically, wound healing was upregulated with SunLec exposure, while cell migration, oxidative stress and angiogenesis pathways were upregulated with SoyLec. DATEM showed increased cell death, regulation of metabolic processes and response to oxygen-related compounds. An increase in type 2 cytokine levels such as IL-4, IL-13 and IL-33 in response to SunLec, while SoyLec induced the production of proinflammatory cytokines (IL-6, IL-18) as well as alarmins (TSLP, IL-33). DATEM induced the production of not only the alarmin, TSLP, but also proinflammatory and cell death-related proteins such as caspase 8, IL-18, DDX58 and peroxiredoxins. Irregular and heterogeneous confocal microscopy staining of ZO-1 was observed after exposure to all three emulsifiers, additionally demonstrating the disruption of the intestinal epithelial barrier. In conclusion, the present study provides direct evidence on the detrimental effects of food emulsifiers, SunLec, SoyLec and DATEM on intestinal epithelial integrity, due to extensive proinflammatory, oxidative stress, tissue healing, angiogenesis and alarmin release of the epithelial cells, namely causing epithelitis. Studies are going on to examine the epithelial damaging and inflammatory effect of emulsifiers on mice in vivo and to verify the related pathways mechanistically in human intestinal organoids using CRISPR/Cas9.

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544 – P2.03.02

PPARβ/ inhibition enhances Mesenchymal Stromal Cell-mediated immunomodulation in the context of Acute Respiratory Distress Syndrome

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Purpose: Mesenchymal Stromal Cells (MSCs) have been utilised in a variety of clinical trials to date, due to their immunomodulatory properties and their low immunogenicity. MSCs are known to require a pro-inflammatory stimulus in order to activate/license them. This makes them an attractive treatment for inflammatory diseases such as Acute Respiratory Distress Syndrome (ARDS); a condition in which there is an abundance of pro-inflammatory mediators available for MSC licensing. The PPARβ/*δ* nuclear receptor has been thought to impact MSC functionality. In the context of ARDS, there are many natural ligands of PPARβ/*δ* in the patient micro-environment that could bind to this ubiquitously-expressed receptor on the MSCs. We sought to investigate the impact of PPARβ/*δ* on MSC immunomodulation, specifically of human monocyte-derive macrophages (MDMs), in the context of ARDS.
Methods: Human bone-marrow-derived MSCs were seeded at a density of $1x10^5$ and stimulated with a synthetic PPARβ/*δ* agonist (GW0742) or antagonist (GSK3787) for 6hrs, followed by cytokines for a further 6hrs. The cells were sent for RNA sequencing, used in a variety of *in vitro* functional assays, or trialled in our LPS-induced Acute Lung Injury (ALI) mouse model.
Results: Our results showed that PPARβ/*δ* inhibition drastically improved MSC-immunomodulation of human MDMs, and upregulated immunomodulatory genes such as *ptgs1*, *icam1* and *ccl2*. These genes were downregulated when the cells were exposed to either the synthetic agonist, or human ARDS serum samples containing natural ligands of PPARβ/*δ*. However, when PPARβ/*δ* was inhibited prior to exposure to the ARDS serum, we could redeem the upregulation of these immunomodulatory genes. RNA sequencing datasets further highlighted the pathways that had been altered, showing clear differences in the NFkB pathway, with many NFkB-regulated genes being enhanced upon inhibition of PPARβ/*δ*.
Conclusion: PPARβ/*δ*-activation negatively impacts MSC immunomodulation, and inhibiting or removing it prior to MSC therapy, in the context of ARDS, may be beneficial.

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1169 – P2.03.03

Differential FLS populations define Rheumatoid and Psoriatic Arthritis pathotypes via their functional response to distinct immune regulators

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Purpose: To identify phenotypic and functional characteristics that define distinct fibroblast (FLS) populations and immune regulators in Rheumatoid Arthritis (RA) vs Psoriatic Arthritis (PsA).
Methods: ScRNAseq was performed on 88,953 RA and PsA FLS from intact synovial-biopsies and FLS populations defined by advanced bioinformatic analysis. Multiparametric flow-cytometric analysis (22-markers) was performed on RA and PsA patient synovial-biopsies to examine FLS phenotype/function. FLS were sorted into $THY1^+$ vs $THY1^-$ FLS, cultured and supernatants analysed by MSD-Multiplex assays. Following ligand-receptor interaction analysis, FLS were cultured with IL-1β (1ng/ml), TGF-β (10ng/ml) alone or in combination. Metabolic-capacity was assessed using Seahorse-XFe-technology and glycolytic markers, mitochondrial fission/fusion proteins (DRP1/MFN1/MFN2) and endoplasmic reticulum (ER) stress proteins quantified by real-time PCR and/or immunofluorescence.
Results: ScRNAseq demonstrated 11-distinct FLS populations in RA and PsA, with differential clusters frequency observed with $THY1^+$FLS dominant in RA vs $THY1^-$FLS dominant in PsA. Flow-analysis of $PDPN^+$FLS demonstrated significant increases in $HLADR^+$, YAP^+, $Cad11^+$, and $pS6^+$ FLS in RA (all-$p<0.05$), whilst CD55 was increased in PsA-FLS ($p=0.0079$). Further flow-analysis identified 6-main FLS populations. RA displayed enrichment of $THY1^+CD34^+CD55^-FAP^+$ and $THY1^+CD34^+CD55^-FAP^+$ FLS ($p=0.0093$), while PsA displayed enrichment in $THY1^+CD34^-CD55^+FAP^+$ ($p=0.02$) and $THY1^-CD34^-CD55^+FAP^+$ FLS ($p=0.0013$). HLADR and Cad11 were significantly higher in RA subpopulations. $THY1^+FAP^+$FLS demonstrated higher expression of angiogenic mediators VEGF-A, VEGF-C, and Flt1 compared to $THY1^-FAP^-$FLS. ScRNAseq receptor-ligand-interactions demonstrated that T-cell-derived TGF-β and macrophage-derived IL-1β synergistically drive the transcriptional profile of RA $THY1^+FAP^+$FLS. At a functional level IL-1β/TGF-β synergistically promoted IL-6, IL-8, MCP-1, ICAM-1, VEGF and MMP3 secretion from RA-FLS ($p<0.05$). Furthermore, IL-1β/TGF-β synergistically induced a glycolytic phenotype in RA-FLS, with synergistic induction of basal glycolysis ($p<0.05$), proton efflux-rate ($p<0.05$), % PER-glycolysis ($p<0.05$) and compensatory-glycolysis ($p<0.001$). This was paralleled by significant increases in GLUT-1, HIF1a and PKM2 (all $p<0.05$) and ER-stress genes XBP1S, BIP and ATF6. Reduced mitochondrial size ($p<0.05$), with an increase in mitochondrial fission protein DRP-1 ($p<0.05$) was also demonstrated.
Conclusion: Distinct FLS populations with unique functional properties and regulators were identified in RA and PsA. Deeper understanding of FLS subsets and blockade of specific immune-stromal cell-interactions may offer new therapeutic intervention in RA and PsA.

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1245 – P2.03.05

Investigating the impact of PPARβ/δ on Mesenchymal Stromal Cell immunometabolism

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Purpose: The immunomodulatory potential and regenerative properties of mesenchymal stromal cells (MSCs) have been utilised in cell-based therapies for immune-mediated disorders. However, clinical trials using MSCs for the treatment of acute respiratory distress syndrome (ARDS) have been disappointing and only effective in around 40% of patients. Differences in inflammatory microenvironments in ARDS patients are thought to play a factor in MSC efficacy. Interestingly, ligands of peroxisome proliferator-activated receptor-delta (PPARβ/δ) are highly expressed in the ARDS patient environment. Recent evidence suggests that PPARβ/δ modulation alters MSC function and metabolic profile. In this study we evaluated the implications of PPARβ/δ on MSC metabolism and immunosuppressive ability *in vitro*.
Methods: Human bone marrow derived MSCs were seeded at a density of $1.2x10^5$, stimulated with PPARβ/δ agonist (GW0742) or antagonist (GSK3787) followed by pro-inflammatory cytokines TNFα and IFNγ. Gene and protein expression profiles were investigated by RNA Sequencing (RNA-Seq), qPCR and flow cytometry. The immuno-metabolic profile of hBM-MSCs pre-treated with PPARβ/δ agonist or antagonist was established using SCENITH assay.
Results: RNA-Seq data showed that MSCs stimulated with pro-inflammatory cytokines in the presence of a PPARβ/δ agonist or antagonist leads to differential changes in key metabolic pathways associated with MSC immunosuppression. Altered gene expression of *slc2a1* and *hk2* following pro-inflammatory cytokine stimulation suggests upregulation of glycolysis. PPARβ/δ activation leads to increased mitochondrial dependence and expression of *cpt1a*, the rate-limiting enzyme required for fatty acid oxidation. Conversely, PPARβ/δ antagonism is associated with an increase in glycolytic genes and *ptgs2* expression following stimulation with TNFα, both associated with MSC immunosuppressive ability. Functionally, PPARβ/δ antagonism correlates enhanced suppression of human monocyte derived macrophages by MSCs *in vitro.*
Conclusion: Differences in the metabolism of MSCs in the presence of PPARβ/δ agonist or antagonist and stimulation with pro-inflammatory cytokines dictate their immunomodulatory potential. Inhibition of PPARβ/δ to enhance MSC function and metabolic capacity has the potential to improve clinical outcomes for the treatment ARDS.

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1299 – P2.03.06

Characterization of PRIME cells identified in circulation of Individuals-At-Risk and patients with rheumatoid arthritis

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Purpose: A recent study has identified CD45-CD31-PDPN+ 'pre-inflammatory mesenchymal' (PRIME) cells in the circulation as predictive of flare in patients with Rheumatoid Arthritis (RA). However, little is known about the functional capacity of 'PRIME' cells. We aim to identify and phenotype PRIME cells in the circulation and site of inflammation (joint) in healthy controls (HC), RA, and in 'individuals-at-risk' (IAR) of developing RA.
Methods: Multiparametric flow-cytometry analysis (23-color panel) was performed on peripheral blood mononuclear cells (PBMC) from HC, IAR, and RA patients. RA patients were further stratified between ACPA+ vs ACPA-. PRIME cells were identified as CD45-CD31-PDPN+ and subsequent analysis of phenotypic markers were quantified by flow-cytometry. Frequency and characteristics of PRIME-cells from circulation were compared to synovial-tissue cells by flow-cytometry.
Results: Increased frequency of PRIME cells was observed in RA PBMCs compared to HC with a significant increase seen in IAR. RA PRIME cells had trending increase of frequency/expression of proliferation (Ki67) and activation (FAP) markers compared to HC. Frequency/expression of adhesion and immunoregulatory markers CD34 ($p=0.08$),-CD44,-ICAM-1,-HLA-DR and CD200R1 were increased RA PRIME cells compared to HC. A stepwise increase in frequency of markers FAP,-CD200R1 and CD34 was shown from HC>IAR>RA. Stratification of RA patients demonstrated increased PRIME frequency ($p<0.01$) and expression of CD44,CD34, and CD200R1 in ACPA+ compared to ACPA-. These phenotypic changes were mirrored by a shift in the metabolic-profile of 'PRIME' cells where a stepwise increase in the expression of the key metabolic sensor mTOR was demonstrated from HC>IAR>RA. CD55 (lining-layer FLS-marker) was not expressed on PRIME cells across disease status, however, CD90 (sub-lining FLS-marker) was observed. Synovial-tissue had significantly higher frequency of PDPN+ cells compared to PBMC ($p<0.01$). Frequency of CD55,-CD90,-pAKT,-YAP, and CD34 were significantly higher in IAR and RA synovial-tissue compared to circulatory PDPN+ cells (all $p<0.05$). In contrast, Cadherin11,-ICAM-1, and CD200R1 were significantly higher on circulating PDPN+ cells compared to synovial-tissue (all $p<0.05$).
Conclusions: PRIME cells could function as a potential biomarker in IAR and an indicator of disease severity in RA. Understanding their functional role in RA pathogenesis could provide additional therapeutic targets.

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1347 – P2.03.07

The inflamed joint microenvironment affects endothelial cell function through soluble mediators and cell-cell interactions

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Purpose: While common pathogenic mechanisms exist between PsA and RA, distinct vascular morphology has been observed, with PsA displaying a tortuous, dilated, irregular shaped morphology compared to a straight regular branching pattern observed in RA. The aim of this study is to examine the effect of the PsA and RA joint microenvironment on endothelial cell function.
Methods: PsA and RA patients underwent key-hole joint arthroscopy and synovial biopsies were obtained. PsA and RA synovial fibroblasts (FLS) were isolated and grown to passage 1-5. PsA and RA FLS supernatants were harvested and referred to as conditioned media (CM). Endothelial cells (EC) were co-cultured with PsA/RA FLS or CM, and pro-inflammatory mediators were quantified by real-time PCR and flow cytometry.
Results: Gene expression of IL-6 and ICAM-1 in EC was increased in response to both PsA/RA FLS and CM. Both PsA CM and RA CM induced MCP-1 and MMP-2 expression in EC, with no effect observed in response to RA/PsA FLS. VCAM-1 expression was decreased in EC in response to both PsA CM and RA CM, however, was increased in RA FLS-EC co-culture. Key angiogenic growth factors VEGF and Ang-2 were increased by either RA/PsA CM and FLS, in addition to the matrix degrading enzyme MMP3. Either co-culture of PsA/RA FLS or PsA/RA CM with EC induced the frequency of key chemokine receptors CXCR3 and CXCR4 on EC, an effect that was more pronounced for CM vs FLS co-culture, particularly for PsA CM. Both RA FLS and RA CM decreased the frequency of CXCR5. Only co-culture with PsA and RA FLS induced the expression of ICAM-1, with no effect observed for PsA or RA CM. Both PsA/RA FLS and CM decreased VCAM-1 expression on EC, similar to that observed at the gene expression level.
Conclusion: PsA and RA FLS/CM induce angiogenic, chemokine, adhesion molecule and matrix degrading enzymes on EC, with differential effects for some mediators observed in response to PsA vs RA joint microenvironments. Furthermore, differences were observed between EC-FLS vs EC-FLS CM co-cultures, suggesting cell-cell contact and soluble mediators both influence the EC pathogenic phenotype.

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1357 – P2.03.08

Defining the effects of trained innate immunity and metabolic reprogramming on the immune functions of airway epithelial and fibroblast cells

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Background: Exposure to an infection or a vaccine can induce heterologous effects mediated by myeloid cells termed 'trained immunity' resulting in enhanced innate immune responses to subsequent infections. The interconnected effects of trained innate immunity on structural cells in the lungs is unknown. Given the critical role for cellular metabolism in the function of innate cells, targeting metabolism through aerosolised delivery of immunomodulatory drugs during infection may be clinically beneficial for difficult to treat respiratory infectious disease such as Tuberculosis. However, a significant knowledge gap remains around metabolic manipulation on the innate-structural cell axis in the lung which amplifies inflammation leading to the clearance of infection, but can also cause collateral damage to the delicate lung tissue.
Aims and Methods: Human lung epithelial (A549) and fibroblast cells (MRC-5) were exposed to conditioned medium from trained monocytes which were restimulated with *Mycobacterium tuberculosis* or untrained controls. To examine if cellular metabolism is associated with the immune functions, A549 and MRC-5 cells were treated with inhibitors of metabolic pathways prior to being stimulated with conditioned medium or innate immune cytokines. The production of IL-6 and IL-8 were quantified by ELISA. The expression of ICAM and HLA-DR were determined by flow cytometry.
Results and Discussion: Conditioned medium from trained monocytes increased IL-6, IL-8 and ICAM expression from A549 and MRC-5 cells. Additionally, IL-1β and TNF, produced by trained monocytes, act synergistically to promote IL-6 and IL-8 by A549 and MRC-5 cells. This synergistic effect is mediated in part, by glycolysis as inhibiting glycolysis with 2DG significantly attenuated the synergistic effects of IL-1β and TNF. Moreover, inhibiting fatty acid metabolism using etomoxir significantly increased IL-6 production induced by the synergistic IL-1β and TNF suggesting that fatty acid oxidation may indirectly limit an excessive inflammatory loop between myeloid and structural cells in the lung.
Conclusion: Trained innate immunity propagates an inflammatory phenotype in surrounding structural cells in the lung microenvironment and immunometabolism is a key regulatory point in the innate-structural network in the lung. This is an important finding as metabolic pathways are a tractable target to develop inhalable host-directed therapies to promote respiratory health.

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1412 – P2.03.09

Human thymus epithelial stem cells - the pillars of T cell development

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Purpose: T lymphocyte development relies on the functional thymus epithelial cells. We continue our study based on the previous results showing the existence of epithelial cell precursors within the postnatal human thymus as it has been described in the mouse model. We investigate the regenerative capacity of the epithelial cell precursors and continue our study on their characterization. However, some of the markers that were used for stem cell enrichment in the mice as Plet-1 have not been described in the human thymus.
Methods: Therefore, we previously described a technique for stem cell enrichment and *in vitro* 3D culture conditions, to assess the number of stem cells obtained as it has been shown for the neuronal cells. The spheroid forming cells are putative epithelial cell precursors that have to be tested for their functional capacity to promote T lymphocyte development from hematopoietic stem cells either *in vitro* by means of re-aggregation cultures or *in vivo* by transplantation into an animal model system.
Results: Since we previously showed that spheroid bodies express genes typical for mature thymic epithelial cells - KRT5, KRT8, AIRE, CD205, FoxN1 and EpCAM, but also genes HOXA3, PAX1, PAX9, and EYA1 active during early development, in the present work we tested the expression of these antigens *in situ* on thymus sections of children of different ages. Since we found in our previous work a certain proportion of cells expressing also PDGFRbeta, we further analyzed thymus resident progenitor cells being of epithelial or mesenchymal origin.
Conclusion: Our research uncovers some previously unknown characteristics of the elusive progenitor populations and their position and frequency in the intact human thymus.

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1778 – P2.03.10

A bacterial metabolite with gut and skin epithelial barrier enhancing and anti-inflammatory activities

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Gut microbiota protects the gastrointestinal system's homeostasis by producing antimicrobial substances, vitamins, and metabolites, which aid in strengthening the gut epithelial barrier, inhibiting opportunistic pathogen colonisation and regulating the immune system. In addition, same bacterial products may have prominent roles in skin barrier homeostasis. With this perspective, we identify a gut microbiome metabolite (compound X) that improves gut epithelial barrier development speed and integrity as demonstrated in gut-on-a-chip Caco-2 3D cultures. The surface application of 0.25, 1- and 4-mM doses of compound X significantly increase the gut epithelial barrier integrity compared to control ($P<0.0001$) by almost 2 times (Transepithelial Electrical Resistance-TEER), during the gut development phase of the barrier. Transcriptomic analysis shows an upregulation of gene expression associated with xenobiotic metabolism, ion, water, glucose, and amino acid transport pathways. It increases the gene expression of the tight junction protein, claudin-1 and displays anti-inflammatory activity, as demonstrated by decreased chemokines (CXCL5, 10 and 11, CCL20, 23 and 25, CSF-1 and MCP-1) and IL-1 α levels detected by proximity extension assay in media. Untargeted proteomics analysis reveals that compound X upregulates glycolysis, tricarboxylic acid cycle and oxidative phosphorylation and downregulates lipid metabolism pathways-related proteins. A 6-day pretreatment with compound X successfully prevents proinflammatory cytokine (TNF-α, IFN-γ and IL-1β) driven gut epithelial barrier impairment. Next, we assess its effects on skin epithelial barrier integrity with an *ex vivo* skin tissue model. Treatment of systemic circulation-relevant doses of compound X (0.25,1 and 4 μM) rescues surfactant (cocoyl methyl glucamide; CMG)-induced skin epithelial barrier damage within 24 hours. In addition, it reverses the inflammation caused by CMG, as demonstrated by decreased IL-18, CSF-1, PRDX-3 and PD-L1 protein levels. In conclusion, our data highlights compound X, as a promising agent for preventing, rescuing, and treating gut and skin epithelial barrier impairment. Studies are going on to confirm the associated pathways mechanistically by using CRISPR/Cas9 in human intestinal organoids and to check the mitigation effect of compound X on surfactant-treated skin of mice *in vivo*.

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2042 – P2.03.11

The fetal liver stromal compartments and hematopoiesis

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Purpose: During fetal development, multiple waves of hematopoietic cells emerge in the yolk sac generating erythro-myeloid progenitors, and in the intraembryonic vasculature giving rise to multipotent progenitors (MPPs). The MPPs differentiate, expand and establish the adult hematopoietic stem cell (HSC) pool in the fetal liver (FL), supported by a specialized stromal compartment, before they migrate to the bone marrow. Different stromal cells have been shown to interact with HSCs/MPPs. A better characterization of the fetal liver microenvironment during the development would provide better understanding of fetal hematopoiesis, new insights into hematologic disorders that originate in the fetus and expansion of HSC for transplantation.
Methods: we isolated different stromal cell components and optimized a 3D culture system such that they maintain the expression of structural proteins and cytokines/chemokines. We used this system to analyze individual or combinations of stromal populations for their capacity to maintain and/or expand hematopoietic stem and progenitor cells (HSPCs).
Results: Erythroid, myeloid and lymphoid cells were observed when stromal cells co-cultured with $CD45^+$ FL cells in the 3D culture system, an expansion of up to 4-fold of HSPCs was also observed, while HPSCs decreased when co-cultured $CD45^+$ FL cells in 2D culture system and co-cultured with LSK cells alone in 3D culture system.
Conclusion: The 3D culture system that we developed supports differentiation of erythroid, myeloid and lymphoid cells and HPSCs expansion from $CD45^+$ FL cells. Co-culture of LSK with other hematopoietic cells improve LSK expansion.

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P2.04 GENETIC AND ENVIRONMENTAL TRIGGERS OF AUTOIMMUNITY

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156 – P2.04.01

Clinical features and outcome in two paediatric patients with anti-MDA5 and anti-Ro52 antibodies after viral infection

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Background: Dermatomyositis (DM) is a rare autoimmune connective tissue disease included within the idiopathic inflammatory myopathies that is characterized by the presence of specific skin lesions and myositis. However, these lesions have been evidenced in patients without muscle disease, thus giving rise to another subgroup called amyopathic dermatomyositis. This entity is strongly linked to the presence of antibodies to melanoma differentiation-associated gene 5 (MDA5), which is associated with a poor prognosis due to rapidly progressive interstitial lung disease (RP-ILD).
Methods: We analyze two paediatric patients under follow-up at Hospital Universitario La Paz between 2020 and 2024 with positive anti-MDA5 antibodies and amyopathic dermatomyositis. Clinical presentation, immunological findings, treatments and clinical outcome are reviewed.
Results: The patients were two girls who presented at 11 and 10 years old with skin lesions compatible with dermatomyositis and fever in the context of tonsillitis and EBV infection, respectively. Interstitial lung disease appeared less than three months after this episodes. Presence of anti-MDA5 and anti-Ro52 antibodies as well as elevated inflammatory mediators were detected. They initiated corticosteroid therapy. After poor response, different immunosuppressants were added sequentially. The use of plasmapheresis did not improve the outcome. In addition, both patients suffered different nosocomial infections. The first patient died 6 months after debut due to multi-organ failure in the context of septic shock whereas the second one developed a macrophage activation syndrome and has recently started extracorporeal membrane oxygenation therapy (ECMO) as a bridge to recovery.
Conclusion: Anti-MDA5 antibodies are extremely rare, especially in paediatric population. It is essential to actively search for ILD if they are found, since it frequently appears in the first months after diagnosis and is the main risk factor in these patients. Viral infection could act as a trigger that leads to increased levels of MDA5 and loss of tolerance. Furthermore, positive anti-Ro52 antibodies are associated with worse prognosis. Evidence suggests the use of combined immunosuppressive therapy and plasmapheresis in unresponsive cases, although in our patients the decrease in antibody titre did not correlate with clinical recovery. An improved knowledge of the pathogenesis is still needed to develop more effective targeted therapies.

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205 – P2.04.02

Increased phosphorylation indicates continuous antigen-mediated autoreactive B-cell activation in rheumatoid arthritis

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Purpose: Many human autoimmune diseases (AIDs) are hallmarked by persistent autoreactive B cells. Unlike B cells against recall antigens, autoreactive B cells may continuously encounter their antigen. Currently, it is unknown whether this maintains continuous activation. As such, rheumatoid arthritis (RA) is hallmarked by B cells targeting post-translationally modified proteins, for instance anti-citrullinated protein antibody (ACPA)-expressing B cells. Here, we sought to develop an antigen-specific flow cytometry staining approach to assess intracellular signaling pathways in ACPA-expressing B cells, isolated directly *ex-vivo* from patients with the prototypic human AID RA.
Methods: Immortalized human B-cell lines specific for tetanus toxoid (TT) or ACPA were used for protocol development. PBMCs of 10 RA patients were subsequently analyzed using spectral flow cytometry and various B-cell differentiation markers, antigen-biotin-streptavidin tetramers, and intracellular (phosphoflow) markers. TT-specific B cells served as antigen-specific comparators.
Results: Antigen-specific staining of B-cell lines induced protein phosphorylation, even when cells were kept strictly on ice. This was prevented by adding a fixation step prior to antigen-specific staining. Using this approach, ACPA-expressing memory B cells (MBCs) from RA patients displayed enhanced expression of Ki-67 and increased SYK-, BTK-, AKT- and S6-phosphorylation compared to TT-specific MBCs.
Conclusion: Our approach allows simultaneous detection of antigen-specificity and protein phosphorylation on the single cell level. The data reveal that autoreactive B cells in RA, in contrast to B cells against recall antigens, display enhanced phosphorylation of signaling molecules downstream of the BCR, pointing towards continuous antigen-mediated activation of autoreactive B cells in RA.

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356 – P2.04.03

Complete description of the Three Pathways of the Complement system in patients with Rheumatoid Arthtitis

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Purpose: Complement system is a major player in innate immunity and an effector arm of the humoral immune system so inadequately controlled complement activation may underlie the pathogenesis of several immunomodulated processes. Rheumatoid arthritis (RA) is the most common chronic form of inflammatory arthritis, resulting from complex interactions between genes and environment, leading to a breakdown of immune tolerance and to synovial inflammation in a characteristic symmetric pattern. The implication of complement in the etiopathogenesis of RA has been proposed but the role of both functionally and individual complement components levels has not been fully characterized. This study aims to comprehensively evaluate the relationships between the functional levels of the three complement cascades and specific elements within these pathways with the characteristics of RA, such as disease activity and the presence of rheumatoid factor or anti-citrullinated protein autoantibodies.
Methods: A cross-sectional study was conducted, including 430 patients with RA who met the 2010 ACR/EULAR classification criteria. A detailed assessment of various components of the complement system belonging to all three cascades, including enzymatically generated fragments and serum regulators, was performed using functional complement assays and individual elements assessments through multiplex detection.
Results: C-reactive protein, complement system products and functional complement assays were found to be positively correlated**.** The analysis revealed that disease activity had a positive correlation with the functional test of the classical pathway specially with the products of the terminal complement pathway. However, the presence of rheumatoid factor and anti-citrullinated protein antibodies was linked to lower levels of specific complement components of the classical pathway and C3a and C4b.
Conclusions: The study findings highlight the complex relationship between disease activity and the complement system in RA. Higher or moderate disease activity indicate upregulation of the complement system components whereas the presence of rheumatoid factor and/or anti-citrullinated protein antibodies is associated with complement consumption. This relationship suggest the potential utility of targeting the complement system in new therapeutic approaches for RA. Future research is warranted to explore the clinical implications and therapeutic opportunities of modulating the complement system in RA management.

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468 – P2.04.04

Loss of thymic interferon signature in autoimmune regulator-deficient mice

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Autoimmune regulator (AIRE) plays a crucial role in the establishment of immune tolerance. AIRE orchestrates the display of tissue restricted antigens to T cells developing in the thymus, mediating elimination of self-reactive thymocytes. The devastating consequences of dysfunctional AIRE are seen in patients with autoimmune polyendocrine syndrome type 1, who develop multi-organ autoimmunity and harbour a range of tissue-specific and cytokine autoantibodies. AIRE's contribution to self-antigen presentation in the thymus has been thoroughly studied, but a comprehensive picture of how loss of AIRE affects development, maturation and function of T cells, and other immune cell populations in the thymus is still largely lacking. Furthermore, how tolerance to cytokines is lost and to what degree the thymus and AIRE are involved in this process, remains an open question.
To address these aspects, we performed an exploratory analysis using single-cell RNA sequencing of transcriptomes and adaptive immune receptors of immune cells isolated from the thymi of six-week-old AIRE-deficient and wild-type (WT) mice, with two male and two female mice in each group.
Our comprehensive dataset encompassing a total of 93,118 thymic immune cells, shows similar distribution of the immune cell subsets in AIRE-deficient and WT mice. Careful annotation of thymocytes using recognized markers expressed at different maturation stages, allowed us to track their thymic developement. Subsequent differential gene expression analysis revealed a strong downregulation of interferon-stimulated genes (ISGs) within the mature single-positive CD4 and CD8 cells, regulatory T cells and NKT cells in AIRE-deficient mice, in line with previous studies suggesting an involvement of AIRE on constitutive type I interferon (IFN) production in the thymus, and the influence of type I IFNs on thymocyte maturation. Diminished expression of ISGs was also observed in hematopoietic thymic antigen presenting cells (APCs), along with downregulation of gene sets related to antigen processing and presentation, suggesting that thymic IFNs might influence antigen-presenting capabilities of thymic APCs.
Our study supports the previously reported role of AIRE on constitutive thymic IFN production and the influence of IFN signalling on the maturation of thymocytes

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506 – P2.04.05

The role of bacterial lipopolysaccharides and gut microbiota dysbiosis in type 1 diabetes

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Type 1 diabetes (T1D) is an autoimmune disease characterized by T-cell mediated destruction of insulin producing β-cells. Possible environmental factors predisposing to T1D include gut dysbiosis. Earlier studies have reported higher abundance of *Bacteroides* species in Finland compared to Karelia (Russia), and it was shown that *Bacteroides* species synthetize a lipopolysaccharide (LPS) structurally distinct from that of *Escherichia coli*, a species more abundant in infants in Karelia with lower T1D incidence. Functional differences were also reported, as *Bacteroides* LPS failed to induce endotoxin tolerance. We recently reported that dysbiosis induces the expression of a chemokine CXCL10 in islets via elevated levels of circulating LPS, attracting activated leukocytes into islets. Elevated levels of CXCL10 in T1D have been observed both in clinical studies and in animal models.
Here we studied the effects of a single exposure of NOD mice to LPS, whereas endotoxin tolerance was modelled with multiple exposures. TNFα production by peritoneal macrophages was determined with ELISA, and T-cell and dendritic cell populations were analyzed with flow cytometry. Moreover, the expression of CXCL10 in pancreatic islets was determined with immunofluorescence staining.
Escherichia coli LPS was more efficient in stimulating macrophage TNFα production and dendritic cell activation compared to *Bacteroides vulgatus* LPS. Similar trend was observed in T-cells and their activation markers. Islet CXCL10 expression was higher in *E. coli* LPS treated NOD mice. In endotoxin tolerance experiments, unlike in macrophages from *B. vulgatus* treated mice, *E. coli* LPS exposure resulted in lower TNFα production compared to controls. Furthermore, *E. coli* LPS resulted in lower levels of CD8+ T-cells recognizing islet specific antigen compared to *B. vulgatus* LPS and control. Similarly, *E. coli* LPS induced tolerization of dendritic cells. Statistically significant differences were also observed in CXCL10 expression after two doses of *E. coli* LPS.
This study provides evidence for the role of endotoxins from two common intestinal bacterial species, *E. coli* and *B. vulgatus*, in T1D related autoimmunity. The two endotoxins differ in terms of immunogenicity— in contrast to *B. vulgatus* LPS, *E. coli* LPS is a potent activator of the immune system and induces the development of endotoxin tolerance.

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656 – P2.04.06

The propensity of B cells to infiltrate the multiple sclerosis brain: impact of genetic variation and environment.

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Purpose: The interplay between Epstein-Barr virus (EBV) and specific genetic risk variants is assumed to alter the B-cell functional program in patients with multiple sclerosis (MS). Recently, our group revealed that CXCR3$^+$ B cells are preferentially recruited to the central nervous system (CNS), correspond with local antibody production and are promising targets for next-generation Bruton's tyrosine kinase (BTK) inhibitors such as evobrutinib (EVO) in MS. Here, we explored the contribution of environmental and genetic risk factors to the inducibility of brain-homing CXCR3$^+$ B cells in MS and examined whether this could be targeted by EVO.
Methods: B-cell subsets were analyzed in blood of monozygotic twin pairs discordant for MS using mass cytometry to study the impact of environmental factors. In addition, human B-cell lines and primary B cells were selected based on the presence of an MS risk SNP in *IFNGR2* (rs9808753) to examine the IFN-γ signaling pathway using flow cytometry, phosphoflow and immunoblotting. *In vitro*-stimulated blood B cells were compared between MS patients and controls and treated with or without EVO.
Results: Amongst all B-cell subsets analyzed, frequencies of CXCR3$^+$ class-switched memory B cells were reduced in genetically identical twins with MS vs unaffected co-twins. In B-cell lines carrying the *IFNGR2* risk SNP, STAT1 phosphorylation was more pronounced after addition of IFN-γ, the main trigger of CXCR3$^+$ B cells. The IFN-γ pathway was constitutively activated in EBV-infected B cells, showing upregulation of IFNGR1, IFNGR2 and phosphorylated STAT1. In primary B cells, CD40 ligand-induced surface expression of IFNGR2 and not IFNGR1 was attenuated by EVO. In B cells from MS patients, specifically for risk SNP carriers, STAT1 phosphorylation was highest in the transitional subset (CD38^{high}CD27$^-$) and T-bet transcription was more induced by IFN-γ.
Conclusion: The twin data show that circulating CXCR3$^+$ B cells are influenced by environmental factors in patients with MS. The work also provides further evidence that EBV interacts with genetic risk variants to induce these types of B cells to enter the CNS, a process that may be prevented by BTK inhibition in MS.

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678 – P2.04.07

Methyl-rich diet suppresses the development of lupus-like symptoms in humanized mouse model of the disease

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Systemic lupus erythematosus (SLE) is a chronic autoimmune disease with complex organ and system involvement. Although the hallmark of the disease is the presence of antinuclear antibodies, other serological markers and clinical symptoms may vary between patients, making the manifestation of SLE heterogeneous in nature. Although genetic predisposition is necessary for SLE to appear, epigenetic factors also played central role in this process. Particular methyl-rich micronutrients, such as methionine, betaine, choline, folic acid and vitamin D, take part in the mechanisms of DNA methylation. We have previously reported that the administration of high amounts of those methyl-rich components in the diet of lupus-prone MRL/*lpr* mice resulted in decreased anti-dsDNA antibody and proteinuria levels and in protected renal structures. In the present study we report that the methyl-supplemented diet also ameliorates the development of SLE and in NSG/Rag2-γc- mice humanized with lupus patients' PBMCs which defines this diet as novel possible therapeutic agent with immunomodulating potential.
Two groups of female NSG/Rag2-γc- mice were engrafted with PBMCs from SLE patients. The first group was put on a normal rodent diet, the other – on methyl-supplemented diet. Two groups of control non-humanized mice were also put on either of the diets. The experimental animals were monitored for 8 weeks with blood and urine samples being collected once a week. At the end of the dietary course the mice were sacrificed, and kidneys were examined for glomerular pathology.
The results showed a decrease in anti-dsDNA antibody and proteinuria levels in the mice put on the supplemented diet, compared to the mice put on normal diet. In addition, histopathological changes in the structure of the glomeruli were observed in the kidney of mice fed with the normal diet but not the supplemented group.
The observed beneficial effect of the methyl-rich diet may be related to the potential modulation of DNA methylation levels and subsequent changes in gene expression. These results point to the importance of DNA methylation as one of the major epigenetic factors responsible for the progression of SLE as well as to its implication as a powerful immunomodulating tool.

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696 – P2.04.08

Exploring the reduced cytokine production capacity of lymph node T cells observed during the earliest phases of rheumatoid arthritis

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Purpose: Rheumatoid arthritis (RA) is a chronic inflammatory disease with an unknown aetiology. Autoantibody positive RA-risk individuals with no clinical inflammation provide a model to study the earliest phases of autoimmunity. We hypothesize that already before RA development, due to loss of tolerance and prolonged antigen exposure, T cells become dysfunctional. Previous work from our group highlights a decreased ability of LN T cells to produce cytokines during the earliest phases of RA even without clinically apparent disease. To determine possible mechanisms responsible for this reduced cytokine production capacity we aimed to further characterise and compare the phenotype and transcriptomic profile of LN CD4 T cells from RA, RA-risk and healthy controls (HC).
Methods: Lymph node biopsies were collected through ultrasound guided inguinal LN needle biopsy after which lymphocytes were isolated and stored in liquid nitrogen. Upon thawing, CD4+ T cells were sorted from 12 donors (4 RA patients, 4 RA-risk and 4 healthy volunteers) and gene expression profiles were analysed using a NanoString nCounter gene panel focussed on exhaustion. For detailed cellular phenotyping a fifteen colour flow cytometry panel was performed on fresh LN cell suspensions (10 RA patients, 5 RA-risk and 3 PsA patients) and analysed in a supervised (FlowJo) and unsupervised manner (FlowSOM, R).
Results: Flow cytometry analysis revealed a trend towards a skewing from effector to naïve CD4 T cells in RA and PsA compared to RA-risk. Preliminary analysis uncovered a loss in Eomes+Tbet+ naïve CD4+ T cell frequency, in RA compared to RA-risk. NanoString nCounter analysis uncovered an increased interferon gene signature in LN CD4 T cells of RA-risk compared to HCs with a similar trend for RA. We also observed a trend towards an increase in co-inhibitory receptors including TIGIT, LAG3 and CTLA4 for RA CD4 T cells compared to HCs.
Conclusions: This interferon signature combined with increased co-inhibitory receptor expression and skewing of memory populations within the LN may contribute to the reduced capacity of CD4 T cells to produce cytokines. Future studies are needed to elucidate the original trigger of this interferon signature, which may be the result of chronic antigen exposure.

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704 – P2.04.09

Evaluation of soluble immune checkpoint proteins in systemic lupus erythematosus and Sjögren's syndrome patients

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Purpose: Systemic lupus erythematosus (SLE) is an autoimmune disease with multisystem involvement ranging from mild mucocutaneous manifestations to multi-organ involvement, including severe central nervous system complications. Sjögren's syndrome is a chronic autoimmune disease characterized by lymphocytic infiltration of the exocrine glands, resulting in dryness of the mouth and eyes due to salivary and lacrimal gland involvement. The aim of this study is to investigate the protein levels of soluble immune checkpoint proteins in serum samples from SLE and Sjörgen's syndrome patients to elucidate the potential of immune checkpoint blockade for the treatment of the aforementioned diseases.
Methods: This study was approved by the local ethics committee of Yeditepe University Hospitals (Approval number: 1631). The patient group consisted of patients diagnosed according to the 2019 EULAR/ACR diagnostic criteria for SLE patients and the 2016 ACR-EULAR diagnostic criteria for SS patients. Patients were informed verbally before peripheral blood samples were collected, and patients signed an informed consent form. The control group consisted of adult subjects without autoimmune diseases. For evaluation of soluble immune checkpoint proteins, serum samples were collected and stored at -80˚C in a freezer. Protein expression levels of the soluble immune checkpoints CD25, 4-1BB, B7.2(CD86), TGF-B1, CTLA-4, PD-L1, PD-1, TIM-3, LAG-3, and Galectin-9 were evaluated with the BioLegend LEGENDplex HU Immune Checkpoint Panel 1 (10 plex) using a Beckman Coulter DxFLEX flow cytometry system.
Results: Our results revealed a statistically significant difference in the expression levels of Galectin-9, LAG-3 and TIM-3 between the SLE group and the control group. Moreover, a strong correlation between PD-1/PD-L1 and TIM3/Galectin-9 levels was observed in SLE patients. No statistically significant difference was detected between the Sjögren's group and the control group in terms of the expression levels of the proteins investigated.
Conclusion: Even though further studies involving larger patient groups are needed, our results suggest that therapeutic options targeting PD-1/PD-L1 and TIM3/Galectin-9 axes may provide benefit in the treatment of SLE.
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717 – P2.04.10

How does SARS-CoV-2 affect the immune system of lupus nephritis patients? – a two-year perspective

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Purpose: Lupus nephritis (LN), a renal manifestation of systemic lupus erythematosus, is an autoimmune disease characterized by altered activity of the immune system and kidney dysfunction. The correlation between infections, primarily viral ones, and the incidence of lupus and the disease symptoms aggravation has been widely discussed in the literature. During the COVID-19 pandemic, many LN patients were at higher risk of severe outcomes of SARS-CoV-2 infection. The lack of data regarding this virus's long-term impact on the patient's immune systems caused many concerns. Our purpose was to evaluate whether COVID-19 infection could significantly affect the immune system of LN patients, thus influencing the course of the disease.
Methods: We performed an extensive flow cytometry analysis of circulating immune cells, including T cells, B cells, and dendritic cells (DC), from LN patients. Patients were divided into groups based on the time that passed from their COVID-19 infection, either 6 or 24 months post-SARS-CoV-2 exposition. All the data were compared to LN with no COVID-19 infection in the history.
Results: LN CoV-2(+) patients were characterized with significant alterations in the T-cell memory compartment, as they had higher levels of effector memory T cells (TEM) and central memory T cells (TCM) compared to patients without the COVID-19 infection. Moreover, the percentages of TEM were lower in LN CoV-2(+)6m compared to LN CoV-2(+)24m. The latter group presented a decrease in the percentage of TEMRA and Treg cells. Little differences were observed in the B cell memory compartment, except for the decrease in non-switched memory B cells. This subpopulation was reduced in the general LN CoV-2(+) group, with a pronounced decrease in LN CoV-2(+)6m compared to LN CoV-2(+)24m. Regarding DCs, the changes related to post-infection time were detected in the cDC2 subpopulation, showing the reduction of these cells in LN CoV-2(+)24m patients.
Conclusions: Several interesting alterations were spotted between LN CoV-2(-) and LN CoV-2(+) patients and between LN patients at different post-COVID-19-infection time points. These data suggest that COVID-19 may affect the immune system of lupus patients in a longer perspective.

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722 – P2.04.11

Transfer of IgG of Long-COVID patients induces subgroup-specific symptoms in mice

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Introduction: Over 10% of individuals develop chronic symptoms after SARS-CoV-2 infection, termed Long-COVID. Currently, the disease's pathophysiology is unknown and there are no effective therapies or objective diagnostic tests available. Autoimmunity is one of the proposed mechanisms of Long-COVID, but it is unclear whether autoimmunity is causal in the disease's progression and whether autoantibodies can be used as Long-COVID biomarkers. In this study, we aimed to investigate the causal link between autoimmunity and Long-COVID. Second, we aimed to determine whether specific autoantibodies can be used as a diagnostic biomarker for Long-COVID.
Methods: Long-COVID patients, identified by WHO criteria, were assessed at least 6 months post non-hospitalized COVID-19. Long-COVID and healthy control plasma were subjected to cytokine and neurodegenerative marker quantitative analysis, proteomic analysis, alongside purification of IgG for comprehensive 'autoantibodyome' analysis for over 21,000 autoantigens. Total IgG was purified from patient and control serum, pooled (by group), and intraperitoneally injected into mice, which were then subjected to behavioral tests.
Results: Based on interferon and neurodegenerative markers, three Long-COVID subgroups were identified, each showing proteome signatures enriched in distinct pathways. Strikingly, intraperitoneal injection of pooled IgG of the three Long-COVID groups induced pronounced subgroup-specific behavioral symptoms in mice when compared to mice injected with IgG from healthy controls. IgG from patients with higher muscle protein levels reduced the locomotor activity of mice. IgG from patients with elevated leukocyte activation proteins strongly induced rapid pain sensory hypersensitivity in mice, and IgG from patients with higher neuronal damage marker levels induced delayed sensory hypersensitivity behavior. Autoantibodyome analysis revealed approximately 50 autoantigens highly specific for Long-COVID patients that also associated with main Long-COVID symptoms.
Conclusions: These findings provide compelling evidence for a causal role of IgG autoantibodies in Long-COVID. Furthermore, it provides proof-of-concept for an *in vivo* model for Long-COVID that could be used for treatment testing. Finally, the identified autoantibodies comprise a promising biomarker for Long-COVID, which we are currently incorporating into a multiplex assay to develop an objective diagnostic test for Long-COVID.

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796 – P2.04.12

Immune parameters of patients with systemic lupus erythematosus after COVID-19 infection

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Purpose: Systemic lupus erythematosus (SLE) patients constitute a group of individuals with an increased risk of infections and infection-related mortality. Therefore, during the global SARS-Cov-2 pandemic, SLE patients were particularly vulnerable to SARS-CoV-2 infections. Moreover, several studies have shown that, compared to other patients, SLE patients seem to develop more severe manifestations of COVID-19 infection, with higher rates of hospitalization, invasive ventilation requirements, or death. Although there are many articles in the scientific literature about the impact of SARS-CoV-2 infection on immunological parameters, they largely concern previously relatively healthy people. We know little about how SAR-CoV-2 affects SLE immunology. Therefore, this study aimed to evaluate the immune parameters after SARS-CoV-2 infection in SLE patients.
Methods: We analyzed subpopulations of peripheral blood cells collected from patients with the renal manifestation (lupus nephrititis, LN) of SLE. LN patients were divided into two subgroups: those unexposed to SARS-CoV-2 and those who had COVID-19 6 months earlier. We analyzed basic subpopulations of T cells, B cells, monocytes, dendritic cells (DCs), and serum cytokines using flow cytometry.
Results: LN patients were characterized by a decreased percentage of CD3+CD4+ cells and an increased percentage of CD3+CD8+ cells. Both groups had a higher percentage of T cells with HLA-DR expression. LN CoV-2(+) patients had a higher percentage of plasmablasts (PB) and a lower percentage of non-switched memory (NSM) B cells compared to LN CoV-2(−) patients or HC. LN patients had a higher percentage of total monocytes compared with HC. LN CoV-2(+) had a higher percentage of classical monocytes than LN CoV-2(−) patients and HC. They also had a higher percentage of intermediate monocytes than HC. No difference was observed in the percentage of non-classical monocytes and DC subpopulations. LN CoV-2(−) patients had higher serum IL-10 than HC.
Conclusions: LN patients are characterized by disturbances in the basic immunological parameters of the blood. However, patients who have had COVID-19 present some abnormalities in B-cell and monocyte compartments.
This work was supported by The Polpharma Scientific Foundation (Poland) grant “The immune system of patients with lupus nephritis post SARS-CoV-2 infection” (no. 1/XX/2021).

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821 – P2.04.13

Childhood Systemic Lupus Erythematosus

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Background: Systemic lupus Erythematosus is a chronic, multisystem autoimmune disease having variable clinico pathological features. According to published data, childhood onset SLE has more severe organ system manifestations in the form of nephritis, cytopenias and neuro psychiatric disease compared to adult variant.
Aim: The objective of this case series is to review and summarize clinico pathological features, laboratory findings and treatment outcomes of childhood onset systemic lupus erythematosus in children presenting to a tertiary healthcare facility in Pakistan. We also aim to compare our study with other published data on cSLE.
Materials and methods: Patients <18 years presenting to Shifa International hospital paediatrics' department diagnosed with childhood systemic lupus erythematosus according to EULAR/ACR 2019 criteria for SLE were evaluated. Clinical data with laboratory findings and treatment outcome was collected according to a structured proforma by interviewing patients/guardians and by reviewing patients' medical record.
Results: All participants were female with median age of 11 years (age range 4.5-15 years). The most prominent clinical presentation was fever followed by rash. Lupus nephritis was diagnosed in 50% patients, proteinuria being the most prominent presenting feature in them. Low haemoglobin level, low serum C4 levels and positive Anti-nuclear antibody were most common laboratory finding present in all patients followed by positive anti-nucleosome and anti-double stranded DNA antibodies.
Conclusion: Childhood onset systemic lupus erythematosus has variable clinical and laboratory presentation with a female preponderance. Fever, rash and lupus nephritis was the most frequent clinical presentation. Anemia, positive ANA followed by positive anti nucleosome and ds DNA antibodies were the most common laboratory abnormality.

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884 – P2.04.14

Detection of autoantibodies anti-RNP70 in the differential diagnosis and classification of connective tissue diseases

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Purpose: The clinical relevance of anti-U1-snRNP antibodies detection for the diagnosis of Mixed Connective Tissue Disease (MCTD) is known and is part of its diagnostic criteria, but, to date, the involvement of its 3 main antigens (RNP70, RNP-A and RNP-C) has not been established. Moreover, anti-U1-snRNP antibodies frequently appear in other connective tissue diseases such as Systemic Lupus Erythomatosus (SLE), Undifferentiated Connective Tissue Disease (UCTD), Overlap Syndrome (OS), Scleroderma (SC) and Dermatomyositis (DM). The aim of this study is knowing the clinical significance of the autoantibody anti-RNP70 in the diagnostic and classification of these connective tissue diseases and them clinical symptoms.
Methods: A retrospective study (2008-2023) in 146 patients with autoantibody anti-U1-snRNP with or without anti-RNP70 (determined by Line-Blot and confirmed by Fluoro-Enzyme Immunoassay (FEIA)) was conducted. Based on the clinical history 7 groups were classified: MCTD (24), SLE (51), UCTD (13), OS (9), SC (2), DM (2) and Unconfirmed Diagnosis and/or lack of follow-up (NA) (45). The clinical symptoms studies were: Raynaud's disease, hand edema, Pulmonary Hypertension (PHT) or Diffuse Interstitial Lung Disease (DILD), synovitis, myositis and kidney or central nervous system involvement.
Results: Anti-RNP70 antibodies were detected in 64 patients (43.8%). Their frequency distribution for each diagnostic groups showed statistically significant differences (p=0.00037): 19/24 MCTD (79.2%), 17/51 SLE (33.3%), 5/13 UCTD (38.5%), 7/9 OS (77.8%), 0/2 SC (0%), 1/2 DM (50%) and 15/45 NA (15%). Most of OS group with anti-RNP70 (6/7, 85.7%) had MCTD pathology along with SLE (3/6, 50%) or SC (3/6, 50%). Furthermore, in the symptom analysis, patients with MCTD pathology with positive anti-RNP70 presented PHT/DILD (40%) and/or hand edema (44%), while for those with MCTD pathology but with negative anti-RNP70 these symptoms were absent, thus implying statistically significant differences with p=0.032 and p=0.022 respectively.
Conclusion: In our population, the increase in the appearance frequency of anti-RNP70 antibodies could be used as a differential diagnostic marker of MCTD compared to other connective tissue diseases studied. Furthermore, the presence of these autoantibodies could be associated with a worse prognosis of the disease due to the greater frequency of appearance of PHT/DILD symptoms and hand edema.

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918 – P2.04.15

Clinical relevance of duodenal lymphogram as a diagnostic tool for celiac disease in adults

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Introduction: Celiac disease (CD) is an autoimmune pathology characterized by lesions in the duodenal mucosa triggered by gluten ingestion. Duodenal lymphogram analyzes the subpopulations of intraepithelial lymphocytes (IELs), including total IELs (tIELs), TCR-γδ+ IELs, and CD3- IELs, and has been proposed as a complementary test for the differential diagnosis of CD in adults.
Objective: To compare the duodenal lymphogram of celiac patients on a gluten-containing diet (GCD), celiac patients on a gluten-free diet (GFD), and non-celiac individuals (digestive pathology different from CD).
METHODOLOGY: 120 patients were recruited and divided into: I) celiac patients on GCD (n=16), II) celiac patients on GFD (n=27), and III) non-celiac individuals (n=77). Duodenal lymphogram study was conducted on duodenal biopsy samples using flow cytometry. Cut-off points were set as follows: tIELs ≥15%; TCR-γδ+≥15%; and CD3-≤6%. Statistical analysis was performed using non-parametric Kruskal-Wallis tests. Values were expressed as mean±standard deviation ($p \leq 0.05$).
Results: When comparing duodenal lymphogram subpopulations among the three groups, celiac patients on GCD showed significantly higher tIELs values compared to those observed in celiac patients on GFD (18.18±6.35 versus 13.48±6.31, p=0.05), and non-celiac patients (11.81±9.28, p=0.001). Comparing TCR-γδ+ values, the mean values obtained in celiac patients on GCD (31.93±12.72) and celiac patients on GFD (24.59±9.08) were significantly higher than in non-celiac patients (9.55±7.51) ($p<0.0001$). Conversely, CD3- values were significantly lower in celiac patients on GCD (2.93±4.43) and celiac patients on GFD (7.70±9.71) compared to those obtained in non-celiac patients (17.10±13.49) ($p<0.0001$).
Conclusion: The results emphasize the utility of duodenal lymphogram in the differential diagnosis of CD with other digestive pathologies. Diet is a crucial parameter to consider as some lymphocytic subpopulations are not altered by it (TCR-γδ+ and CD3-), while others are (tIELs).

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1002 – P2.04.16

Exploring the Impact of EBV Infection on T-bet Expression in B Cells and Its Influence on T Cell Activation and Migration

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Epidemiological evidence suggests that EBV infection in susceptible individuals triggers enhanced EBV-specific immune responses and asymptomatic CNS damage preceding multiple sclerosis (MS). However, the extent of cross-reactive immune responses and the mechanism by which EBV infection leads to CNS damage remain unclear. Studies on lymphocyte infiltrates in post-mortem MS brain tissue indicate that a subset of B cells expressing the T-box transcription factor (T-bet) may significantly influence CNS autoimmunity. Depending on genetic MS predisposition, EBV could potentially induce pathogenic T-bet^{+} B cells, making them a plausible contributor to this disorder. Therefore, our aim is to further elucidate the role of T-bet^{+} B cells that arise during EBV infection and their potential contribution to the pathogenesis of MS.
To investigate the impact that EBV infection has on T-bet expression in B cells, we mimic de novo infection in primary B cells *in-vitro* and monitor longitudinal changes of T-bet expression during the infection. To assess the functional capacity of EBV transformed T-bet^{+} B cells, T-bet^{high} and T-bet^{low} cells were separated from EBV transformed lymphoblastoid cell lines (LCL) and functional assays were performed.
We could show that EBV infection induces upregulation of T-bet expression in infected B cells during the early stages of infection *in-vitro*. With T-bet positivity reaching 30% percent in certain donors. Based on CXCR3 expression cells from the same donor could be sorted into a T-bet^{high} and T-bet^{low} fraction. Migration assay demonstrated that exposure to supernatant from LCL expressing high T-bet results in increased migration of total T cell numbers with preferentially activated T cells migrating compared to exposure to supernatant from low T-bet expressing LCLs.
These results indicate that EBV infection may initiate the formation of a T-bet^{+} B cell subset. This subset has the capacity to recruit activated T cells, and potentially act as antigen presenting cells promoting the development of cross-reactive and MS autoantigen-specific T cells in predisposed individuals, thereby contributing to MS pathology.

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1059 – P2.04.17

Zbtb20 as a regulator in the development and activation of the immune system in wild type and heterozygous animals.

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Zbtb20 is part of the Zbtb gene family. It plays a significant role in the individual development, as high genetic activity can be found during the late stages of astrocytes and chondrocytes genesis, during the formation of the thyroid gland, the liver regeneration and in the regulation of the circadian rhythms. Recent studies have shown the effect of the gene on the generation and differentiation of different lymphocyte populations. A Cre-lox recombinant mouse model was developed as the Zbtb20 gene was removed and some of the animals were complete knock-outs (homozygous, -/-). Animals with partially retained Zbtb20 activity (heterozygous animals, +/-) do not show visible changes and defects in the major systems and organs.
Heterozygous animals (+/-) were crossed in order to obtain 3 genetic types (+/+, +/- and -/-). Animals from the different genotypes were analyzed for a change in the quantity of the immune cells, their activating capacity and the histological structure of their primary and secondary lymphoid tissues. The purpose was to determine the function of the Zbtb20 gene in the development of the immune system and to define the effect of the zygosity on the immunological parameters.
Animals of different age and genotype were selected for the study. Sera were isolated and ELISA screening for IgA, IgG and IgM antibodies was performed. Flow cytometry of spleen and bone marrow was assayed for the CD19+, CD3+, CD4+, CD8+, Sca-1 and c-KIT surface markers. Cell proliferation analysis was made as well as cytological and histological evaluation of the immune organs.
Our results show different profile of the immune system in homo- (+/+) and hetero- (+/-) zigous animals. A difference in the Sca-1 and c-KIT cells populations was observed. Similar results were found regarding the proliferating capacity of the cells.
Based on the data we can speculate that different expression of the ZBTb20 gene may induce an abnormal response in conditions like autoimmunity and cancer.

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1073 – P2.04.18

Real-life implementation of multiple autoantibody detection for Connective Tissue Disease in a single referral center in Northern Spain

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Purpose: Connective tissue diseases (CTD) are driven by autoimmune processes that affect connective tissue, causing skin and organ damage. Most rheumatic diseases have a variable course, including from mild to severe affections. Therefore, the detection of multiple autoantibodies involved in CTD could help to appropriately stratify patients and disease management. The aim is to evaluate the activity of a fully automated digital system utilizing particle-based multi-analyte technology (PMAT) in the detection of autoantibodies.
Methods: During the first year of PMAT implementation, 3,985 tests were executed on 2,983 different patients, with a mean age ± 2 SD of 54.53 ± 35.29 years and a ratio female/male of 3.5/1. From the total of patients, 2,114 of them were tested only once and 779 were tested more than one time. All patients had previously an anti-nuclear test positive with an indirect immunofluorescence test using HEp-2 cells. Levels greater than 40 IU/mL for ds-DNA and 5 FLU for all extractable nuclear antigens (ENA) were considered as positive, as indicated by manufacturer. In addition, medical records of 83 patients with at least 4 different ENA specificities, and 86 patients with more than 20 FLU of anti-DSF70 (control group) were collected.
Results: Out of 2,983 patients, 1,896 (63.5%) tested negative for all the specificities, 239 (8%) were positive for anti-dsDNA, 859 for anti-DFS70 (28.8%) and 1017 (34.1%) patients were positive for ENA screening. The highest prevalence in ENA screening was anti-Ro60 (18.8%), followed by anti-Ro52 (11.4%), anti-RNP (10.8%), anti-La (7.9%), anti-Scl70 (6.7%), anti-CENP (5%), anti-RiboP (1.8%), anti-Jo-1 (0.5%) and anti-Sm (0.7%). The most frequent diagnoses in the group with multiple ENA specificities were systemic lupus erythematosus (50.6%) and Sjogren's syndrome (34.9%) whereas in the control group, 90.7% of patients did not have a CTD diagnosis. In both groups, the most frequent clinical manifestations involve the joints, skin and cytopenia.
Conclusions: CTD multiparametric test gives multiple information compared to single antigen assay which is important in the growing world of CTD that comprehends a wide range of diseases. Different specificities show different clinical subsets that will help to obtain a correct risk stratification and different clinical approaches.

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1109 – P2.04.19

The role of sclerodermatosis/myositis-related autoantibodies detected by immunoblot in the diagnosis of systemic autoimmune rheumatoid arthritis diseases in 410 patients from a single referral center

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Purpose: In clinical practice, immunoblot assays are being used more frequently as a diagnostic tool for systemic autoimmune rheumatic diseases (SARDs). The objective is to assess the contribution of extended analysis of autoantibodies (aAbs) related to scleroderma/myositis disorders found by immunoblot to the diagnosis of patients with SARDs.
Methods: From November 2017 to November 2023, we examined all medical and analytical records of patients at our center who had positive immunoblot results related to scleroderma or myositis. The high suspicion of SARDs in patients with nonspecific symptoms led to the request of these assays.
Results: 410 patients (127 men/283 women; mean age 58.21 ± 16.29 years) were positive for at least 1 aAb, 69 of them were positive for 2 aAbs. Main clinical features at the time of immunoblot requests were: arthralgia/arthritis (n=192), Raynaud's phenomenon (n=165), rash (n=64), myopathy (n=56) and sicca syndrome (n=50). During follow-up, 82 patients were diagnosed with overlap myositis, 75 with scleroderma, 52 with other inflammatory diseases, 41 with interstitial pneumonia with autoimmune features (IPAF), 36 with undifferentiated connective tissue disease (UCTD), 20 with Sjögren's syndrome, 18 with dermatomyositis, 17 with systemic lupus erythematosus and finally 1 with necrotizing myositis. In 68 patients the diagnosis of SARD was finally ruled out. Interstitial lung disease (ILD) was present in 133 patients, being particularly frequent in those with anti-PL12 (p=0.017) in comparison to the rest of the specificities. Cancer was detected in 35 (8.53%) patients, 20 of them were anti-Ro52+ (p=0.01). Furthermore, it was found that high creatine kinase (CK) levels correlated with the probability of being affected by myopathy (p=0.013).
Conclusion: For patients with a high clinical suspicion of SARDs, immunoblot assays are very helpful in the diagnosis process. While some aAbs, such as anti-Mi2 and anti-Th/To, remain to be nonspecific, other Abs including anti-PL12 and anti-Ro52 are particularly helpful in detecting SARDs patients with associated ILD and cancer, respectively.

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1236 – P2.04.20

The peripheral MAIT cell TCRα/β repertoires alter with age of psoriasis vulgaris patients.

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Purpose: Psoriasis vulgaris (PV) is a chronic autoinflammatory skin disease characterised by dysregulated T-cell activation. Adaptive αβT cells are regularly involved, but a role for innate-like, mucosal-associated invariant T (MAIT) cells has also been proposed. These unconventional cellular players act against non-peptide antigens in MR1-restricted manner, using semi-invariant TCRVα7.2 T cell receptor (TCR). Typically, a limited number of TRAV (TRAV1-2) and TRAJ (TRAJ33, TRAJ12 or TRAJ22) genes is used, together with a more diverse panel of TRBV/D/J segments. Novel evidence, however, supports a greater MAIT TCR diversity both in terms of alternative TRAV/J gene usage and higher TRBV clonality, but the relevance of these findings for PV remains to be established.
Methods: To this end, we used cell sorting (FACSAria) and bulk-RNASeq (Archer Immunoverse™HS TCR assay, NextSeq Illumina platform) with MiXCR, VDJTools and Immunarch pipelines to decipher TCRα/β repertoires of flow-sorted MAIT cells (MR1-5-OP-RU-tetramer$^+$ TCRVα7.2$^+$) from 26 PV patients and 12 healthy controls.
Results: In total, 12219 unique TRA and 60478 TRB clonotypes were observed in PV patients and controls, with similar and predominant expression of TRAV1-2-TRAJ33/20/12 and TRBV20-1/TRBV6-4/TRBV6-1/TRBV6-2/TRBV4-2 variants. The TCRβ chain was highly polyclonal and private, contrasting oligoclonal and frequently shared TCRα repertoires. No significant differences in diversity, V/(D)/J gene usage, and clonotype distribution were observed in case-control comparisons. Age, however, emerged as a negative predictor of TRA and TRB repertoire size in PV (TRA:Spearman's rho=-0.466, p=0.017; TRB:rho=-0.530, p=0.005), but not healthy subjects. A decline of the low-frequent clonotypic variants in PV (Efron-Thisted; TRB:rho=-0.55, p=0.003, TRA:rho=-0.47, p=0.015) underlined the age-related reduction of TRA/TRB variability. The diminished TRB diversity was further accentuated by an increased proportion of hyperexpanded clonotypes (rho=0.76, p=5.64E-06), occupying >5% of the total repertoire. Hyperexpanded TRB clonotypes in PV did not overlap between individual repertoires and displayed diverse CDR3 properties, supporting influence of individually unique epitopes in MAIT repertoire arrangement.
Conclusion: Our results support substantial diversity of MAIT TCRβ repertoire and highlight the influence of age in shaping the diversity of TCRα and TCRβ repertoires in psoriasis. The earlier onset of reduced MAIT TCR repertoire diversity in PV may signify complex underlying mechanisms, warranting further investigations.

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1308 – P2.04.21

Diagnostic efficacy of anti-SSB versus anti-SSA antibodies in Sjögren's Syndrome and other connective tissue diseases

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Purpose: In healthcare practice, anti-SSA is frequently found without clear clinical suspicion. The aim of this study is to assess whether the appearance of anti-SSB in patients with anti-SSA antibodies improves the effectiveness of differential diagnosis of Sd. Sjögren's versus other connective tissue disorders.
Methods: A retrospective study (2022-2023) in 241 patients with autoantibody anti-SSA (anti-Ro60 and/or anti-Ro52), of whom 62 also had anti-SSB specificity (determined by Fluoro-Enzyme Immunoassay and confirmed by Line- blot) was conducted. Based on the clinical history, patients were classified into 3 diagnostic groups: Sjögren's Syndrome (SS, n=112) (with or without other connective pathologies), Other Connective Tissue Diseases (OCs, n=61) and Absence of Connective Tissue Diseases (NCs, n=68). Within these groups, the frequency of occurrence of the different specificity combinations was studied, being: anti-Ro52 with and without anti-SSB (3 and 53, respectively), anti-Ro60 with and without anti-SSB (3 and 44, respectively) and anti-Ro60/Ro52 with and without anti-SSB (56 and 82, respectively).
Results: The frequency of appearance of anti-SSA or anti-SSA along with anti-SSB antibodies in each diagnostic group showed statistically significant differences (p=0.000023). Thus, patients with anti-SSA along with anti-SSB had SS 69.4%, OCs 21% and NCs 9.7%, while those without anti-SSB had SS 38.5%, OCs 26.8% and NCs 34.6%. When examining the distribution of anti-SSA specificity (anti-Ro52, anti-Ro60, or anti-Ro52/Ro60) along with anti-SSB in each diagnostic group, statistically significant differences were found (p=0.00015). The SS diagnosis was made in 69.6% of patients with anti-Ro52/Ro60 and anti-SSB, and in 100% of patients with anti-Ro52 and anti-SSB. Those those with anti-Ro60 and anti-SSB showed a uniform frequency of occurrence in each diagnostic group (33.3%).
Conclusion: In our population, the isolated detection of anti-Ro60 and/or anti-Ro52 antibodies is not always associated with the diagnosis of connective tissue diseases. The presence of anti-SSB along with anti-SSA antibodies has demonstrated greater relevance for the diagnosis of Sjögren's syndrome than the presence of anti-SSA alone or along with other autoantibodies. This may entail an increase in unnecessary referrals to specialist consultations, which could be avoided if requests for antinuclear antibodies were more closely aligned with the patient's clinical presentation.

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1310 – P2.04.22

The TCR repertoire of circulating γδ T lymphocytes in psoriasis vulgaris varies with age and disease severity

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Purpose: Psoriasis vulgaris is a recurring autoinflammatory skin disease that often manifests systemically. The underlying immune aberrations are predominantly driven by T cells, with emerging evidence suggesting a potential role for innate-like γδ T cells. Healthy peripheral γδ T cell repertoires are largely composed of Vγ9Vδ2 cells, yet alterations in the diversity and composition of the γδTCR repertoire in PV remain largely unknown.
Methods: We applied bulk TCRSeq analysis (Archer Immunoverse™-HS TCR, NextSeq Illumina coupled with MiXCR, VDJTools and immunarch pipeline) to comparatively investigate the characteristics of the TCRγ and TCRδ repertoires from flow-sorted, peripheral γδ T cells (20 PV patients and 15 healthy controls).
Results: A largely public TRG repertoire was discovered alongside a highly polyclonal and private pool of TRD transcripts, with CDR3δ length varying with TRDV1 (15-23 amino acids), TRDV2 (14-19 aa) and TRDV3 (13-23 aa) gene expression. Common TRDV2/TRDD3/TRDJ1 and TRGV9/TRGJP transcripts were predominantly expressed in both groups, while TRDV1, TRDV3 and various TRGV/J expressing clonotypes dominated the rest of the repertoire, without significant differences in case-control comparisons. The TRD/TRG repertoire diversity however, significantly declined with increasing age and PASI index in the PV group, largely due to incremental increase in hyperexpanded clonotype frequencies (comprising > 5% of the repertoire), ultimately affecting TRG/TRD clonotype richness and evenness. The variety of TRDV1 and TRDV3 clonotype compartments was significantly altered in PV, likely reflecting TRDV1 clonotype expansion (Inverse Simpson Index; median (PV vs. CTRL): 3.71 vs. 7.20, p=0.037, Mann-Whitney test) and reduced TRDV3 clonotype heterogeneity (Shannon-Wiener Index; 3.91 vs 9.85, p=0.036). Moreover, an age-related increase in CDR3δ length was notably evident in the TRDV1 compartment of severely affected PV patients (Spearman's rho= 0.828, p= 0.006), suggesting potentially important effects of age and PV on TCRδ repertoire shaping. Healthy controls, conversely, exhibited increased TRDV1 and TRDV3 diversity with age.
Conclusion: Our findings underscore a complex, multilayered influence of age and disease severity on γδ TCR repertoire diversity and CDR3 characteristics, particularly in the TRDV1 and TRDV3 compartments. This intricate interplay between ageing, disease severity, and TCR repertoire dynamics in PV emphasizes the need to further investigate the underlying mechanisms.

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1318 – P2.04.23

Clinical correlation between anti-centromere antibodies (CENP-B) and autoimmune diseases

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Purpose: Anti-centromere antibodies (ACA) are useful biomarkers in Limited cutaneous systemic sclerosis (lcSSc) and can precede its clinical diagnosis by years. They are also found in other autoimmune diseases (AID) like primary biliary cirrhosis (PBC), Sjogren's disease (SjD), lupus erythematosus, rheumatoid arthritis, isolated Raynaud phenomenon and in subjects without AID.
Our aim is to describe the clinical relationship, in our setting, between ACA (CENP-B) and the presence of AID.
Methods: In a retrospective study, 473 patients (389 female, 48 male) positive for CENP-B (CENP-B+) were reviewed (2008-2023). CENP-B was detected using indirect immunofluorescence (IFI) on HEp-2000 cells and confirmed by LineDot. The data were collected from their medical record.
Results:

- 25.6% of patients were diagnosed with lcSSc. Nine were diagnosed during the follow-up (average time: 5.4 years after the initial detection). The rest were already symptomatic when the first determination was made.
- There are two peaks in CENP-B detection, between the ages of 51-60 (18.8%) and 71-80 (20%).
- The highest proportion of lcSSc patients is found between the ages of 61-70 (32.9%). The percentages are similar across age groups from 30 to 80, ranging between 27% and 32.9%, decreasing at extreme ages.
- 13.7% of patients have another AID besides lcSSc. The most frequent are PBC (5.6%) and SjD (3.7%). 21 out of 26 patients with PBC are positive for antimitochondrial antibodies. 12 out of 16 patients with SjD are positive for SSA.
- 57.9% of patients have no AID. 42% CENPB+ patients have not been evaluated by a clinical specialist, or it is not reflected in the medical record.

Conclusion: CENP-B predominantly manifest in middle to advanced-aged individuals. While the majority of them are asymptomatic, this age group also experiences a higher prevalence of lcSSc diagnoses. In our setting, CENP-B also manifest, albeit less frequently, in other AID. ACAs have proven to be predictive of lcSSc, so perhaps asymptomatic patients should receive follow-up. A significant proportion of patients without AID have not undergone autoimmune disease screening. Considering the significant number of asymptomatic individuals, it is important to request antibody determination based on clinical suspicion.

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1320 – P2.04.24

Impact of COVID-19 on the prevalence of ANA positivity within the Indian population

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Purpose: Since the Covid 19 infection, the prevalence of Antinuclear Antibodies (ANA) positive has grown. By comparing ANA data from six different years - 2017 to March 2020(pre-Covid years), and April 2020 to March 2023 (post-Covid years) - this study examines the ANA positivity rate.
Methods: ANA Indirect Immunofluorescence Assay (IFA) data for six different years - April 2017 to March 2020, representing the pre-COVID era, and April 2020 to March 2023, representing the post-COVID era are analyzed and compared in this retrospective study based on a number of characteristics, such as age, gender, prevalence rate, positivity rate by grade, and ANA patterns.
Results: Both the overall number of suspected cases and the number of ANA-positive cases increased significantly in the post-Covid era April 2020 to March 2023, rising by almost 20%. Age-related increases in positivity rates were seen, and both years showed a preponderance of females. In both eras, nuclear speckled patterns continued to be the most prevalent.
Conclusion: Our study underscores the significant role of immune modulation in the development of autoimmunity during the post-COVID pandemic period. This modulation may occur through mechanisms such as Molecular Mimicry, the production of autoantibodies upon exposure to viral epitopes through the formation of neutrophil extracellular traps (NET), or Toll-like receptor (TLR) pathways of immune modulation.

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1433 – P2.04.25

Diagnosis of antiphospholipid syndrome based on 2023 ACR/EULAR versus 2006 Sydney criteria, in a population of patients from Cádiz, Spain

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Purpose: Antiphospholipid Syndrome (APS) is an autoimmune thrombophilic condition with vascular and obstetric manifestations and anti-phospholipid autoantibodies (apL Ab). A descriptive cross-sectional study was conducted to compare the number and clinical features of patients who meet the criteria for APS based on the 2023 ACR/EULAR classification vs the previous 2006 Sydney classification.
Methods: The population submitted to our Hospital for analysis of apL Ab during 2022 and 2023 was included in the study. Laboratory parameters (lupus anticoagulant, anticardiolipin antibodies and anti-B2-glycoprotein I antibodies) were determined and clinical data were registered. Anticardiolipin and anti-B2-glycoprotein I immunoglobulin G and M (IgG and IgM) antibodies were determined by chemiluminescence. Values above 20 IU/mL was considered moderate-positive and values above 200 IU/mL were considered high-positive. According to ACR/EULAR criteria, a total score ≥ 3 in both laboratory and clinical domains is required to classify a patient as having definite APS.
Results: From 302 patients who had at least two laboratory determinations separated by at least 12 weeks, 157 (52%) did not meet the ACR/EULAR or Sydney criteria; a total of 145 (48%) patients were evaluated following the 2023 and 2006 APS criteria. 46 (31.7%) patients met the Sydney classification criteria -average age 51.4 years, 33 (71.7%) females and 13 (28.3%) males-. However, only 26 (17.9%) patients met the ACR/EULAR classification criteria -average age 47.8 years, 19 (73%) females and 7 (27%) males-.
Conclusion: According to the new ACR/EULAR APS criteria, 20 patients (43.5 % of our cohort) previously diagnosed with APS following the Sydney classification did not meet the required score to be classified as APS.
This result depended mainly on the lack of real value of IgM apL Ab for APS diagnosis according to the 2023 criteria, because these 20 patients were found to have moderate and high titres of IgM apL Ab, but not IgG apL Ab.
In summary, a detailed analysis should be performed in order to not misdiagnose primary or secondary APS according to 2023 ACR/EULAR classification criteria.

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1468 – P2.04.26

Establishing the optimal cut-off value to detect clinically relevant figures of anti-citrullinated protein antibodies for the diagnosis of rheumatoid arthritis

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Introduction: Anti-citrullinated protein antibodies (ACPA) present a good sensitivity/specificity balance for the diagnosis of rheumatoid arthritis (RA). Its determination was included in the 2010 ACR/EULAR diagnostic criteria. These criteria assign 2 points to those 'low-positive' ACPA values -higher than the upper limit of normal (ULN)- while 3 points are assigned to 'high-positive' ACPA values -higher than 3 times the ULN-.
Purpose: To determine the optimal cut-off value for ACPA that allows us to detect clinically significant high-positive values more accurately in a cohort of ACPA-positive patients.
Patients and methods: A cohort of 168 patients from the Hospital U Puerta del Mar, Cádiz, Spain with positive ACPA results by chemiluminescence (reference values: 0.0-5.3 U/ml) were included in the study. Rheumatoid factor (RF) was determined by immunoturbidimetry. Clinical features were registered. Through ROC curve analysis, the optimal cut-off value was determined. Objective values, likelihood ratio and Cohen's κ coefficient with RF were calculated and compared with those obtained with the theoretical cut-off value outlined in the 2010 ACR/EULAR criteria, who recommends 3 times the manufacturer's recommended ULN.
Results: An optimal cut-off value for ACPA of 38.3 U/ml was obtained, showing a specificity (S) and positive predictive value (PPV) of 91% and 95% respectively. Additionally, this value presents a positive likelihood ratio (+LR) of 8.56 and a Cohen's κ coefficient of 0.4, indicating substantial agreement between ACPA and RF determinations. These values were higher than those obtained using the theoretical cut-off value of 3 times the ULN (16.0 U/ml) which showed a lower S (61%), PPV (82%), +LR (2.26) and Cohen's κ coefficient (0.3).
Conclusion: Our results indicate that a cut-off value 7.2 times the ULN has a high specificity and positive likelihood ratio for detecting ACPA-positive values of clinical significance for the diagnosis of RA.
In summary, our results highlight the importance of determining in each laboratory optimal cut-off and +LR values to improve diagnostic accuracy.

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1624 – P2.04.27

A RARE CASE OF RHEUMATOID ARTHRITIS AND SARCOIDOSIS CO-OCCURRENCE

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Introduction: Rheumatoid arthritis (RA) and sarcoidosis are autoimmune diseases that can affect multiple organ systems, including the lungs. While pulmonary involvement is common in both conditions, the coexistence of RA and sarcoidosis is rare. This case introduces a patient with RA who developed symptoms of sarcoidosis following treatment with anti-TNF medication.
Case presentation: A 42-year-old male with a 22-year history of RA initially received conventional disease-modifying antirheumatic drugs. Years back while being treated with methotrexate, hydroxychloroquine, and mycophenolate, RA progressed systemically, leading to a initiation of anti-TNF therapy. Subsequently, he developed enlarged mediastinal lymph nodes and nodular lung infiltrates. Sarcoidosis was diagnosed based on non-caseating granulomas in transbronchial lung biopsy specimens. Possible diagnosis of anti-TNF related sarcoid like reaction, anti-TNF treatment was stopped and he continued RA medications with short term low dose steroids. During the current presentation, the patient reported he has discontinued his RA medications and claimed to have been in remission for several years. He admitted to our clinic with a severe cough, sputum and shortness of breath. With mediastinal enlarged multiple lymph nodes, bilateral lung infiltrates and with previously diagnosed biopsy proven sarcoidosis, he started methylprednisolone 1mg/kg. His symptoms were revealed consequently after the steroid treatment.
Discussion: The co-occurrence of RA and sarcoidosis is rare, making this case noteworthy. The unique aspect of this case is the onset of sarcoidosis symptoms following treatment with anti-TNF medication. Unlike typical anti-TNF-related sarcoid-like reactions, the patient's lung involvement persisted even after discontinuation of anti-TNF therapy. This case underscores the importance of recognizing the rare coexistence of RA and sarcoidosis. Increased awareness of this rare association can aid in timely diagnosis and appropriate management of affected individuals.

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1701 – P2.04.28

Characterization of a model of vitiligo skin depigmentation in C57BL6/J mouse tail via immunisation with tyrosinase-related protein 2 (TRP2)

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Vitiligo is an autoimmune skin disorder which affects 0.5-1% of the general population. Vitiligo and the associated depigmentation is mediated by CD8+ T-cell immunogenicity against melanocytes. Based on this mechanism, various mouse models have been developed. We characterized an existing vitiligo model, induced by immunisation with melanocyte tyrosinase related protein 2 peptide 180-188 (TRP2), LPS and CpG oligodeoxynucleotides.
C57BL6/J mice received 4 immunisations with all the above, once a week. Tail skin depigmentation was evaluated by visual scoring. As JAK inhibitors (JAKi) are efficacious in vitiligo, mice were treated with oral baricitinib from wk 9 after the last immunisation, when at least 20% of tail skin was depigmented. Abundance of total CD8+ and T resident memory cells (TRMs) was monitored in the skin by spectral flow cytometry, at different time-points after immunisation (wks 1, 5, 9 and 17). Gene expression analysis was performed on tail skin homogenates on wk 17, by real-time qPCR.
TRP2-induced mice developed skin depigmentation on the tail from wk 5, which progressed to a 20%-30% average depigmentation on wks 7 to 9. Increased CD8 T-cell infiltration was seen in vitiligo mice from wk1 compared to control mice (26% *vs* 2% of total CD45+), and was still evident at wk17 (8% *vs* 1%). The % of CD8+TRM was also constantly higher than in control mice. Murine skin which was actively depigmenting (adjacent to white areas) showed a similar gene expression profile to human vitiligo skin. This included higher expression of mediators with a documented importance in human vitiligo such as CD8a, Cxcl10, IFNg, Gzmb and TNF. The JAKi, baricitinib partially decreased depigmentation (25% reduction) and resulted in lower inflammatory gene expression, however, no reduction in infiltrating CD8+ T-cells was seen. In conclusion, our results show that this model recapitulates several features of human vitiligo and responds to a JAKi, making it suitable for evaluating novel drugs for vitiligo.

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1706 – P2.04.29

The impact of peripheral T follicular cells in the diagnosis of ANCA associated glomerulonephritis

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Purpose: T cells play crucial role in the pathogenesis of autoimmune diseases such as ANCA associated vasculitis-glomerulonephritis (AAV/GN) by secreting immune mediators and helping B cell-mediated long-lived humoral immunity development. Specific T and B cells subsets can be used as biomarkers of patients with AAV/GN. We aimed to prospectively compare the predictive ability of such subpopulations in patients with active AAV/GN.
Methods: Flow cytometry was applied in the peripheral blood of 29 subjects. 15 AAV/GN patients (M/F 6/9), Age:62.1(28-82)years, MPO/PR3/Negative ANCA: 10/4/1) and 14 healthy controls (HC), to estimate T-cells subsets: CD4, T-folicular cells (Tfol), T-regulatory cells (Tregs), Tfh, Tfh1, Tfh2, Tfh17, and T-folicular regulatory cells(Tfr) and B-cells subsets: CD19, IgD(+)CD127(-), IgD(+)CD127(+), IgD(-)CD127(+) and IgD(-)CD127(-).
Results: Compared to HC, AAV/GN patients had reduced CD4 (698.8 vs 368.8, p=0.04) but increased proportion of Tfol, and both Tfh1 and Tfh2 cells (7% vs 12%, p=0.05, 8.4 vs 11.2, p=0.03, 13.3 vs. 45.9, p=0.09, respectively). Both Tfr (p=0.048) and Tfh (p=0.023) were also significantly higher in AAV/GN, compared to HC.
CD19 levels were lower in AAV/GN compared to HC, with reduced all subpopulation, though of not-statistical significance.
Conclusion: Specific T-cells subsets can be measured in AAV/GN patients' peripheral blood. Subpopulations such as Tfh and Tfr can be used as biomarkers to diagnose patients with active disease and their role in the pathogenesis of AAV/GN must be studied in detail to determine if they can become targets for new therapies in the future.

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1750 – P2.04.30

Genetic landscape of ulcerative colitis: possible novel genetic variants in MST1 in two familial clinical cases

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Ulcerative colitis (UC) is a chronic inflammatory disorder of the intestinal tract secondary to immune dysregulation. In some cases, the pathology is associated with autoimmune liver diseases, such as primary sclerosing cholangitis (PSC), autoimmune hepatitis (AIH) or their combination.
In the present study, two fourth-degree related individuals (cousins) with UC associated with AIH were used as a starting point. The family history included multiple autoimmune diseases in the common kinship line (vitiligo, psoriasis, rheumatoid arthritis, AIH and systemic lupus erythematosus).
The objective was to identify possible common gene variants in both individuals predisposing to the common clinical picture. For this purpose, a massive next-generation sequencing study was carried out with a panel of genes involved in diseases related to the immune system, as well as HLA typing to identify associated alleles.
This study revealed ten common SNP variants located in the *MST1* gene (chromosome 3, NC_000003.12), all of them in heterozygosis. Of all the variants observed with possible clinical relevance, only one was previously described and reported as a variant of uncertain significance (*MST1*:c.767A>C). Of the remaining variants, two of them were *missense* variants (*MST1*:c.55C>T; *MST1*:c.3781G>C), and the remaining one was a +3 variant of a splicing donor site (*MST1*:c.94+3A>G). Non-shared variants in the *CARD9* gene, associated with UC and PSC, were also observed in both patients.
Regarding the HLA allele study, it revealed that both patients shared a haplotype. In addition, both had inherited risk alleles of the *DRB1* locus for UC (*DRB1*07:01* and *DRB1*13:01*) from the non-common line of descent.
These preliminary results point to the possibility of the existence of as yet undescribed variants in *MST1* with very low population frequency that, together with other genetic risk factors, have defined the clinical picture in a familial series with clustering of autoimmune diseases. The next steps lead to the sequencing of the progenitors of the studied individuals of the common kinship line, as well as the determination of the serum levels of the gene product to determine if there may be an impact in the MSP-RON pathway.

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Increase in the concentration of granzymes, perforin and granulysin in patients with systemic lupus erythematosus

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Purpose: Systemic lupus erythematosus (SLE) is a chronic autoimmune disease. Although their prognosis has improved over the last decades, the mortality rate in these patients is 4.5 times higher than in the general population, so it is important to know the molecular, cellular, and immunological mechanisms involved in the development of the disease. CD4 and CD8 T cells play a relevant role in SLE; they present greater hyperreactivity and secretion of proinflammatory cytokines, contributing to tissue damage and exacerbation of the disease. The activation of CD4 T cells promotes their differentiation into cell populations such as Th1, Th2, Th17, producers of IFNγ, IL-4 and IL-17 respectively. While CD8 mainly secrete IFNγ, granzyme B (GZMB), perforins and granulysin. Deregulation of the secretion of these cytokines in SLE has been explored in patients and animal models. However, studying their prevalence together and in relation to disease activity may result in the differentiation of pathological subpopulations of T cells, such as exhausted T cells.
Methods: Peripheral blood samples were collected from patients with active and inactive SLE. For serum determination of cytokine concentration, the commercial LEGENDplex™ Human CD8/NK Panel (13-plex) kit was used. Cellular events were acquired using the MACSQuant Analyzer 10 cytometer (Miltenyi Biotec, Germany), and the results obtained were analyzed with LEGENDplex software. As a control group, samples were obtained from healthy individuals matched by age and sex with respect to the SLE group.
Results: In this study we investigated the concentration of 13 cytokines (IL-17A, IL-2, IL-4, IL-10, IL-6, TNF-α, Fas, FasL, IFN-γ, Granzyme A, Granzyme B, Perforin, Granulysin) related to the immune response of patients with SLE. We studied the behavior of cytokines in patients with active and inactive SLE and identified a subset of SLE patients with increased levels of granzymes, perforin and granulysin compared to healthy subjects.
Conclusion: Chronic inflammation in SLE promotes exposure to autoantigens that can maintain the constant activation of autoreactive clones of T cells. The study of the cytokines addressed here could be important for monitoring the possible cell populations involved in the production of these, as well as for patient monitoring.

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1788 – P2.04.32

Stratification of inflammatory arthritis using immunophenotyping

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This project aims to enhance early diagnosis of inflammatory arthritis (IA) subtypes, such as rheumatoid arthritis, psoriatic arthritis, osteoarthritis and spondyloarthritis. IA subtypes may be difficult to differentiate early in the diagnostic workup with current diagnostic parameters, while delayed targeted treatment can lead to rapid joint destruction. This project aims to utilize immune-cell phenotyping of synovial tissues from inflamed joints to stratify IA subtypes.
We are collecting peripheral blood, synovial fluid and biopsies from patients referred to a large outpatient clinic that receives the majority of undiagnosed IA patients in Oslo (Diakonhjemmet Hospital). Our study will include a minimum of 10 patients from each IA subtype and will leverage a novel multi-parametric spectral analyzer and a >35-parametric self-designed panel that includes T-cell, B-cell, and myeloid cell markers for deep immunoprofiling.
By correlating the immunological profiles with clinical diagnoses and disease severity, we hope to identify unique immunophenotypic patterns specific to each IA subtype. The comparison of immune composition across synovial fluid, synovial biopsy, and blood will not only provide a more comprehensive picture but also provide information on which sample type provides the best diagnostic yield. To develop a clinically relevant diagnostic test, we will assess whether a smaller flow-cytometric panel, based on key markers from the initial findings, can be used to stratify the IA subtypes.
In conclusion, our ultimate goals are to use these immunopathological signatures to stratify IA subtypes, with a particular focus on the underutilized synovial fluid samples, and to improve personalized treatment approaches in IA.

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1837 – P2.04.33

Explorative study on the role of EBV-mimicry autoantibodies in Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) and post-COVID syndrome

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Purpose: Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) is a debilitating multi-systemic chronic disease. Patients suffer from worsening symptoms after mild to moderate exertion named post-exertional malaise (PEM). Key symptoms are fatigue, pain, cognitive, autonomic, and flu-like symptoms. ME/CFS is triggered by various infections, e.g. EBV or SARS-CoV-2. There is evidence for autoimmunity in a subset of patients. The role of autoantibodies (AAB) including regulatory G protein-coupled receptor AABs was shown in previous studies, and underpinned by first clinical studies of AAB depletion. In the BMBF-founded research network (IMMME), we aim to investigate the role of AABs in ME/CFS and post-COVID syndrome (PCS).
Methods: To investigate the serum IgG response against EBV and potential human mimicry epitopes, we developed a novel cytometric bead array (peptide-CBA). Thereby peptide-coated beads allow a multiplex high throughput cytometric analysis of up to 15 peptide-specific IgG responses in one measurement. In the screening cohort, 44 PCS patients with 25 of them fulfilling the diagnostic criteria for ME/CFS, 34 post-infectious ME/CFS patients, and 31 healthy control subjects (HC) diagnosed at the Charité Fatigue Center were analyzed.
Results: PCS and ME/CFS patients showed more frequent serum IgG responses against several EBV peptides and the corresponding mimicry peptide sequences of human autoantigens compared to HCs. Furthermore, the level of serum IgG against mimicry epitopes correlates with disability severity assessed by Bell score, the restriction in physical functioning measured by SF-36, and the severity of the disease and several key symptoms, pointing towards a functional role of these antibodies.
Conclusion: The correlation shown between the level of peptide-specific IgG response in PCS and ME/CFS patients with disease severity provides first evidence for the role of EBV mimicry and that these AABs may contribute to pathological processes in ME/CFS and PCS, which needs to be investigated in further studies. Moreover, the diagnostic potential of mimicry peptide-specific IgG patterns has to be studied in larger cohorts.

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1971 – P2.04.34

Using genetic risk and EBV infection status to decipher the disease-inducing B cell in Multiple Sclerosis

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Purpose: Although the cause of multiple sclerosis (MS) remains unknown, several single nucleotide polymorphisms (SNPs) linked to disease susceptibility could affect B cell homeostasis in MS. This may be shaped by the B cell-lymphotropic Epstein-Barr virus (EBV). Besides for EBV-infected T-bet$^+$ B cells, a novel subset of B cells expressing cytotoxic molecule granzyme B (GzmB) and transcription factor Runx3 has been recently implicated in MS. Notably, the coding SNP *Runx3* is associated with MS. The goal of this work is to elucidate the interaction between EBV and genetic variants and how this influences T-bet$^+$ B cells to identify pathogenic signatures for MS.
Methods: We analysed anti-EBV (EBNA1, VCA, EA) IgG levels in 706 serum samples from MS patients and 107 controls. Genotyping was performed on 432 MS patients, followed by imputation and weighted genetic risk scores (wGRS), including several P-value thresholds with the discovery GWAS study (IMSGC, 2019), resulting in 175 SNPs. We performed functional analysis of Runx3 and GzmB in *ex vivo* human B cells using spectral cytometry and BCR- and T helper cell-dependent *in vitro* cultures.
Results: We found an association between the risk SNP *Runx3* and the anti-EBNA1 IgG levels in patient serum (p=0.02). Furthermore, we found an upregulation of GzmB in approximately 50% of naïve B cells after 2 days, especially under BCR-stimulating conditions. This was a transient effect, which coincided with both Runx3 and T-bet expression. To assess the overall impact of genetic variants on B cells, we performed wGRS and we can stratify the MS patients based on EBV infection and genetic risk profiles.
Conclusion: These findings suggest that GzmB expression is transiently induced when naïve B cells first encounter an antigen. Our current working model consists of a primary EBV infection potentiates this effect by triggering Runx3 and T-bet expression, which may increase their survival and further maturation into pathogenic subsets before MS onset. Integration of these data with RUNX3 genotype, wGRS and EBV infection status may further characterize the impact of the genetic variants on GzmB expression in T-bet+ B cells.

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1989 – P2.04.35

Identification of Epstein-Barr Virus microRNAs in blood in multiple sclerosis

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Purpose: Multiple sclerosis (MS) is an immune-mediated disease. Epidemiological studies indicate that Epstein-Barr Virus (EBV) infection is necessary for development of MS, but other factors are also involved. The EBV genome encodes 44 microRNAs (miRNA), which are molecules of ~20 nucleotides that regulate mRNA with hundreds of targets. MiRNAs are found in immune cells and their secreted exosomes, enabling systemic distribution. The role of ebv-miRNAs in MS is currently unknown, we therefore investigated ebv-miRNAs in MS patients with high and low disease activity, their correlation with biomarkers of inflammation, and the effect of MS treatments on ebv-miRNA.
Methods: Total RNA was extracted from whole blood (WB) and plasma exosomes (PE) from 50 treatment-naïve relapsing-remitting MS (RRMS) patients at baseline and one-year follow-up. Forty-three ebv-miRNAs were measured by qPCR using custom TaqMan Array Cards. Granzymes and cytokines were measured in WB by qPCR using TaqMan Assays. Serum IL-6 and neurofilament light (NfL) was measured by SIMOA. Age-adjusted NfL levels and presence of active magnetic resonance imaging brain lesions classified high and low disease activity. FDR-adjusted q-value<0.1 for correlations and p-value<0.05 for group comparisons was accepted.
Results: In the low compared to high disease activity groups, significantly higher levels of ebv-miR-BART20-5p and ebv-miR-BART11-3p in WB, and ebv-miR-BART5-3p in PE were observed, while opposite for ebv-miR-BART12 in PE.
In WB, positive correlations were observed between granzyme A (*GZMA*) and ebv-miR-BART19-3p; granzyme H (*GZMH*) and ebv-miR-BART20-5p; interleukin-10 (*IL-10*) and ebv-miR-BART20-5p; IL-6 and ebv-miR-BART3-5p; interferon- γ (*IFN-γ*) and ebv-miR-BART19-3p, while a negative correlation was found between *IL-10* and ebv-miR-BART12. In PE, positive correlations were found between *GZMA* and ebv-miR-BHRF1-3p; *GZMH* and ebv-miR-BART8-3p; *IL-1β* and ebv-miR-BART10-3p, and negative correlations were observed between *GZMH* and ebv-miR-BART13-5p; and ebv-miR-BART14-3p; IL-6 and ebv-miR-BART11-5p; *IFN-γ* and ebv-miR-BART13-5p. Furthermore, Teriflunomide significantly lowered ebv-miR-BART8-3p (n=13).
Conclusion: The profiling of ebv-miRNAs in exosomes and WB indicate a role in MS pathogenesis. Correlations with granzymes and inflammatory cytokines suggest ebv-miRNAs involvement in the activity of cytotoxic T and NK cells. The results also indicate anti-viral effects of Teriflunomide affects the levels of ebv-miRNA, and the presence of exosomal ebv-miRNAs shows a high regulatory potential.

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2152 – P2.04.36

Anti-Ro52 positivity is associated with poorer lung function at diagnosis in connective tissue disease and idiopathic inflammatory myopathy associated interstitial lung disease

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Purpose: Recent cohort studies suggest an association with anti-Ro52 antibodies and more progressive ILD in CTD-ILD and IIM-ILD. This study investigates the significance of anti-Ro52 positivity in an Irish cohort of these patients.
Methods: This is a monocentric 7-year retrospective cohort study of CTD-ILD and IIM-ILD patients. 781 extended myositis panel results were screened from 2016 to 2022 to identify a suitable cohort. PFT data was analysed to explore the significance of anti-Ro52 positivity.
Results: 41 CTD-ILD and IIM-ILD patients (19 male, 22 female) were included. The mean age was 66.7 (std +/- 13.2) and a mean disease duration was 4.4 years (std +/- 2.6). 14/41 patients were anti-Ro52+. The anti-Ro52+ cohort had a significantly lower baseline DLCO at diagnosis; 41.70% vs. 55.41% (p = 0.009). Baseline FVC predicted was numerically lower in the anti-Ro52+ cohort but did not reach statistical significance; 75.78% vs. 92.83% (p = 0.083). After standard of care treatment, the anti-Ro52+ cohort had poorer FVCs; 72.16% vs. 97.06% (p = 0.028). There was no difference in all-cause mortality with 3 deaths among the anti-Ro52+ cohort and 4 among the anti-Ro52- cohort (p = 0.673). No associations between anti-Ro52 status and smoking, malignancy or ILD pattern on CT were found. Patients under 65 presented with lower baseline FVC compared with those over 65 irrespective of anti-Ro52 status; 75.45% vs. 95.6% (p = 0.026).
Conclusion: Anti-Ro52 antibodies are associated with a lower baseline DLCO in CTD-ILD and IIM-ILD patients. Patients under 65 also presented with a more markedly reduced FVC than more elderly patients, perhaps reflecting the fulminant nature of disease onset in this demographic.

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2157 – P2.04.37

Investigating biological age and ELOVL2 expression in ANCA-associated vasculitis

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ANCA-associated vasculitis (AAV) is an autoimmune disease that effects the small blood vessels resulting in systemic inflammation and pathologies of the kidney, lung, and skin. AAV tends to effect people later in life than other autoimmune conditions, and occurs more often in people assigned male at birth. Within an aging immune system, genetics and lifetime environmental exposure drive the process of Inflamageing, where a key feature of this process is epigenetic changes. These epigenetic changes can be used to predict "biological age", a measure of how cellular processes associated with aging change as this process takes place. The links between these phenomena and autoimmune disease have not been fully explored, and specifically the role biological age plays in relation to the pathogenesis of AAV has not yet been described. Furthermore, determining whether this process is a sex-specific or sex-determined factor will be essential for improving our understanding of the factors driving AAV disease pathogenesis, relapse, and remission. In as-yet unpublished work within the Bourke lab, the biological age of AAV patients has been quantified with an ELOVL2 DNAm model, and demonstrated to be accelerated when compared to healthy controls, with treatment modulating this acceleration. Additionally, the expression of ELOVL2 as measured with qPCR has been demonstrated to be downregulated in active AAV patients when compared to healthy controls. Herein, we demonstrate that this difference is specific to disease state, where active AAV patients demonstrate significantly lower ELOVL2 expression compared to their remission counterparts, as well as to healthy controls. Additionally, we demonstrate that there is no significant sex difference in ELOVL2 expression in active nor remission AAV patients. This work suggests that biological age may be contributing to the pathogenesis of AAV, and consequently ELOVL2 expression changes may be specific to AAV disease state. More research is required to tease out the potential mechanisms by which biological age may be contributing to AAV pathogenesis, how the effect of treatment relates to both biological aging and AAV pathogenesis, and to investigate whether this phenonomen is AAV specific.

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2162 – P2.04.38

The presence of myositis specific and myositis associated antibodies does not alter prognosis in idiopathic pulmonary fibrosis

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Purpose: Myositis specific antibodies (MSA) and myositis associated antibodies (MAA) can be detected in pulmonary involvement in systemic autoimmune rheumatic diseases. The prognostic effect of these antibodies in idiopathic pulmonary fibrosis (IPF) however remains unclear.
Methods: This is a monocentric 7-year retrospective cohort study of IPF patients from 2016 to 2022. IPF patients with MSA/MAA antibodies were age and sex matched 1:1 to IPF patients without antibodies. The 2 groups were analysed by PFTs, all-cause mortality, and presence of malignancy.
Results: 17/109 (15.6%) IPF patients (12 male, 5 female) with MSA/MAA antibodies were identified. Anti-Mi2-β was detected in 6/17 (35.3%). The mean age at diagnosis was 71.9 (std +/- 8.6) and the mean disease duration was 3.79 (std +/- 2.81) years. There were no differences between groups in disease duration, smoking history, or prior malignancy. There was no difference in baseline FVC between the MSA/MAA group and the antibody negative group; 90.68% vs. 89.86% ($p = 0.911$). The baseline DLCO was also the same; 60.20% vs. 58.49% ($p = 0.810$). There was no difference in the decline in FVC predicted per year between the MSA/MAA group and the antibody negative group; -2.09% vs. -2.24% ($p = 0.965$). There was also no difference in the decline in DLCO per year either; -5.65% vs. -6.24% ($p = 0.866$). There was no difference in all-cause mortality with 5 deaths in the MSA/MAA group and 7 in the antibody negative group ($p = 0.720$).
Conclusion: MSA/MAA antibodies do not alter disease trajectory in terms of lung function or all-cause mortality in IPF. Larger longitudinal studies are needed to determine if MSA/MAA antibodies in IPF are prognostically important or merely epiphenomena.

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2181 – P2.04.39

Assessment of myositis specific/associated autoantibodies during the COVID-19 pandemic and post pandemic period. The experience of a tertiary care hospital

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Background and aims: Inflammatory myopathies are an important complication associated withSARS-CoV-2 infection or vaccination. Recognition of this phenomenon led to an increase in the rate at which testing for Myositis-specific/associated autoantibodies (MSAs/MAAs) was requested by clinicians during the pandemic, with simultaneous rise in positivity against specific antigenic targets. The aim of this study is the registration, analysis and assessment of therequested tests for MSAs/MAAs, in a cohort of patients with suspected myopathy, during the pandemic period until nowadays (2020-2023), compared to the pre-COVID period (2018-2019).
Methods: 432 serum samples (group I, period 2018-2019) and 1068 serum samples (group II, period 2020-2023) were analyzed for MSAs/MAAs during the diagnostic work-up for Idiopathic Inflammatory Myopathy (IIM). The Immunoblotting Assay (IB-Euroimmun panel) was used to determine the MSAs/MAAs specificity (anti-PL-7, PL12, EJ, OJ, Mi-2, SRP, PM/Scl, Ku, TIF1g, MDA5, NXP2, SAE1, Ro52).
Results: A gradual increase in requested parameters was observedin the 3-year period following the pandemic compared to the pre-pandemic group, while the highest number of requested specific antibodies was recorded within the last year. Positive samples were recorded at a rate of 3.6% and 2.8% for the years 2018 and 2019, respectively, while the positivity for the years 2020, 2021, 2022 was recorded at 5.5%, 8.8%, and 9.2%, respectively. Especially for the post COVID year (2023) both, samples for investigation and their positivity (8.9%), have been kept steadily increased. Anti-Jo1 and anti-Ro52 were the most predominantly detected antibodies, while significant diversity was observed in terms of antibody specificity against the other various antigenic targets.
Conclusions: Our study demonstrated a gradually increasing frequency of requests for MSAs/MAAs testing during the pandemic and post-pandemic period, as well as a substantial rise in their positivity rates. This might be related to increased clinician vigilance towards the importance of detecting these autoantibodies. In any case, further investigation into the possible interlink between COVID-19 infection or vaccination and the development of IIM, is warranted.

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P2.05 IMMUNE DEFICIENCIES

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337 – P2.05.01

STAT3 haploinsufficiency induces an increase in IgE+ B cell development

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Autosomal dominant hyper IgE syndrome (AD-HIES) is a primary immunodeficiency caused by heterozygous mutations in the signal transducer and activator of transcription 3 (STAT3). Patients with AD-HIES are characterized by recurrent bacterial and fungal infections and high levels of IgE in serum, however the cause of increased IgE serum levels is not known. We created a mouse model of *Stat3* heterozygosity ($Stat3^{+/-}$) to study the humoral immune response and plasma cell development, considering that some mutations in STAT3 could have a haploinsufficient effect and not a dominant negative as it has been described for most of the STAT3 mutations.
The STAT3 protein analysis in $Stat3^{+/-}$ mice showed 50% reduction compared to WT animals, confirming the heterozygosity expected on this mouse model. Flow cytometry analysis showed that the $Stat3^{+/-}$ mice have a normal lymphocyte and myeloid development. The analysis of the serum immunoglobulin levels showed no differences in IgM, IgG1, IgG2b, IgG2c, IgG3 and IgA between WT and $Stat3^{+/-}$ mice. Interestingly, IgE levels were significantly enhanced in $Stat3^{+/-}$ mice compared to their WT counterparts, resembling the phenotype of AD-HIES patients.
To evaluate the role of STAT3 haploinsufficiency in the humoral immune response, we immunized mice with the T cell-dependent antigen NP-KLH, and 7 days later we detected an increase in the NP-specific IgE serum levels of $Stat3^{+/-}$ mice, while there was a reduction in the NP-specific IgM and IgG1 serum levels of $Stat3^{+/-}$ compared to $Stat3^{WT}$ mice. Moreover, Germinal Center IgE+ cells were significantly increased in $Stat3^{+/-}$ mice compared to their WT counterparts. Similar results were observed when the animals were immunized with the T cell-independent antigen NP-LPS.
Finally, naive B cells from $Stat3^{+/-}$ mice cultured with anti-CD40 and IL-4, in the absence of IL-21, showed a significant differentiation towards IgE+ plasma cells, in comparison to $Stat3^{WT}$ cells, with enhanced IgE levels in the supernatant of $Stat3^{+/-}$ cultures, supporting the role of IL-21 as a negative regulator of IgE+ plasma cells.
Together, these data highlights the relevance of STAT3 haploinsufficiency in humoral immunity and open new insights in understanding the role of STAT3 in IgE plasma cell differentiation.

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351 – P2.05.02

Sex Differences Influence Circadian and Circannual Variation in Circulating Alpha-1 Antitrypsin

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Purpose: Alpha-1 antitrypsin (AAT) is an acute phase protein with a broad range of anti-inflammatory and anti-protease functions. AAT deficiency (AATD) is a genetic condition which causes emphysema and liver complications. Daily (circadian) and seasonal (circannual) differences, and sexual dimorphism in circulating AAT levels have been reported in healthy populations. The aim of this project was to investigate circannual and sex differences in circulating AAT in an Irish cohort with AATD and healthy controls.
Methods: The national Targeted Detection Programme (TDP) is the diagnostic and screening body for AATD in Ireland. We analysed 20 years of TDP data, measuring AAT levels and diagnosing AATD by phenotype (or genotype). Data were segregated by date of test, and also by sex and age. Statistical analysis was carried out using GraphPad Prism.
Results: 19,437 complete, anonymised data entries were exported from the TDP. Of these, 11,655 were healthy (MM), 4,364 had a moderate deficiency (MZ), and 350 had a severe AAT deficiency (ZZ). Healthy MM individuals showed seasonal variations in AAT levels, with a nadir in autumn. This pattern persisted in moderately deficient MZ patients between March and September, but was lost in severe AATD (ZZ). This pattern of seasonal and circadian variation was only evident in MM males (n=5,800), with MM females (n=5,855) consistently maintaining significantly higher AAT levels year-round (median 1.46g/L vs 1.42g/L, $p<0.0001$). We also noted a discordant pattern of AAT variation between the sexes, which broadly mapped to the onset of puberty (ages 10-15) before converging upon menopause (ages 45-50), suggesting that variation in circulating AAT may correlate with oestrogen.
Conclusion: Despite consistently higher AAT levels in healthy females throughout life, healthy males showed greater circadian and circannual variation in circulating AAT levels. How these variations are linked to sex hormones and inflammatory cytokines is currently under investigation.

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456 – P2.05.03

Mild phenotype of PSTPIP1-associated myeloid-related proteinaemia inflammatory (PAMI) syndrome: case report. A challenging diagnosis

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Case presentation: A two-year-old girl was referred to the immunology department for immunodeficiency suspicion due to persistent neutropenia, anaemia, growth failure, lymphocytosis and recurrent cervical abscesses. Several tests had been previously carried out without any pathological results (oxidative burst assay, MPO expression, leuko-lymphocytic inmunophenotype, anti-neutrophil antibodies). Further humoral and cellular immunity studies did not show any relevant alterations, apart from the already detected neutropenia. Next-generation sequencing was performed using a panel comprising 549 immune system disease-causing genes. The genetic analysis revealed a heterozygous autosomal dominant pathogenic variant (c.748G>A, p.Glu250Lys) in exon 11 of *PSTPIP1* (NM_003978.4, NP_003969.2), previously described in individuals with PAMI syndrome. Eventually, serum zinc and calprotectin concentrations were quantified and both were highly increased, as well as the inflammatory markers LDH and CRP, data consistent with the diagnosis of PAMI syndrome.
PAMI syndrome is a rare autoinflammatory disorder caused by specific mutations (E250K or E275K) in *PSTPIP1* gene, which is involved in T-cell and phagocyte activation, cell migration, and pro-inflammatory cytokine production, among other biological functions. Clinically, the disease is mainly characterised by the presence of anaemia, neutropenia, cutaneous and osteoarticular manifestations, hepatosplenomegaly, failure to thrive, lymphadenopathy and chronic systemic inflammation. However, the phenotype can vary from mild presentations to severe cases. Our patient has not manifested any osteoarticular symptoms, skin lesions or hepatosplenomegaly so far. Regarding laboratory parameters, high serum levels of zinc and calprotectin (MRP8/14 or S100A8/A9) are the distinctive traits to differentiate PAMI syndrome from other *PSTPIP1*-**associated inflammatory diseases (PAID).**
Conclusion: This case provides an example of the difficulties that clinicians have to face when diagnosing PAMI syndrome, given its rarity and wide spectrum of clinical phenotypes. It also highlights the importance of considering this disorder in patients with undefined systemic inflammation and neutropenia, as well as emphasises the role of genetic screening and zinc and calprotectin serum concentrations. Segregation study is currently being carried out.

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466 – P2.05.04

Clues for diagnosis of hidden primary immunodeficiencies in B-cell lymproprolipherative diseases: a logistic regression approach

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Purpose: The distinction between primary (PID) and secondary (SID) immunodeficiencies, particularly in the setting of late-onset PIDs and their relationship to hematological B-cell lymphoproliferative disorders (B-CLPD), is increasing blurred. In this work, we aimed to analyze and define the clinical and laboratory variables of SID to B-CLPD patients, identifying overlaps with late onset PIDs, which could potentially improve diagnostic precision and prognostic assessment.
Methods: We studied 40 clinical and laboratory variables in 151 SID to B-CLPD patients. These were classified as "possible PID" when the sum of serum free light chain (sFLC) kappa and lambda concentration was below 20mg/L. Bivariant statistical analysis and *rpart* method were performed to obtain a tree decision model containing the most discriminative variables.
Results: Significant statistical differences were found in 17 out of 40 variables analyzed, including 5 clinical and 12 laboratory biomarkers. Suspected PID patients showed higher frequencies of childhood recurrent and severe infections, as well as recurrent respiratory infections preceding B-CLPD diagnosis, a family history of B-CLPD and better remission rates than non-suspected PIDs. Laboratory findings showed significantly lower sFLC, immunoglobulin concentrations, as well as lower total leukocyte, B-cell and NK-cell counts at baseline. The *rpart* model developed a decision tree with 3 major variables (childhood infections, recurrent respiratory infections prior to B-CLPD diagnosis, and sFLC sum), creating a possible diagnostic route that could allow clinicians to classify B-CLPD patients into suspected SIDs or PIDs, with high sensitivity (98%) (95%CI (0.9348, 1.0000)), specificity (87%) (95%CI (0.6571, 0.9783)) and accuracy (95%) (95% CI (0.8874, 0.9868). Surprisingly, this tree-decision model classified as possible PID 75% of the 151 B-CLPD patients.
Conclusion: Significant clinical and immunological variables could aid in the difficult task of identifying late-onset PIDs among SID to B-CLPD, emphasizing the clinical utility of a comprehensive immunological history and work-up. While "possible PID" seem to have a higher grade of hematological remission, yet suffer more frequent and severe infections, and different tumor behavior, raising the importance of early, tailored diagnostic and treatment strategies for personalized patient management and follow up.

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560 – P2.05.05

Inhibiting autoreactive CD8+ T-cells in Type 1 Diabetes using blocking anti-CD8β antibody to inhibit pancreatic β-cell islet destruction

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Purpose: Type 1 diabetes (T1D) is an autoimmune disease, which induces selective pancreatic β-cell destruction in the pancreas. Insulin injections are provided to patients to control blood sugar levels. However, the underlying immune mediated damage remains untreated. We aim to develop an approach to block autoreactive CD8 dependant T-cells, using the YTS 156.7.7 monoclonal anti-murine CD8β antibody to inhibit β-cell destruction.
Methods: Initially, we generated F(ab)2 fragments to remove the depletion effect *in vivo*. The F(ab)2 fragments were tested using both an *in vitro* and *in vivo* proliferation assay, to measure the impact on NY8.3 autoimmune T-cell proliferation. In addition, Balb/c mice were injected with either whole YTS156.7.7 antibody or F(ab)2 fragments of YTS156.7.7 to examine the level of CD8 depletion mediated *in vivo*. The biological half-life of F(ab)2 fragments was measured using blood samples taken over 48 hours.
Results: Whole YTS156.7.7 was shown to deplete the CD8 population *in vivo*, whereas F(ab)2 fragments did not mediate depletion. We also demonstrated the ability of F(ab)2 fragments to block autoreactive CD8 T-cell proliferation both *in vitro* and *in vivo*, and measured the serum half-life of the F(ab)2 fragment (30.1 hours) in Balb/c mice. In addition, we have showed that there is no significant difference between the blockade mediated by the whole YTS156.7.7 antibody compared to the F(ab)2 fragments in inhibiting IFNγ release in an ELISA assay. The blockade of T-cell activation of primed CL4, high affinity, and primed NY8.3, autoreactive T-cells, was only observed at lower pulsed P815 concentration, 10ng to 1pg. However, unprimed NY8.3 T-cells were blocked at the highest peptide concentration.
Conclusion: The use of blocking anti-CD8β F(ab)2 fragments eliminated the *in vivo* depletion of CD8 T-cells mediated by the whole antibody. Also, it was found that the use anti-CD8β YTS156.7.7 F(ab)2 fragments is a promising approach to inhibit the release of IFNγ by diabetogenic NY8.3 T-cell and to inhibit their proliferation *in vivo* within polyclonal NOD mice.

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571 – P2.05.06

Defects in T cell distribution and function in WHIM Syndrome patients

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Introduction: The WHIM Syndrome (WS) is a rare inherited immunodeficiency caused by a gain-of-function mutation in the *CXCR4* gene. The patients suffer from human papillomavirus-induced warts, hypogammaglobulinemia, recurrent infections, myelokathexis, and severe B and T cell lymphopenia. Due to the rarity of the disease, the immune responses remain poorly characterized in WS patients. However, some studies were suggestive of inefficient adaptive immune responses and these patients have a heightened risk of developing cancer.
Objective: To understand how CXCR4 gain of function may impact immune responses we aimed to assess both the distribution and function of T cells in French WS patients.
Methods: Immune populations were analysed using a 40-color spectral flow cytometry panel from frozen peripheral blood mononuclear cells (PBMCs) of four WS patients and healthy donors (HD). One patient was studied before and after one year of treatment with a CXCR4 antagonist. CellTrace Yellow staining of PBMCs was performed to determine the proliferation after 3 days of stimulation with anti-CD3/anti-CD28 antibodies. PBMCs were stimulated *in vitro* with PMA/Ionomycin for 6 hours to evaluate TNFα, INF-γ and IL-2 release by intracellular staining.
Results: Our results reveal abnormal T-cell distribution in WS patients with an increased CD4/CD8 ratio, a decrease in naive T cells and $CD31^+$ recent thymic emigrants mirrored by an increase in effector memory cells. These defects were normalized upon treatment with a CXCR4 antagonist. Furthermore, we report an increased expression of the activation marker CD25 on CD4 T cells and the exhaustion markers CD95 and KLRG-1 on CD4 and CD8 T cells. At the functional level, WS T cells exhibited a decreased proliferation and an enhanced capacity to secrete IFN-γ, TNF-α and IL-2 upon stimulation. This increased cytokine release seems to be normalized after one year of treatment with a CXCR4 antagonist.
Conclusion: Both phenotypic and functional T cell defects were observed in WS patients, potentially contributing to the inefficiency of immune responses to infections and vaccines.

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795 – P2.05.07

The different faces of inborn errors of immunity with case examples

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Purpose: Inborne errors of immunity (IEIs) are generally considered rare monogenic disorders of the immune system. Immune dysregulation (autoimmunity, autoinflammation, lymphoproliferation, and malignancy) and immunodeficiency (susceptibility to infection) represent two sides of the same coin and are not mutually exclusive. Based on this information, we aimed to present the different faces of IEIs with case examples.
Case 1: A 12-year-old boy presented with complaints of recurrent otitis media, neutropenia, and lymphopenia when he was 2 years old. Severe neutropenia, CD4+ T cell, CD19+ B cell lymphopenia and hypogammaglobulinemia were determined. Whole exome sequencing (WES) was performed and a missense mutation was identified in the MSN gene on the X chromosome.
Case 2: A 7-year-old girl presented with fever, dehydration and vaginal ulcers at the age of 13 months. He was diagnosed with T-B+NK+ combined immunodeficiency, severe inflammatory bowel disease (IBD), and arthritis. Homozygous mutation in CORO1A and NLRP6 genes and heterozygous mutation in NLRP 3 gene were determined by WES.
Case 3: A 16-year-old male patient, who had been followed up with the diagnosis of chronic ITP since the age of 2.5, was admitted due to recurrent fever, diarrhea and recurrent aphthous stomatitis when he 7 years old. Chronic thrombocytopenia, autoimmune thyroiditis, B cell lymphopenia, autoinflammation and hypoglobulinemia were determined. WDR1 homozygous mutation was detected by WES.
Case 4: A 16-month-old male patient was admitted on the 14th postnatal day. During the examination, widespread eczematous lesions on the skin, mucositis in the mouth, and flatness at the root of the nose were observed. Thrombocytopenia, eosinophilia, sepsis, cow's milk protein allergy, D. Coombs positivity, proportionally low CD3 and CD4 T cells were determined. ARPC1B mutation was detected in molecular testing.
Conclusion: In this article, we wanted to show the different faces of IEIs that occur with different clinical and laboratory results, such as bone marrow failure, autoinflammation, inflammatory bowel disease and autoimmunity. On this occasion, we would like to emphasize that the path to the diagnosis of IEI is primarily through awareness and looking at the patient from a holistic perspective.

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883 – P2.05.08

LOGISTIC REGRESSION FOR THE STUDY OF COMMON VARIABLE IMMUNODEFICIENCY BIOMARKERS

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Purpose: Early diagnosis of common variable immunodeficiency (CVID) remains challenging despite advancements in genetic and functional studies. Laboratory findings play a critical role but can sometimes contribute to diagnostic inaccuracies, without any markers that are pathognomonic of the disease. The aim of this study was to overcome the complexities associated with the diagnosis by evaluating the individual and combined performances of various biomarkers in accurately identifying CVID using logistic regression models.
Methods: We evaluated retrospectively 88 patients from Immunology Service of Hospital Clínico San Carlos (Madrid): 27 CVID patients, 23 selective IgA deficiency (SIgAD) patients, and 20 patients with secondary immunodeficiency (SID) due to active haematological malignancy (chronic lymphocytic leukemia (CLL) or multiple myeloma (MM)). We also studied 18 healthy controls. Routine clinical and analytical data (immunoglobulin levels, B-cell and T-cell levels and B-cell subpopulations, and specific antibody responses) were collected. We evaluated soluble BCMA (sBCMA) levels and serum free light chains (sFLC, κ and λ) in our patients' cohort. We also performed different statistics analysis including a series of logistic regression models.
Results: At first, we found that sBCMA levels and the sum κ+λ were significantly lower in CVID patients, which validates previous studies. Indeed, these biomarkers showed high sensitivity (Se) and specificity in our cohort (Se 88.89% Sp 86.96%; Se 88.00% Sp 100% respectively). We also evaluated associations between the different CVID-related biomarkers (switched-memory B cells (smB), sFLCs, sBCMA and VISUAL score). We found that the sum κ+λ was positively correlated with sBCMA levels but not with other biomarkers such as smB. As a final step, we confronted all the studied biomarkers in our patients' cohort to develop the best-fit algorithm for CVID diagnosis. The decision tree model was the most consistent and robust, with specific antibody responses and the sum κ+λ being the best diagnostic biomarkers.
Conclusion: Our exploratory study suggests that combining the measurement of specific antibody responses with the sum κ+λ may improve the early diagnosis of CVID and consequently lead to a decrease in disease morbidity and mortality.

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911 – P2.05.09

Biomarkers in patients with haematological malignancy in response to polyclonal gammaglobulin replacement therapy

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Patients affected with B-proliferative syndromes (BPS), specifically multiple myeloma (MM) and chronic lymphocytic leukaemia (CLL), typically have a deficit in antibody production that makes them susceptible to recurrent or severe infections, and immunoglobulin replacement therapy (IGRT) is indicated.
To date, there are no studies on the use of GnRTIs on the progression of BPS or biomarkers for monitoring secondary immunodeficiency (SID).
It has been shown that TRIg is able to reprogram macrophages towards a more pro-inflammatory state, which could slow down infectious and recurrent processes. We currently have a total of 16 patients, separated into three groups, four patients with CLL, eleven patients with non-Hodking's lymphoma (NHL) and one patient with monoclonal gammopathy of uncertain significance (MGUS). The experimental development will consist of four analytical processes for each patient, first at the start of treatment, then after seven days, then after six months and finally after one year.
Preliminary results in 16 patients showed a significant increase in the population of M-MDSCs after TRIg infusion at six hours (13.37±13.17 vs. 26.62±16.52; p= 0.008). Moreover, in 3 of them this increase was maintained at 7 days. A significant increase in classical monocytes (82.38±15.04 vs 86.03±18.81; p=0.049) and a significant decrease in intermediate monocytes (13.60±10.63 vs 8.07±14.08; p=0.008 and non-classical monocytes (4.53±2.46 vs 1.79±2.72; p=0.001) was also observed.

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972 – P2.05.10

Flow cytometry-based protocols for the assessment of B lymphocyte activation after CpG stimulation: a diagnostic approach for primary immunodeficiencies

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More than half of primary immunodeficiencies are associated with impaired antibody production, although the disease mechanisms are still largely unknown. The first signs usually appear in childhood and early diagnosis is important to initiate appropriate treatment and prevent severe infections. Functional activation tests of B cells are important to assess the function of the humoral immune system in patients with primary immunodeficiency. Based on in vitro functional tests to determine proliferation potential, differentiation to plasmablasts and immunoglobulin secretion after stimulation of B cells with CpG, we can reliably assess the activation capacity of B cells. CpG activates the B lymphocytes in a T cell-independent pathway. Proliferation is determined by measuring the incorporation of fluorescently labeled EdU into the DNA of the dividing cells. The study included two distinct groups: 25 healthy volunteers and 6 adult patients diagnosed with common variable immunodeficiency (CVID). We have shown that the B cells of healthy individuals proliferate after stimulation, differentiate into plasmablasts and secrete immunoglobulins. However, in patients diagnosed with primary immunodeficiency, B cell proliferation, plasmablast differentiation and immunoglobulin secretion are reduced or absent. To introduce the in vitro functional test into routine practice, we determined the optimal conditions for stimulating B cells with CpG. We also determined the normal values for the proliferation of B cells, naive and memory B cells and plasmablasts in healthy individuals for adults and children.

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976 – P2.05.11

Chediak-Higashi syndrome with associated hemophagocytic lymphohistiocytosis in 3-month-old male patient

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Hemophagocytic lymphohistiocytosis (HLH) is a severe hyperinflammatory condition caused by a permanent proliferation and activation of lymphocytes and macrophages, resulting in the characteristic clinical symptoms of inflammation. In recent years, genetic defects affecting lymphocyte cytotoxicity have been identified as the main cause of primary HLH. One of these primary forms of HLH is Chediak-Higashi syndrome (CHS). This is a rare and severe immunodeficiency characterised by partial oculocutaneous albinism (OCA), recurrent infections, mild hemorrhage, neurological dysfunction and lymphoproliferative disorder. CHS is caused by mutations in the *LYST* gene, which codes for a lysosomal trafficking regulatory protein, leading to decreased cytotoxic activity and degranulation of NK lymphocytes. Within CHS, a more severe early-onset form (first months of life) and a late-onset attenuated form have been described.
We present a 3-month-old male patient admitted to the pediatric ICU of our hospital for persistent fever over 40ºC, splenomegaly and mild respiratory symptoms. Phenotypically, the patient had partial OCA. In laboratory tests, the patient presented pancytopenia (with severe neutropenia), hyperferritinemia, hypofibrinogenemia and hypergammaglobulinemia. In the peripheral blood smear, vacuolated neutrophils with large azurophilic granules were observed and in the bone marrow smear, purplish inclusions were observed in the lymphoid series, as well as evidence of hemophagocytosis. Functional studies showed an increase in activated CD8+ T cells and a decrease in cytotoxic activity of NK cells. These data were sufficient to diagnose HLH (according to the criteria of the HLH-2004 protocol), and taking into account the patient's OCA, the main diagnostic suspicion was Chediak-Higashi syndrome with associated HLH. Finally, the genetic study was performed, finding two pathogenic variants in the *LYST* gene. After confirming the diagnosis of CHS, the patient began the search for a hematopoietic progenitor donor, as transplantation is the only curative option for this disease.

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1000 – P2.05.12

Synovial histopathological stratification reveals a relationship between pathotypes and clinical presentation in osteoarthritis.

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Osteoarthritis (OA) is the most common joint disease worldwide, mostly affecting people aged 65 and more. It is characterized by cartilage degradation, formation of osteophytes and synovitis, causing pain and disability. There is currently no curative treatment available, and only symptomatic treatments exists. The mechanisms underlying OA development remain poorly understood, but synovitis has been shown to play a major role in the initiation and progression of the disease. The current gold standard Krenn scoring of synovial inflammation, based on intimal thickness, stromal cell density and lymphocyte infiltration do not reveal relevant association between synovitis score and clinical data. We recently identified three histological pathotypes based on the nature and distribution of synovial immune cells infiltration, namely pauci-immune (PI), diffuse-myeloid (DM), and lympho-myeloid (LM). Here, we aim to study how pathotypes relate to clinical and radiographic changes in OA, and reveal their specific transcriptomic signature.
Synovial tissue, tibial plateau cartilage and infrapatellar Hoffa's tissue (fat pad) from 94 OA patients (8 PI, 43 DM and 43 LM) were collected at the time of joint replacement together with clinical data, and a subset of 15 (3 PI, 6 DM and 6 LM) synovial tissues were analysed by bulk RNA-sequencing. Synovial pathotypes were determined by immunohistochemistry, fat pad inflammation was characterized using a relevant score set up by our group following H&E staining, and cartilage degradation was assessed using the OARSI score. Our results confirmed the non-redundancy between pathotypes categorization and the gold standard Krenn score. Moreover, 78% of the patients with LM pathotype presented important radiographic joint damage (Kellgren Lawrence score = 4), compared to only 60% of the DM and 57% of the PI synovium. In addition, synovial pathotypes differently associated with fat pad inflammation. However, at this stage of the disease, cartilages were highly degraded and OARSI scores did not correlate with synovial inflammation. Importantly, preliminary RNA-sequencing analysis revealed pathotype-specific transcriptomic signatures, paving the way for the development of personalized therapeutic strategies. Altogether, our results confirm the relevance of OA patient's stratification based on synovial pathotypes.

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1008 – P2.05.13

Discovering the T-Cell Receptor Repertoire in APECED Patients

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Adaptive immune receptor repertoire sequencing has become a powerful tool in studying immune responses and their correlation with various health conditions. This study explores the T-cell receptor (TCR) repertoire within individuals diagnosed with polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) to investigate potential links with melanocytes and melanoma antigens. APECED patients seem to be protected against cancer, except for squamous cell carcinoma of the oral cavity and oesophagus, often linked to chronic candidiasis on mucosal surfaces. This protective mechanism is considered to arise from a diverse TCR repertoire targeting autoantigens.
The primary objective of this research is to evaluate the prevalence of melanocytes/melanoma-specific TCRs within the circulating T-cell populations of APECED patients. We aim to identify unique facets of the immune response specific to this autoimmune disorder by comparing these results with data from healthy individuals. Additionally, we seek to classify each melanocyte/melanoma-specific TCR according to T-cell functional subtypes to gain deeper insights into the roles of distinct T-cell populations in developing autoimmune processes.
To achieve these goals, eight T-cell subpopulations were isolated from each individual (ten patients and ten age-matched controls), and TCR beta-CDR3 deep sequencing was conducted, followed by the analysis of T-cell subpopulations for each melanocyte/melanoma-specific TCR. Since autoantigen-specific T-cells are scarce, we stimulated peripheral blood mononuclear cell (PBMCs) samples to increase the autoreactive population. Then, single cells were sorted and amplified for TCR alpha and beta sequencing, and repertoire overlap analysis was conducted among patients and various cell types, along with distributions of V or J genes, including clustering and PCA, etc.
This study aims to investigate potential therapeutic implications in the interplay between immune responses, autoimmune disorders, and melanoma. By deciphering the mechanisms underlying self-tolerance and immune surveillance against cancer, we aspire to identify novel targets for therapeutic intervention. Ultimately, the insights gained from this study could be used to develop personalised treatments for autoimmune diseases and melanoma, which could revolutionise current treatment approaches.

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1061 – P2.05.14

Immune Dysregulation, Hypopigmentation and Silvery Hair Syndromes, Single Centre Experience

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Purpose: Chediak-Higashi syndrome (CHS) is a rare autosomal recessive disorder characterized by immune dysregulation, neurological dysfunction, hypopigmentation of the skin and hair, and a bleeding tendency. Other diseases with partial albinism and immunodeficiency are Griscelli syndrome (GS), Elejalde syndrome, and Hermansky-Pudlak syndrome (HPS). Associated immunological and neurological defects and a predilection for hemophagocytic lymphohistiocytosis (HLH) make them a distinctive entity in pediatric practice. Haematopoietic stem cell transplantation (HSCT), which is the treatment of the diseases, is known to improve clinical and hematological symptoms, but does not prevent progressive neurological deterioration or oculocutaneous albinism.
Methods: The follow-up files of six patients with CHS and GS who were admitted to Ege University Department of Paediatric Immunology between 2009 and 2023 were retrospectively analyzed.
Results: Two of the six patients were diagnosed with GS and four with CHS. The mean age at diagnosis was 34 months (2 months–167 months). One patient was presented with HLH, the presenting symptoms of the others were fever, rash, recurrent pulmonary infections, hair findings and gait disturbance. All patients had pigmentation disorder in the form of grey hair colour change. Three of the patients had retarded neuromotor development (NMG) stages. Four of them developed HLH during the follow-up. Four patients underwent HSCT. One patient died before HSCT and one after HSCT. One is in process of HSCT.
Conclusion: Early diagnosis and treatment of HLH, which is a remarkable cause of mortality in the disease, and HSCT from HLA-matched donors improve the prognosis in patients with Chediak-Higashi and Griscelli syndromes.

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1080 – P2.05.15

Will sarcoidosis help us understand granulomatous disease in Common Variable Immunodeficiency (CVID) patients?

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Purpose: Granulomatous Lymphocytic Interstitial Lung Disease (GLILD) occurs in about 20% of CVID patients, with a negative prognostic impact. Disease induction and maintenance mechanisms are still unclear. GLILD shares histological and some clinical features with sarcoidosis, another rare multisystemic inflammatory disease of unknown etiology. Recent studies demonstrated the relevance of innate immunity in granulomas formation in sarcoidosis. Macrophages, in particular, were identified as microenvironmental influencers, with the mTOR pathway playing a key role in their polarization and activity. Little is known about the role of macrophages in GLILD, and *in-vitro* models are lacking. Thus, we sought to investigate similarities and differences between GLILD and sarcoidosis, starting from multinucleated giant cells (MGC) formation and mTOR activation and aiming to develop a 3D *in-vitro* model.
Methods: Monocytes from THP-1 cell line and from peripheral blood of sarcoidosis, CVID without GLILD, GLILD patients and healthy donors were stimulated 7 days with GM-CSF and 7 days with IL-4 to obtain MGC. Cells underwent immunofluorescence staining and images were analysed to evaluate the fusion index, MGC% and area. By Western Blot we evaluated P-S6/S6total as marker of mTOR pathway activation while CD301 due its involvement in IL-4-induced MGC formation. A 3D model was developed by human bone fragments decellularization and recellularization with macrophages. 3D digital reconstructions were performed by confocal microscopy.
Results: We obtained MGC from THP-1 and from primary macrophages of all sources. We found a statistically higher MGC% and fusion index in GLILD and sarcoidosis compared to the non-granulomatous controls; sarcoidosis MGC presented the smallest cell area. Healthy MGC showed a statistically higher CD301 expression and S6 phosphorylation compared to the non-treated macrophages. Experiments are ongoing with patients' macrophages. THP-1 and primary macrophages were also able to adhere on the bone-based 3D scaffold, paving the way for MGC studies in a 3D setting.
Conclusion: Macrophages from GLILD and sarcoidosis patients show a similar in vitro behavior, compared to heathy donors and CVID patients without GLILD. *In-vitro* models might help to unravel GLILD etiopathogenesis, being the 3D model a potential platform to design patient tailored therapies in such a rare disease.

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1094 – P2.05.16

Human IL12RB1 deficiency reveals essential roles of IL-12 and IL-23 in innate and adaptive immune responses

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Purpose: *IL12RB1* deficiency is the most frequent cause of Mendelian Susceptibility to Mycobacterial Diseases. We recently described the case of an adult patient with disseminated *M. xenopi* infection diagnosed with *IL12RB1* deficiency. Since IL-12Rβ1 is shared between IL-12 and IL-23 receptors, its deficiency impairs leukocytes' response to both cytokines. *IL12RB1* deficiency has already been studied at cellular level, however there is still paucity of data showing how it affects leukocytes' development and functionality at molecular level.
Methods: PBMNC from an *IL12RB1* deficient patient were studied by flow cytometry and single-cell RNA sequencing. Genome-wide DNA methylation on $CXC3^+$ $CD4^+$ and $CD8^+$ T cells was evaluated by sequencing.
Results: Leukocytes' development in the bone marrow showed no abnormalities. In the circulation, $CD4^+$ T cells displayed reduced absolute numbers and impaired IFN-γ production and CXCR3 expression. Additionally, we demonstrated by scRNAseq that the cluster of CXCR3+ CD4+ T cells with a TEMRA phenotype was functionally impaired in the *IL12RB1*-deficient patient, with significantly reduced expression of cytotoxic genes. A similar finding was observed also for $CD8^+$ T cells, which exhibited marked contraction of the memory cell cluster characterized by expression of cytotoxic genes. Gene set enrichment analysis showed downregulation of immune system-related pathways in both $IL12RB1^{-/-}$ CD4+ and CD8+ T cells. Genome-wide DNA methylation study performed on $CXCR3^+$ $CD4^+$ and $CD8^+$ T cells further demonstrated that *IL12RB1* deficiency affects the epigenome of T cells with type I effector functions. NK cells displayed reduced circulating absolute numbers and belonged to the classical $CD56^{dim}CD16^+$ subset. However, among these cells we did not observe expansion of the memory $NKG2C^+$ cluster, characterized by a distinct transcriptional signature, that has been described upon CMV infection. Since the patient is CMV^+, this finding demonstrates that IL-12 and/or IL-23 are fundamental for the development of adaptive-like NK cells *in vivo*. B cells were also reduced in the circulation and exhibited contraction of the memory cluster. Finally, monocytes demonstrated significant downregulation of gene signatures related to IFN signaling and antigen processing capability.
Conclusion: Collectively, these data demonstrate that IL-12Rβ1 deficiency significantly impairs the functionality of both innate and adaptive branches of immunity.

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1116 – P2.05.17

Obesity leads to reduced Vaccine Responses to SARS-CoV-2 in Male C57BL/6 Mice

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Background: Obesity poses an increasing health problem worldwide. Obesity predisposes to a wide-range of diseases and is connected to immunological disorders. Previous studies indicate that obesity influences the immune response to certain vaccines and impairs their efficacy or longevity of protection. During the COVID-19 pandemic, obese individuals belonged to the high-risk group for severe diseases; the impact of the COVID-19 vaccination was less well investigated.
Methods: C57BL/6 mice were fed a high-fat or standard diet for 18 weeks. After ten weeks, mice were immunized intramuscularly: 1) twice with different doses of COVID-19 origin mRNA vaccine, 2) three times with 0,25µg against OMICRON XBB.1.5 in three-week intervals. After the last vaccination, spike-specific antibodies in sera and mucosae (bronchoalveolar lavage (BAL)), and the spike-stimulated cytokine response from spleen cells were measured. The distribution of the spike-specific lymphocytes in the spleen was investigated by flow cytometry.
Results: In the 0,25µg vaccination group against the original strain, we measured a significantly decreased level of spike-specific IgG in sera of obese compared to lean animals. Due to the fast mutation rate of the virus, further experiments were performed using mRNA vaccine against the Omicron XBB1.5. After three vaccinations, a downregulation of spike-specific antibody levels in sera and a significant reduction in the BAL was noted in obese compared to lean mice. Despite higher B cell levels, the percentage of spike-specific memory B cells in obese mice was reduced compared to lean animals. Splenocyte stimulation showed induced IL-2 levels in obese mice only. Additionally, obese vaccinated mice exhibited significantly higher levels of Tregs in the spleen.
Conclusion: In obese mice, three times vaccination of mRNA vaccine was necessary to induce a spike-specific antibody response comparable to lean mice. The spike-specific antibodies were significantly lower at the mucosal site, suggesting a higher vulnerability against infection in obese mice. Also, spike-specific memory B cells were reduced, showing that long-term protection might be impaired in obese animals. Finally, the finding that IL-2 in the obese mice was induced and that the Tregs population was increased compared to lean mice suggests a mechanism of immune impairment.

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1259 – P2.05.18

Clinical impact of a non-canonical splicing variant in NFKB1 gene

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Purpose: We report the case of a 25 years-old patient with common variable immunodeficiency (CVID) presenting recurring infections (influenza A and invasive neumucocical pneumonie) since last year with sinusitis, abscess, molluscum contagiosum, asthma splenomegaly, and severe periodontitis. The patient refered infectious episodes such as acute media otitis and suspicion of allergic reactions in childhood.
Methods: His blood tests had revealed agammaglobulinemia IgG2 <2 mg/dL (242-700), undetectable IgA and IgM < 10 mg/dL. The patient was qualified for immunoglobulin replacement therapy. There was no vaccine response, and the immunophenotype revealed lymphopenia T, B, and NK. The genetic analysis revealed an intron variant (c.2125-9 G>A) next to the splice region of *NFKB1* gene, outside the canonical splicing region. The genetic variant is in heterozygosis, not present in healthy controls and not described in association with NFKB1 haploinsufficiency. It has been classified as uncertain significance but Alamut algorithm indicates that it is very likely to alter splicing, giving rise to a new acceptor site in the intron 18 that that would affect the processing of exon 19.
Results: After analyzing the patient's mRNA to test for splicing alterations, a new splicing site produced an mRNA with the insertion of 7 nucleotides, resulting in a frameshift and a premature stop codon G709I*4.
It was performed mutational analysis of this variant in their progenitors and the brother; it was found also in the father who refered pharyngitis, oral aphthosis and candidiasis in childhood. As an adult, he suffers from recurrent pyorrhea. He showed decreased serum IgG1 298 mg/dL (382 -929) with <8% of switched memory B cells (CD27+IgD-) .
Conclusion: We identified an intron variant outside the canonical splicing region in the *NFKB1* gene leading to a new splicing site and a premature stop codon classified finally as pathogenic: the phenotype of both patients (father and son) showed the coexistence of both humoral immunodeficiency and recurrent infections.
Loss-of-function NFKB1 variants are the most common monogenic cause of CVID, a primary immunodeficiency, characterized by primary hypogammaglobulinemia and increased infections susceptibility.

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1364 – P2.05.19

Common variable immunodeficiency not associating recurrent infections

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Introduction: Common variable immunodeficiency is the most common symptomatic primary immunodeficiency. It is a heterogeneous group of clinical manifestations due to dysregulation of the immune system, generating an increased risk of recurrent infections, autoimmunity and lymphoproliferative syndromes.
Results: We report the case of a 50-year-old patient with a history of recurrent sinusitis. He presented Hodgkin's lymphoma with nodular sclerosis at the age of 28 and diffuse large B cell non-Hodgkin lymphoma associated with Epstein Barr virus at the age of 46, treated with chemotherapy and local radiotherapy, achieving complete remission in both cases. Subsequently, he developed a relapse of diffuse large B-cell lymphoma at the age of 49, treated with chemotherapy and autologous transplantation. During first lymphoma diagnosis, the patient presented sustained IgG, IgA and IgM hypogammaglobulinemia, decreased immunoglobulin production in vitro, absence of response to vaccines and decreased memory B lymphocytes. In view of these data, common variable immunodeficiency was suspected and substitutive treatment with intravenous immunoglobulins was started. In addition, CD4/CD8 ratio was inverted with CD4+ T cell lymphocytopenia, requiring prophylactic antibiotics. CD132 (common gamma chain) expression of CD4-CD8 T lymphocytes, NK and B lymphocytes without alterations. Autoimmune thrombocytopenia was associated. The genetic study detected a heterozygous variant in TNFRSF13B gene (exon 4, c.542C>A), with pathogenicity prediction, associated with common variable immunodeficiency type 2, and a hemizygosis variant in IL2RG gene (exon 7, c.918C>A) of uncertain significance.
Conclusion: Common variable immunodeficiency associates greater risk of developing lymphoproliferative syndromes, probably being the clinical debut of the disease. Treatments for hematological diseases can mask an underlying primary immunodeficiency. In a young patient with a history of lymphoproliferative syndrome, it is important to perform a complete immunological study and follow up to distinguish a primary immunodeficiency from a secondary one. Acquiring special relevance the asymptomatic patients, the lack of typical symptoms as recurrent infections can lead to late diagnosis of the immunodeficiency.

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1373 – P2.05.20

Activated phosphoinositide 3-kinase delta syndrome type I in a patient with significant neurodevelopmental delay

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Purpose: The purpose of this abstract is to share an unexpected result that we encountered from an 8-year-old female patient while performing a Next Generation Sequencing (NGS) analysis, targeting genes associated with intellectual disability and hypotonia. The patient presented with proximal hypotonia of the shoulder girdle, recurrent febrile & afebrile seizures, intellectual disability and dysphagia. She first presented with failure to thrive, but ultimately achieved adequate weight and height. At 7 months old she was admitted for bronchiolitis, and later for splenomegaly due to an EBV infection. Since then, she has suffered recurrent respiratory infections that occasionally require antibiotics.
Methods: Blood analysis revealed leukocytosis (22,000/mcL, [normal values (NV): 4,800-12,000]) with a predominance of neutrophils (16,000/mcL, [NV: 1,700-8,000]) and a slight elevation of GGT (40U/L, [NV: 5-36]). A slight elevation of IgM (285 mg/dL, [NV: 47-240]) was detected, with IgG (1,128 mg/dL, [NV: 552-1,631]) and IgA (132 mg/dL, [NV: 21-282]) levels within normality. The autoimmunity study was negative and vaccine response was adequate. High-resolution chest CT of the lungs detected cylindrical and saccular bronchiectasis in the right lower lobe.
Exome targeting genes related to hypotonia and intellectual disability was requested.
Results: One heterozygous Single Nucleotide Variant was identified in the PIK3CD gene (NM_005026.5): c.3061G>A (p.Glu1021Lys), which was classified as pathogenic.
Conclusion: The PIK3CD gene encodes for the catalytic subunit p110δ of the phosphoinositide 3-kinase delta (PI3Kδ) enzyme. This protein is mostly present in leukocytes and participates in the metabolism, cellular growth, survival and apoptosis inhibition of T and B cells. Pathogenic gain-of-function mutations in PIK3CD result in the overactivation of PI3Kδ and are associated with Activated phosphoinositide 3-kinase delta syndrome (APDS) type I. APDS is a rare Inborn Error of Immunity (IEI), first described in 2013, with a variable clinical phenotype that is primarily characterized by increased susceptibility to recurring viral & bacterial infections (mainly sinopulmonary), as well as lymphoproliferation and autoimmunity. Our patient was presenting very striking neurological manifestations that drew the clinical suspicion away from IEI. As a result of the NGS, the mutation p.Glu1021Lys was found, allowing for the diagnosis of APDS type I.

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1400 – P2.05.21

Patients with common variable immunodeficiency should be vaccinated against viral infections - insight into cellular and antibody responses

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Purpose: Patients with common variable immunodeficiency (CVID) have a defect in the B cells development leading to decreased production of IgA/IgG antibodies. This reduced/absent concentration is therefore associated with a wide range of clinical manifestations, such as infections and non-infectious complications. Infectious manifestations are effectively managed by immunoglobulin substitution. Although patients with CVID are further characterized by failure to produce specific antibodies after vaccination to protein and/or polysaccharide antigens, vaccination still represents a great benefit for them, such as the formation of memory T cells. Until 2020, mRNA vaccines were only used for research purposes and their immunogenicity was not known in patients with immune disorders who were not included in clinical trials monitoring the effectiveness and safety of vaccines against SARS-CoV-2. We focused on the persistence of antibody and cellular response after vaccination with mRNA (BNT162b - Pfizer, BioNTech) against SARS-CoV-2 and inactivated vaccine (Vaxigrip Tetra 2022/2023 – Sanofi Pasteur) and compared their effectiveness and safety.
Methods: Serum and full blood from 21 CVID patients and 23 age matched healthy controls (HC) was collected at 1, 3, and 6 months after the vaccine administration and used for the detection of SARS-CoV-2/H1N1 IgG antibodies by immunoblot/ELISA methods. The cellular response was detected by cytokine production after stimulation by specific peptides.
Results: 52.4% (n=11/21) of vaccinated patients developed IgG against S –protein (S-RBD) of SARS-CoV-2 and 46% (n=11/21) T cells immune response one month after vaccination. The S-RBD IgG concentration continuously decreased in patients compared to HC and finally, at month 6 after vaccination, only 33% (n = 5/15) of patients had a positive titer compared to 100% of HC. In addition, significantly fewer virus-neutralizing antibodies were detected in CVID patients. The cellular response of patients was comparable to HC. H1N1 IgG concentration and cellular response values in CVID patients will be presented at the congress.
Conclusion: The mRNA vaccine induced the formation of specific antibodies in a large percentage of patients. However, the concentrations of these antibodies declined more rapidly compared to HC.

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1421 – P2.05.22

Clinical, immunological, and genetic features in patients with NF-κB1 and NF-κB2 defects

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Purpose: Inborn errors of immunity (IEIs) encompass various diseases with diverse clinical and immunological symptoms. Precisely characterizing each IEI entity is challenging, as manifestations can be heterogeneous even among patients with the same mutated gene. In this study, we conducted a systematic review of patients documented with NF-κB1 and NF-κB2 defects, two of the most prevalent monogenic IEIs worldwide.
Methods: The search for relevant literature was conducted in databases including Web of Science, PubMed, and Scopus. From these reports, demographic, clinical, immunological, and genetic data were extracted from cases with mutations in *NFKB1* and *NFKB2*.
Results: A total of 430 patients were included in the study, 290 had *NFKB1* mutations, and 140 had *NFKB2* mutations. NF-κB1 and NF-κB2 defects predominant cases (82.7% and 62.5% respectively) initially presented with a CVID-like phenotype. NF-κB1 defect patients often experienced hematologic autoimmune disorders, whereas NF-κB2 patients were more prone to other autoimmune diseases. Viral infections were significantly higher in NF-κB2 defect cases compared to NF-κB1 (P-value < 0.001). NF-κB2 defect patients exhibited a greater prevalence of ectodermal dysplasia and pituitary gland involvement than NF-κB1 patients. Most NF-κB1 and NFκB2 defect cases showed low CD19+ B cells, with NF-κB2 having more cases of this type and low memory B cells being more common in NF-κB1 patients. The identified mutations included H67R and N291Mfs*141 in NFKB1 and R853*, and A867V in NF-κB2, with missense mutations being predominant in NFKB1, and NFKB2 having more cases with nonsense mutations.
Conclusion: Patients with NF-κB2 mutations, particularly p52LOF/IκBδGOF, are at a higher risk of viral infections, pituitary gland involvement, and ectodermal dysplasia compared to those with NF-κB1 mutations. Genetic tests are essential for resolving the initial complexity and confusion surrounding the clinical and immunological features. Emphasizing the significance of functional assays in determining the probability of correlation between mutation and immunological and clinical characteristics of patients is crucial.

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NAD+ as a potential biomarker for chronic lymphocytic leukemia and monoclonal gammopathy of undetermined significance patients with secondary immunodeficiency

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Purpose: Primary immunodeficiencies (PIDs) are genetic disorders affecting immune function, leading to heightened susceptibility to infections and diseases. Secondary immunodeficiencies (SIDs) arise from external factors, such as infections or hematological malignancies such as chronic lymphocytic leukemia (CLL) and multiple myeloma, an advanced stage of monoclonal gammopathy of undetermined significance (MGUS).
We hypothesize that the diminished responsiveness of immune cells to infections and tumors in individuals with immunodeficiencies (ID) could be associated with disruptions in mitochondrial function. Specifically, we postulate that alterations in the levels of the coenzyme nicotinamide adenine dinucleotide (NAD+), which is essential for mitochondrial health, might underlie the compromised state of the immune system. The aim of this study is to assess the NAD+ levels in PBMCs from patients with ID, including CVID, MGUS and CLL, in comparison to those from healthy controls (HC).
Methods: Blood samples were collected from 14 PID patients with CVID; 17 SID patients, including 12 CLL and 5 MGUS patients; and from 19 HC. PBMCs were isolated and NAD+ levels were quantified using a colorimetric assay. Additionally, mitochondrial respiration was studied by measuring the oxygen consumption rate in PBMCs from 3 CLL patients and compared with 3 HC.
Results: Interestingly, NAD+ levels in PBMCs from CVID patients were not significantly different from those in HC (mean values, 7.5 pmole/10^6 cells ±5.38 vs 6.98 pmole/10^6 cells ±2.42; p=0.5773), despite noticeable variability among the CVID patients. Conversely, patients with SID, specifically those diagnosed with CLL and MGUS, exhibited significantly reduced NAD+ levels than HC. CLL patients presented a mean NAD+ level of 3.35 pmole/10^6 cells ±1.46 (p=0.0001), and MGUS patients had a mean of 2.88 pmole/10^6 cells ±2.39 vs 6.98 pmole/10^6 cells ±2.42; (p=0.0028). Furthermore, the PBMCs from the three CLL patients examined displayed an altered mitochondrial bioenergetic profile compared to those from HC.
Conclusion: These data suggest that NAD+ levels could serve as a prognostic and mitochondrial function-related biomarker for diseases such as CLL and MGUS. Our preliminary findings advocate the need to increase our sample size and conduct further research, not only to establish this biomarker, but also to explore novel therapeutic agents.

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1786 – P2.05.24

Exploring SEC61A1 deficiency: a rare genetic cause of common variable immunodeficiency

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Background and Purpose: 53-year-old female patient with no history of consanguinity or recurrent infections during childhood. The patient presents to the emergency department with generalized pruritus and exacerbation of chronic urticaria, with no analytical data suggestive of allergy. In the past year, she had experienced SARS-CoV-2 infection followed by pneumonia, *H. pylori* gastritis, acute conjunctivitis lasting two months, and a new episode of bronchopneumonia, in addition to the urticarial lesions.
The immunological study revealed leukopenia, with slightly decreased levels of NK cells and normal levels of T and B cells, albeit with a moderate reversal of the CD4:CD8 ratio. B cell subpopulations showed markedly reduced levels of non-switched memory B cells and elevated levels of CD21low B cells. Additionally, severe hypogammaglobulinemia and absence of response to polysaccharide vaccines were observed. Abdominal ultrasound revealed mild splenomegaly.
Methods: Exome sequencing with targeted analysis of 464 genes associated with Inborn Errors of the Immune System was performed using the Twist CE2_v2 Kit with the NextSeq 500 sequencing system platform (Illumina). The SOPHiA DDM platform was used for bioinformatics analysis.
Results: One genetic variant present in heterozygosity in the SEC61A1 gene not previously described was identified: a deletion of 2 nucleotides (c.1294_1295del). The genetic variant is considered `Likely Pathogenic´ as it results a premature nonsense codon (p.Ser432Glyfs*4).
Conclusion: SEC61A1 Deficiency is an autosomal dominant (AD) disorder related to Common Variable Immunodeficiency (CVID) primarily characterized by plasmablast alteration. Pathogenic genetic variants within SEC61A1 are extremely rare and have been correlated with distinct pathologies. Initially, also under AD inheritance, it was linked to Tubulointerstitial Kidney Disease, subsequently extending to CVID. Moreover, it has also been associated with severe congenital neutropenia and with pustular psoriasis accompanied by hypogammaglobulinemia. The presence in our patient of recurrent infections, hypogammaglobulinemia, B cell abnormalities and the identification of the genetic variant in heterozygosis in the SEC61A1 gene not previously described lead us to the diagnostic of CVID caused by SEC61A1 Deficiency.

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1805 – P2.05.25

Neutrophil functions in patients with a rare disease: pulmonary alveolar proteinosis

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Purpose: Pulmonary alveolar proteinosis (PAP) represents a rare interstitial lung disease, occurring at a rate of 2-6 per 1.000,000 individuals. The increased mortality associated with infection in PAP patients is closely linked to the presence of autoantibodies against Granulocyte Macrophage-Colony Stimulating Factor (GM-CSF). Whole lung lavage (washing lung with 10-15 litre fluid), stands as the definitive treatment for this severe condition. GM-CSF treatment has shown beneficial for a subset of PAP patients. This study aims to analyse the immunophenotyping and functional analysis of neutrophils in individuals diagnosed with PAP.
Methods: Nine patients with PAP, and ten age- and gender-matched healthy control subjects were included in this study. Isolated neutrophils were stimulated with *Escherichia coli* for phagocytic activity, phorbol myristate acetate for oxidative burst and formyl methionyl-leucyl-phenylalanine for chemotactic activity and analysed by flow cytometry. Given the susceptibility to infections in PAP patients, the ratio of CD3+ T, CD3+CD4+ helper T, CD3+CD8+ cytotoxic T, CD3-CD19+ B and CD3-CD16+CD56+ NK cell ratios were determined using flow cytometry. Additionally, demographic data, biochemical parameters, and complete blood counts were examined.
Results: PAP patients exhibited no significant impairment in vital organ systems. The ratio of lymphocytes to neutrophils, and neutrophil levels did not significantly differ between PAP patients and healthy controls. Furthermore, neutrophil functions, including phagocytic activity, oxidative burst, and chemotactic activity, remained comparable between PAP patients and healthy subjects. These findings suggest that the fundamental functional capacity of neutrophils to combat pathogens and inflammation remains intact in PAP. Notably, percentages of cytotoxic and helper T cells, B cells, and NK cells did not differ significantly between the groups.
Conclusion: Contrary to existing literature indicating impaired anti-microbial functions of neutrophils in PAP patients, our study showed that neutrophil functions in PAP patients were within normal limits. In our study six symptomatic PAP patients had a history of GM-CSF treatment, while the remaining three of them had a non-symptomatic disease. This could have affected the neutrophil functions and might be the underlying reason for the indifference between groups.

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1877 – P2.05.26

The in vitro functional memory B cell response is intact in most patients with Common Variable Immunodeficiency Disorder

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Purpose: Common Variable Immunodeficiency Disorder (CVID) is the most prevalent primary immunodeficiency characterized by defects in B cell maturation or differentiation. Patients suffer from decreased levels of class-switched (CS) memory B cells (MBC) and CS plasmablasts (PB) resulting in hypogammaglobulinemia, poor vaccine responses and inability to produce pathogen-specific antibodies. Therefore, patients are prone to more frequent and severe infections and need life-long Immunoglobulin replacement therapy. The underlying pathogenesis of impaired antibody secreting cell (ASC) differentiation is still poorly understood. Here, we aimed to characterize functional defects of MBC differentiation and to restore ASC generation *in vitro* in CVID patients.
Methods: We examined *in vitro* generation of IgG, IgA and IgM ASC from CVID patients (n=15) and healthy controls (HC, n=10) by stimulating PBMCs for 7 days with a T cell depended (TD) and T cell independent (TI) protocol. Functional MBC response was investigated using ELISpot technique in combination with flow cytometry B cell phenotyping. Quantity and quality of secreted antibodies were assessed by ELISA.
Results: Using our stimulation protocols we were able to drive differentiation of MBCs into class-switched (CS) PB as main source of ASC in all CVID patients, albeit with lower frequencies compared to HC. ELISpot analysis revealed IgG responses in 14/15 CVID patients upon TD stimulation comparable to HC, whereas significantly less patients (n=8) exhibited IgG responses following TI stimulation (p=0.0013). In terms of IgA secretion, both stimulation protocols elicited significantly less IgA secretion in 8/15 patients compared to HC (TI p=0.0286; TD: p=0.0002). All patients had the capability to generate IgM from IgM-only PB induced by TD stimulation and 12/15 from TI stimulation. *In vitro* generated CS PB of CVID patients were shown to secrete protective IgG antibodies against different antigens, including *Streptococcus pneumonia*. Autoantibodies targeting double-stranded DNA were not induced.
Conclusions: Our data indicate almost normal functionality of MBC and IgG/IgM ASC in the majority of patients examined, when subjected to adequate TD stimulatory signals. This opens up future opportunities for potential clinical translation of targeted and adoptive cell therapy in CVID.

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1914 – P2.05.27

The role of LFA-1 and regulatory T cells in skin inflammation

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Purpose: Leukocyte-specifically expressed β2-integrins that are composed of a variable alpha (CD11a-d) and a constant beta (CD18) subunit are essential for cell-cell communication, the extravasation of leukocytes from blood into inflamed tissues, and cell signaling. Humans with reduced or defective β2-integrin expression develop Leukocyte Adhesion Deficiency Syndrome Type-1 (LAD-1). The most prominent clinical symptoms of LAD-1 are profound leukocytosis, high susceptibility to infections and the development of autoimmune diseases. Leukocyte function-associated antigen 1 (LFA-1) is the only β2-integrin family member expressed on T cells. We generated mice specifically lacking LFA-1 on regulatory T cells (Treg) ($CD18^{Foxp3}$) to investigate the cell-specific role of LFA-1 on Treg.
Methods: $CD18^{Foxp3}$ mice were sensitized and challenged topically with oxazolone to induce contact dermatitis responses. Ear and skin-draining lymph node single-cell suspensions were subjected to flow cytometry to assess their immunological phenotype. Enzyme-linked immunosorbent assay was performed to evaluate IgE, IgG1 and IgG2a levels in serum. Single-cell RNA sequencing of splenic Treg and gene enrichment analysis was performed to delineate the role of LFA-1 for the immunophenotype of Treg.
Results: We observed profound systemic T_H2 pathway-based autoinflammation in $CD18^{Foxp3}$ mice that correlated with age showing also massively elevated IgE, IgG1, IL4, eosinophilia and lung tissue damage in the steady state. Furthermore, $CD18^{Foxp3}$ mice developed aggregated T_H2-driven immune responses in a model of allergic contact hypersensitivity associated with an expansion of T_H2-promoting $IRF4^+$ $PDL2^+$ cDC2. Gene expression profiles of LFA-1-deficient Treg revealed elevated expression of T_H2-related receptors and transcriptionfactors (e.g. Gpr15, Klrg1, IL18R, IL33 and Gata3) associated with atopy and characteristic for tissue repair Treg.
Conclusion: Our results suggest that the dysregulated expression of genes associated with suppressive activity in β2-integrin-deficient Treg may cause systemic T_H2-polarization. This in turn may drive the manifestation of the T_H2-dominated phenotype of $CD18^{Foxp3}$ mice. We conclude that LFA-1 deficient Treg are not able to induce a tolerogenic state in DC and to counteract T_H2-driven allergic responses. Altogether, our study demonstrates an indispensable role of LFA-1 on Treg for maintaining immune homeostasis.

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1956 – P2.05.28

Expanding the clinical phenotype of TRAF3 haploinsufficiency syndrome

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TRAF3 (TNF receptor-associated factor 3 protein) is one of the most diverse adaptor proteins of the TRAF family, with roles in various signaling pathways. Autosomal dominant TRAF3 deficiency had been associated to herpes simplex virus-1 encephalitis, and a recent study reported the TRAF3 haploinsufficiency (TRAF3Hl) as a condition including recurrent bacterial infections, autoimmunity, systemic inflammation, and hypergammaglobulinemia.
The purpose of this study was to conduct a genetic re-analysis of our cohort of patients with inborn errors of immunity (IEI), in whom Next-Generation Sequencing (NGS) was previously performed, to identify cases of TRAF3Hl and describe its impact on human immunity through the characterization of clinical and molecular features.
We performed a TRAF3 targeted re-analysis of NGS data from 800 patients with IEI from Hospital Vall d'Hebron (Catalonia, Spain). This involved comprehensive clinical and genetic characterization of identified patients, including familial studies to elucidate inheritance patterns and phenotype-genotype correlations. Additionally, western blot and flow cytometry immunophenotyping were performed to enhance the characterizations.
Three patients (P1, P2 and P3) from two different families with TRAF3Hl were identified in our cohort. Genetic analysis revealed stop-gain heterozygous TRAF3 variants that were de novo in one patient (P3) and inherited (P1) from her symptomatic mother (P2). All patients exhibited recurrent sinopulmonary infections, lymphoproliferation, various types of gastrointestinal complications, and had previously been diagnosed of common variable immunodeficiency (CVID). However, P2 also had clinical manifestations consistent with T-cell dysfunction. Immunological studies showed that all three patients had low absolute CD4+ counts with a low CD4+/CD8+ ratio, impaired B cell maturation, and a profound reduction of naive subpopulations in both, CD4+ and CD8+ T cells. P2 and P3 showed altered lymphoproliferative function, with P2 showing more severe impairment.
Our research highlights the significance of periodically reanalyzing genetic data, especially in response to novel phenotype descriptions. While our patients initially resembled CVID cases, we observed a wider spectrum of phenotypes, including combined immunodeficiency and immune dysregulation. These differences may reflect the phenotypic variability in TRAF3Hl. Our findings broaden the clinical phenotype associated with TRAF3Hl, potentially aiding in the identification of similar cases among patients previously diagnosed with CVID.

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1987 – P2.05.29

AD-HIES patients with impaired STAT3 signalling contain pre-Th17-cells that are activated by opportunistic pathogens to produce IL-10

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Background: STAT3 is critical for T-cell differentiation, and dominant-negative STAT3 mutations cause Autosomal Dominant-Hyper-IgE Syndrome (AD-HIES). AD-HIES patients lack IL-17-producing CCR6^{+}T-cells and present consequently recurrent infections with opportunistic bacteria and fungi.
Objective: We previously identified a population of IL-10 producing CCR6^{+}T-helper cells, and asked if they were STAT3-dependent and pre-committed to a Th17 fate (pre-Th17).
Methods: CCR6^{+}T-cell subsets from the blood of healthy donors and AD-HIES patients were analysed by multi-dimensional flow cytometry for phenotype, cytokine production, differentiation potential and responsiveness to pathogens.
Results: CCR6^{+}T-helper cells that lacked other differentiation markers (CCR6$^{\text{single-positive(SP)}}$), were central memory T-cells (T_{CM}) that expressed some RORγt, but produced only low levels of Th17 effector cytokines. Non-polarized CCR6^{SP}T-cells efficiently acquired IL-17 and IL-22 producing capacities upon TCR stimulation in the absence of polarizing cytokines. IL-12 induced in addition IFN-g and thus Th1/17 differentiation. CCR6^{SP}T-cells produced IL-10 upon TCR stimulation that activated STAT3 and promoted Th17 differentiation in an autocrine manner. In AD-HIES, CCR6^{+}T-cells were strongly and selectively reduced, but CCR6$^{SP}T_{CM}$ and Th1/17-cells were nevertheless present. Residual CCR6^{+}T-cells in AD-HIES were activated *in vivo* and produced increased amounts of IL-10. Moreover, they were activated by antigens derived from opportunistic pathogens to produce IL-10, but not IL-17. Upon stimulation with bacteria they produced also high levels of IL-2 and IFN-g.
Conclusions: Th17 differentiation in AD-HIES patients with impaired STAT3 signalling in response to pathogens is not completely blocked, but arrested at an intermediate stage of pre-Th17-cells, which produce IL-10 and could directly differentiate to Th1/17-cells.

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2017 – P2.05.30

Heterozygous SERPINA1 defects and their impact on clinical manifestations of patients with predominantly antibody deficiencies

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Introduction: Patients with predominantly antibody deficiencies (PAD) display hypogammaglobulinemia and pulmonary and hepatic manifestations irrelevant to autoimmunity. Therefore, additional genetic polymorphisms, may affect their clinical and laboratory phenotype. Alpha-1 antitrypsin (AAT) encoded by *SERPINA1* gene has antiprotease activity along with the ability to modulate both inflammation and apoptosis leading to lung and liver dysfunction and might contribute to PID phenotype.
Purpose: The study was conducted to investigate the possible contribution of *SERPINA1* defects in the clinical phenotype of patients with PAD.
Methods: A total of 80 PAD patients enrolled in the study, including 70 with common variable immunodeficiency (CVID), a patient with combined IgA and IgG subclass deficiencies with a CVID-like clinical phenotype, two patients with pathogenic *CTLA4* mutations and seven patients with unclassified hypogammaglobulinemia (displaying mild to moderate) with recurrent infections and a negative work-up for secondary immunodeficiencies. Genomic DNA was extracted from peripheral blood and a PCR amplification of all five exons (including exon–intron boundaries) of the *SERPINA1* gene was performed. Thereafter, PCR products were purified and sequenced.
Results: *SERPINA1* genetic analysis revealed both pathogenic and non-pathogenic defects in 67 out of 80 participants (84.0%). Among them, 10 patients (12.5%) exhibited pathogenic defects, all in heterozygous state; interestingly, all these patients suffered from CVID. The pathogenic defects include PI*Z variant, PI*S variant, rs61761869, rs1379209512 and rs77598233. The presence of the Z allele (rs28929474) was found in three patients and was significantly associated with liver disease (Odds Ratio: 29.57, p=0.006). Additionally, hepatic complications were observed in two more patients carrying the p.Leu23Gln (rs1379209512) and the p.Phe76del (rs775982338) alleles, respectively. Conversely, no correlation of *SERPINA1* defective variants was observed with respiratory complications, although patients with pathogenic variants exhibit a reduced probability of developing autoimmune diseases compared to those without pathogenic defects (Odds Ratio: 0.17, p-value=0.03).
Conclusion: The present study clearly demonstrates that CVID patients and defective *SERPINA1* variants may display a higher probability of developing hepatic complications. We recommend *SERPINA1* genetic analysis in PAD, in order to identify patients with a higher risk for liver disease.

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2065 – P2.05.31

Lymphocyte subsets analysis in adult patients affected by common variable immunodeficiency

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Common variable immunodeficiency (CVID) is marked by deficient immunoglobulin concentrations and causes recurrent airway and gut infections and several complications.
This study aims to classify CVID patients using the Freiburg and the EUROclass classification, which combines the Freiburg and Paris classifications, by measuring B cell subsets of switched memory (sm) and $CD21^{+low}$ in peripheral blood samples and assessing the correlation between the specific CVID classes and changes in T cell populations. Additionally, we checked Ig titers, C3 and C4 concentrations in each sample, and Anti-Nuclear Autoantibodies (ANA) to identify correlations with CVID patient categories.
We analysed B and T cell subsets by flow cytometry, both as frequency and absolute count, in 25 CVID adult patients treated with Ig replacement therapy.
Results often display a normal B-cell frequency but a significant alteration in the frequency and absolute count of B-cell subsets, compared to the reference ranges, in all the patients. In almost all CVID samples, both follicular-like and regulatory T cells had very low frequency and absolute counts. According to the Freiburg or EUROclass classifications, the ANA positive test is often associated with groups "2 or 1b" and "$smB+21^{norm}$", respectively. Additionally, IgA titres were higher in groups "2" and "smB+21norm". The Freiburg group 1b displays a sm frequency <0,4% and a $CD21^{+low}$ frequency <20%, while group 2 display a sm frequency >0,4%. The EUROclass $smB+21^{norm}$ group displays a sm>2% frequency and a $CD21^{+low}$ frequency < 10%.
In conclusion, improving patient classification could provide helpful insight into the follow-up of CVID patients.

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2106 – P2.05.32

Immunological and genetical characterization of novel homozygous DIAPH1 variants

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Purpose: Diaphanous-related Formin 1 (DIAPH1) deficiency is associated with microcephaly, developmental delay, early infancy epilepsy, vision loss and recently with combined immunodeficiency. The observed immunological defects marked DIAPH1 biallelic loss of function as an Inborn Error of Immunity (IEI). This study aims to investigate and expand the genetical, clinical and immunology data of multiple patients with DIAPH1 deficiency.
Methods: Clinical, laboratory information, genetic analysis and comprehensive immunophenotyping via a Multi-color flow cytometry panel was used to investigate T-cell lymphopenia and B-cell maturation deficits in DIAPH1 deficient patients.
Results: DIAPH1 Biallelic loss of function mutational spectrum included intronic variants. The immunological clinical phenotype is that of a combined immunodeficiency, with recurrent bacterial sinopulmonary, viral and fungal infections. Although basic B-cell, T-cell numbers and serum immunoglobulins may be low or normal, there is a distinct lack of naïve CD4 and CD8 T cells. Markers of senescence on T-cells, such as CD57 and exhaustion TIGIT are typically raised, as is PD-1.
Conclusions: The mutational catalog of DIAPH1 is diverse and diagnosis might be missed in intronic cases. Although the typical clinical features of DIAPH1 are neurological with microcephaly, epilepsy, severe developmental delay and blindness, more subtle immune abnormalities are also a feature, partially in terms of lack naïve T-cells and increased senescence of mature T-cells. The presented abnormalities may promote frequent infections with viruses, fungal and particularly bacterial sinopulmonary infections. Highlighting that indeed DIAPH1 is IEI affecting the adaptive immune response in T cells.

Keywords IEI, DIAPH1, immunodeficiency, T-cell lymphopenia, microcephaly

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2130 – P2.05.33

Associations between prenatal per- and polyfluoroalkyl substances (PFAS) exposure and antibody response to childhood vaccines in Norwegian children

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Purpose: Per- and polyfluoroalkyl substances (PFAS) are immunotoxicants, and there is a need to study their effects in populations with different exposures. We assessed prenatal PFAS exposure and vaccine antibody response in Norwegian school-aged children.
Methods: We combined two sub populations of the Norwegian Mother, Father and Child Cohort Study (MoBa, 2002-2009): HELIX (n=268, aged 7-10 years) and the Norwegian Environmental Biobank II (n=642, 7-14 years). PFAS were measured in maternal 2nd trimester and childhood samples (five different PFAS in HELIX and up to 12 in NEB II). 10 persistent organic pollutants (POPs) were also measured. Antibody responses (IgG) to tetanus, diphtheria and rubella were measured in childhood samples using in-house multiplex immunoassays. We assessed single-pollutant associations between PFAS and IgG, adjusted for covariates, and stratified analyses by sex and age (<12 yrs/ ≥12 years). Analyses were restricted to those with the recommended vaccine doses (DTP 4 doses, MMR 2 doses).
Results: In children under 12 years, per interquartile range (IQR) increase in exposure, prenatal perfluorononanoic acid (PFNA) was associated with higher diphtheria antibodies in boys (β=0.19, 95% CI: 0.02–0.36). In children 12 years and over, perfluorooctanoic acid (PFOA) levels were associated with lower diphtheria antibodies, particularly in girls (β=-0.39, 95% CI: -0.75– -0.02), while perfluorooctane sulfonic acid (PFOS) was associated with lower levels only in boys (β=-0.42, 95% CI: -0.84– -0.01). Additional adjustment for POPs did not affect results. Similar trends were found for tetanus and rubella antibodies.
Conclusion: Prenatal PFAS exposure may have differing effects on the immune response based on child's age and sex, possibly due to puberty. We will further investigate mixture effects and current exposure.

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2199 – P2.05.34

Heterozygous LIG4 gene variant in a patient diagnosed with common variable immunodeficiency: a case study

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Introduction: Common Variable Immunodeficiency (CVID) represents a set of deficiencies in antibodies with a complex, heterogeneous and polygenic nature that cause hypogammaglobulinemia and a defect in the production of specific antibodies. Patient's phenotypes are highly heterogenous due to a high variety of related complications, such as autoimmune manifestations, lymphoproliferation, enteropathy, and lymphoid malignancies, suggesting that CVID could be a common outcome of diverse immune system failures. Exome sequencing is a cost effective approach to identify disease-causing mutations in CVID patients with severe phenotypes. Recently, monoallelic LIG4 missense mutations have been reported as a novel cause of immune disregulation in patients with hypogammaglobulinemia, lymproliferation and autoimmunity.
Objectives: To describe a case of a patient meeting CVID criteria with significant pulmonary and hepatic involvement that presents a novel missense mutation in LIG4.
Case report: A 67-year-old female patient was referred to the Immunology department due to severe hypogammaglobulinemia and longstanding splenomegaly. She experienced recurrent respiratory infections, with some requiring hospitalizations. Additionally, she was monitored for liver cirrhosis and portal hypertension. The patient also had autoimmune hypothyroidism. The analysis showed intense panhypogammaglobulinemia, absence of isohemagglutinins, and low levels of antibodies, including a poor response to vaccination. An inversion in the CD4/CD8 ratio was observed due to TCD4+ lymphopenia. Switched memory B cells were diminished. The leukocyte count was within the normal range, while the platelet count was reduced. Chest CT revealed subcarinal lymphadenopathies, bronchiectasis, and findings suggestive of lymphoid granulomatous disease. Biopsy results showed non-caseating granulomas and lymphoid hyperplasia, raising suspicion of sarcoidosis, although it was not confirmed. Additionally, lymphoproliferative syndrome was ruled out. Recently, the patient has also been diagnosed with cholangiocarcinoma.
Exome sequencing analysis was performed and a novel missense mutation (Leu903Ser) was identified in the LIG4 gene, classified as a variant of uncertain significance (VUS). This mutation lies within the BRCT 2 domain of the protein.
Conclusions: The disease phenotype closely resembles that recently described for the first time as being secondary to heterozygous LIG4 mutations resulting in haploinsufficiency. This suggests that the variant found in LIG4 may play an important role in the observed immunological dysregulation in the patient.

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2239 – P2.05.35

Distinct inflammation profiles in pregnant women living with and without human immunodeficiency virus in Cameroon an their children associate with lower anti-respiratory syncytial virus seroconversion

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Purpose: During HIV disease, several biomarkers of inflammation and immune activation increase and remain elevated despite antiretroviral therapy (ART). Chronic inflammation and immune activation are key features of HIV disease and have been shown to impact both disease progression and various immune responses. The present study aimed to investigate inflammation and immune activation in HIV+ women in Cameroon and their HIV-exposed uninfected children.
Methods: Blood samples from 106 pregnant, HIV+ women receiving ART and 103 pregnant, HIV- women were collected during the 3rd trimester. Additionally, cord blood and peripheral blood samples were collected from the children at birth and at 3 months of age. Soluble biomarkers of inflammation measured in plasma included markers for microbial translocation (IFABP, sCD14) and 21 cytokines or chemokines covering angiogenesis, innate cytokines, Th1, Th2 and Treg cytokines. Frequencies of activated, exhausted and senescent T-cell subsets were measured in PBMCs. In addition, CMV and RSV antibodies were measured in plasma.
Results: While sCD14, IFABP and MCP-1 plasma levels were elevated in HIV+ compared to HIV- pregnant women in Cameroon, no difference was observed for other soluble markers in plasma or for T-cell subsets. Assessment of CMV seropositivity revealed a 90-100% CMV prevalence in the Cameroon cohort, compared to 50% prevalence in Germany. Irrespective of HIV status, participants from Cameroon had significantly higher levels of various plasma cytokines compared to participants from Germany, denoting unusually high grade of inflammation in the HIV-negative women from Cameroon. Moreover, several inflammation measures were positively correlated to each other across participants from Cameroon, denoting distinct profiles within the cohort. This observation was also made in the cord blood of HIV-exposed and -unexposed children. Intriguingly, various pro-inflammatory markers (e.g. IL-1b, IL-6) in cord blood plasma negatively correlated with anti-RSV antibodies in the cord blood. Lastly, HIV-exposed children showed significantly lower RSV seroconversion rate at three months of age compared to HIV-unexposed children.
Conclusion: Our study suggests a link between high levels of inflammation in pregnant mothers in Cameroon, which, irrespective of HIV, may limit the transfer of maternal antibodies and have consequences for long-term immunity in newborns.

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P2.06 IMMUNE EXHAUSTION

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63 – P2.06.01

Evidence of exhausted lymphocytes after the third anti-SARS-CoV-2 vaccine dose in cancer patients

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Introduction: Evidence is scant regarding the long-term humoral and cellular responses Q7 triggered by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) mRNA vaccines in cancer patients after repeated booster doses. The possibility of T-cell exhaustion following these booster doses in this population has not yet been fully studied and remains uncertain.
Methods: In this single-center prospective observational study, we explored the specific humoral and cellular response to S1 antigen in 36 patients with solid malignancies at baseline, and after the second and third doses of the mRNA-1273 vaccine.
Results: A dual behavior was observed: 24 (66.7%) patients showed partial specific IFN-γ response after the second dose that was further enhanced after the third dose; and 11 (30.5%) already showed an optimal response after the second dose and experienced a marked fall-off of specific IFN-γ production after the third (4 patients negativization), which might suggest T cell exhaustion due to repetitive priming to the same antigen. One (2.8%) patient had persistently negative responses after all three doses. Seroconversion occurred in all patients after the second dose. We then studied circulating exhausted CD8+ T-cells in 4 patients from each of the two response patterns, those with increase and those with decrease in cellular response after the third booster. The patients with decreased cellular response after the booster had a higher expression of $PD1^+CD8^+$ and $CD57^+PD1^+CD8^+$ exhausted T cells compared with those with an increased cellular response both *in vivo* and *in vitro*. The proportion of $PD1^+CD8^+$ and $CD57^+PD1^+CD8^+$ exhausted T cells inversely correlated with IFN-γ production.
Discussion: Our preliminary data show that the two-dose SARS-CoV-2 vaccine regimen was beneficial in all cancer patients of our study. An additional booster seems to be beneficial in suboptimal vaccine seroconverters, in contrast to maximal responders that might develop exhaustion. Our data should be interpreted with caution given the small sample size and highlight the urgent need to validate our results in other independent and larger cohorts. Altogether, our data support the relevance of immunological functional studies to personalize preventive and treatment decisions in cancer patients.

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212 – P2.06.02

Effect of Pfizer-BioNTech COVID-19 vaccine booster doses: implications for vaccination strategies in healthcare workers

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Introduction: The booster vaccine against SARS-CoV-2 is indicated in at-risk groups, including those aged 60 and older, chronic illnesses or immunocompromised individuals, and healthcare workers. Recent scientific attention has turned towards dose tolerance and immunological exhaustion, particularly in response to mRNA-based vaccines, which show a notable increase in IgG4 levels with repeated administrations compared to adenovirus-based vaccines. Preclinical trials have indicated a potential decrease in T cell response and an increase in exhaustion markers following repeated antigen exposure. However, there is a scarcity of studies on the effects of COVID-19 booster doses.
Methods: Thus, we aim to investigate both humoral and cellular responses to the fourth dose (second booster dose) of the Pfizer vaccine in 37 healthy individuals with no declared history of COVID-19 symptoms, stratified by age (Young, N=13; Middle-aged, N=14; Old, N=10), 10-12 months after the first booster dose (third dose) of Pfizer vaccine (T1) and 3-5 weeks after the second booster dose (T2). We measured spike protein IgG antibody serum levels and evaluated PBMC cytokine production in response to SARS-CoV-2 spike protein stimulation using multiparametric flow cytometry.
Results: Older individuals showed lower antibody levels initially but experienced a significant increase after the booster dose ($p<0.01$), suggesting they benefit most from additional vaccination. Individuals with lower initial antibody levels exhibited greater fold changes in response to the booster dose. We identified two groups based on their response: vaccine responders (VR) and non-responders (VnR). As expected, VR individuals showed increased antibody levels post-booster ($p<0.0001$). Surprisingly, VnR individuals, predominantly young and middle-aged (87%), exhibited decreased levels. The functionality assay showed similar T cell responses in both groups.
Conclusion: The high levels of IgG spike antibodies presented in some individuals could be blocking the antigen of the vaccine, affecting the humoral response. Despite no signs of immune exhaustion in our cohort, repeated antigen exposure could potentially induce this state, as seen in preclinical trials. These findings suggest that the vaccination schedule for healthcare workers in these age groups should be reconsidered, as the current annual booster strategy could lead to a poorer response to future SARS-CoV-2 infections.

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285 – P2.06.03

Characterizing tumor microenvironment-mediated exhaustion in CD8 T Cells: Insights from a hepatocarcinoma model

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Purpose: CD8 T cells play a pivotal role in tumor immunity; however, within the tumor microenvironment (TME), they often become functionally hyporesponsive (exhausted), hindering anti-tumor responses. Recent studies have identified distinct exhaustion profiles in T lymphocytes, depending on the type of chronic antigenic stimulation they encounter. Despite considerable progress in T cell-reinvigorating immunotherapy using checkpoint inhibitors, only a subset of cancers responds to these treatments. This highlights the need for a deeper understanding of the role played by the tumor microenvironment (TME) in contributing to T cell exhaustion. This project aims to unravel genetic and epigenetic programs guided by the TME during CD8 T cell differentiation toward dysfunctional states.
Methods: We developed a murine hepatocarcinoma model using oncogene-expressing plasmids via hydrodynamic transfection. Plasmids also expressed ovalbumin (OVA), serving as a surrogate tumor-specific antigen, enabling tracking antigen-specific T cell fate. Hydrodynamic injection of ovalbumin-expressing plasmid in the absence of oncogenes drove the expression of OVA in non-transformed (healthy) hepatocytes. Transfer of OVA-specific lymphocytes in these mice allowed us to follow antigen-specific T cell fate in response to the same antigen in the context of healthy or advanced hepatocarcinoma liver environment.
Results: Antigen-specific T cells exhibited exhaustion features (PD1, TOX, etc…) in all mice, but tumor-bearing mice expressed more advanced T cell exhaustion with impaired functionality (reduced IFNg and TNFa production). RNAseq and ATACseq analyses revealed consistent transcriptional and epigenetic signatures of exhausted CD8 T cells, when subjected to tumor or healthy liver chronic antigenic stimulation. Single-cell transcriptomic studies provided further insights into the heterogeneity within exhausted T cells, facilitating the identification of candidate genes for subsequent in vivo and in vitro testing.
Conclusion: Distinct exhaustion profiles associated with advanced hepatocarcinoma highlight a potential for targeted interventions and personalized immunotherapies to alleviate CD8 T cell exhaustion.

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299 – P2.06.04

Exclusive expression of PD-1/TIGIT versus TIM3 differentially associates with metabolic plasticity and polarization of HIV-specific CD8+ T cells

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Purpose: Response of HIV-specific CD8+ T cell from people with HIV (PWH) to dendritic cell (DC) depends on the co-expression of PD-1, TIGIT and TIM3. However, mechanisms underlying these associations remain unclear and could be crucial to improve immunotherapies against HIV-1. In this study, we characterized CD8+ T lymphocytes defined by the expression of these receptors at the transcriptional, metabolic and functional levels.
Materials and methods: PD-1/TIGITDP (PD-1+TIGIT+TIM3-), TIM3SP (PD-1-TIGIT-TIM3+) and TN (PD-1-TIGIT-TIM3-) central memory CD8+ T cells (n=4 healthy donors) induced after 5 days in culture with allogeneic DCs activated with STING and TLR3 agonists were used for transcriptional studies by RNA-seq. Computational analysis of differentially expressed genes (DEG) of each population compared to TN cells was performed using Ingenuity Pathways Analysis. Validation of selected DEG, mitochondrial mass and ROS (MitroTracker and MitoSOX) and polarization in response to Gag peptides (IFNg, IL-13, CD107a) of CD8+T cells was performed using n=25 PWH.
Results: DEG identified in TIM3SP cells (1671) were differentially associated with upregulation of modulators of oxidative phosphorylation, TCA cycle, amino acid and lipid metabolism expression in contrast to PD-1/TIGITDP lymphocytes (866), which preferentially transcribed genes associated with immune exhaustion and mitochondrial dysfunction. Consistently, higher ratios of cells with TCF1hi vs ToxLow positive cells were detected in TIM3SP but not in PD-1/TIGITDP CD8+ T cells from PWH (p=0.0059). Also, PD-1/TIGITDP cells exhibited significantly higher (p=0.002) mitochondrial stress levels defined by ROS accumulation than TIM3SP from PWH. In addition, FACS analysis validated upregulated expression of ARG2 (p=0.0012) and MGST2 (p=0.0674) in TIM3SP from PWH, involved in amino acid and lipid metabolism, respectively. These metabolic patterns associated with higher transcription of genes related to degranulation and Tc1 in TIM3SP, and with higher polarization to Tc2 in PD-1/TIGITDP CD8+ T cells that demonstrated high IL-13 secretion (p=0.002) in response to gag peptide stimulation.
Conclusion: TIM3SP cells represent a less exhausted, metabolically plastic population with cytotoxic profiles compared to exhausted PD-1/TIGITDP cells, predisposed to induce Tc2 responses against HIV-1. Thus, our data may be relevant to improve immunotherapies against HIV.

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387 – P2.06.05

Atf7ip-deletion results in attenuated T cell exhaustion and reduced tumor growth

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ATF7ip is an epigenetic regulator, involved in the formation of the H3K9me3 histone mark and is highly expressed in immune cells. Previously, we have shown increased memory and effector responses in mice with *Atf7ip*-deletion after acute infection with LCMV Armstrong. This enhanced effector and memory response was due to the difference in H3K9me3 deposition at key immune gene loci. Here we interrogate *Atf7ip*-deletion in T cells with chronic infections and cancer.
CD4-Cre/*Atf7ip*$^{fl/fl}$ mice infected with LCMV clone13 showed increased GP33 tetramer positive CD8 T cells compared to *CD4*-Cre/*Atf7ip*$^{+/fl}$ mice. Transfer experiments with congenially distinct P14+/*Atf7ip*$^{+/fl}$ or P14+/*Atf7ip*$^{fl/fl}$ CD8+ T cells in a host mice infected with LCMV clone 13 resulted in significantly higher Tcf1+ *Atf7ip*$^{fl/fl}$ CD8+ T cells and higher cytokine secretion (TNFα and IFNγ) in *Atf7ip*$^{fl/fl}$ CD8+ T cells. Using B16GP33 tumor cells, we found that *CD4*-Cre/*Atf7ip*$^{fl/fl}$ mice had enhanced tumor immune response and survival. Expression profiling with tumor infiltrating lymphocytes (TIL's) revealed higher GZMK positive cells and increased expression of genes responsible for a T cell exhaustion signature in the *CD4*-Cre/*Atf7ip*$^{+/fl}$ mice. Single cell RNAseq using TIL's from *CD4*-Cre/*Atf7ip*$^{fl/fl}$ mice and *CD4*-Cre/*Atf7ip*$^{+/fl}$ mice showed higher CD4+ T cells present in the tumor of *CD4*-Cre/*Atf7ip*$^{fl/fl}$ mice along with enhanced expression of *Il7r*. Flow experiments with these mice confirmed downregulated expression of *Tox* in TIL's from CD4-Cre/ *Atf7ip*$^{fl/fl}$ mice. To understand the intrinsic CD8+ response, we carried out a second single cell RNAseq experiment using a transfer model with P14+/*Atf7ip*$^{+/fl}$ or P14+/*Atf7ip*$^{fl/fl}$ CD8+ T cells co-injected with B16gp33 tumor cells in C57BL/6 mice. We found that the exhaustion cluster was highly depleted in P14+/*Atf7ip*$^{fl/fl}$ CD8+ T cells along with downregulated expression of *Tim3* and an enhanced memory signature.
Based on our findings, deletion of *Atf7ip* may result in increased progenitor population under chronic conditions that results in the anti-tumor response of *Atf7ip*$^{fl/fl}$ CD8+ T cells. These results demonstrate the therapeutic potential of *Atf7ip*-deletion in T cells that can help in improved tumor control and effective T cell-based therapy.

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660 – P2.06.07

NLRP3 activation of macrophages regulates intratumoral T cell dysfunction in a pancreatic cancer model - dual mechanism of NLRP3-mediated cytokine secretion and PD-L1 expression

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Purpose: Intratumoral cytotoxic $CD8^+$ T lymphocytes (CTLs) enter a dysfunctional state called T cell exhaustion, described by the loss of effector function and specific changes in the transcriptional profile. One prominent mechanism that results in T cell exhaustion is mediated by T cellular IL-18 receptor (IL-18R) signaling. IL-18 is activated and secreted by the NLRP3 inflammasome, commonly expressed in macrophages. However, macrophage and T cell interaction mechanisms regarding IL-18R-mediated T cell exhaustion remain unknown. This work will investigate the role of myeloid NLRP3-derived IL-18 on T cell plasticity and motility.
Methods: A 2D coculture system of bone marrow-derived macrophages (BMDMs) with OT-1 $CD8^+$ T cells was used to investigate the role of myeloid NLRP3-derived IL-18 on T cell plasticity in the context of T cell exhaustion. Additionally, to analyze T cell motility using a 3D TruLive light-sheet microscope, we established a murine pancreatic spheroid model of PancOVA cells with BMDMs and OT-1 CTLs. Furthermore, a murine pancreatic cancer model was utilized to characterize the TME of wildtype and *Nlrp3*$^{-/-}$ mice.
Results: *In vitro* activation of the NLRP3 inflammasome by LPS and Nigericin treatment resulted in the secretion of IL-1β and IL-18 in wild-type macrophages but also caused increased levels of PD-L1, rendering those macrophages more immunosuppressant. In a murine pancreatic tumor model, NLRP3-deficient TAMs had low PD-L1 levels compared to wild-type TAMs. Supplementation of IL-1β in cell culture experiments indicated that lack of myeloid-derived IL-1β results in decreased expression of PD-L1 *in vivo* and, thus, a less immunosuppressive phenotype of these TAMs. Additionally, a less prominent subset of exhausted intratumoral $CD8^+$ T cells in the tumors of NLRP3-deficient mice was found.
Conclusion: Our results highlight the NLRP3 inflammasome and NLRP3-derived cytokines as potential regulators of an immunosuppressive tumor milieu. In a pancreatic tumor model, a dual mechanism of NLRP3-mediated cytokines IL-18 and IL-1β and the PD-1:PD-L1 axis was demonstrated to promote intratumoral T cell dysfunction.

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767 – P2.06.08

Dysregulated inflammatory response and poor fracture healing in polytrauma

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Purpose: Bone healing is primarily concerned with treating isolated fractures in orthopedic patients. However, fractures in over 30% of polytrauma patients with multiple injuries exhibit impaired healing. There are significant gaps in our understanding of how fractures heal in this polytrauma environment. We aimed to characterize the temporal local and systemic immune responses to polytraumatic injuries in a polytrauma murine model using four experimental groups including healthy, chest trauma, isolated fracture and polytrauma groups.
Methods: To study the local and systemic immune responses and cytokine expression after injury, we collected serum, bone marrow from uninjured limb, femur, and lungs at 0 h, 6 h, 12 h, 24 h, 72 h & 3W following injury. Immune cell distribution from isolated bone marrow, femur, or lung tissue were assessed with a BD Fortessa 18-color flow cytometer. Femur tissues were collected after 3 weeks to study fracture healing using micro computed tomography, histological staining, immunohistochemistry, torsion test and small angle X-ray scattering.
Results: Our flow cytometry results indicated significantly higher expression of innate immune cells in the polytrauma group compared to other groups locally and systemically. In contrast, the expression of B and T cells was dramatically suppressed in the polytrauma group at 6h following injury and remained low throughout the 3 weeks' timeline of the study. The decreased expression of B and T cells demonstrated an exhaustion of the adaptive immune system response that could be a contributor to fracture nonunion.
Conclusion: Our data confirms the early, dysregulated inflammatory state in polytrauma correlated with impaired fracture healing and the formation of a poor and dysregulated callus in this polytrauma group. Taken together, this study elucidates the role of persistent immune and inflammatory dysregulation that leads to poor fracture healing.

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1072 – P2.06.09

A pseudo-temporal framework analysis of exhausted CD8 T cells (Tex) across PD-1 therapy revealed limited "reinvigorability" of exhausted progenitor cells and underlying regulatory pathways

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Exhaustion of CD8 T cells limits immunotherapy outcomes. Targeting the immune checkpoint PD-1 temporally relieve some functional constraints on exhausted CD8 T cells (T_{EX}) but most patients do not experience long-term benefits. As acquired resistance mechanisms to checkpoint therapy begin to be elucidated, whether cell-intrinsic regulatory mechanisms also restrict durability of T_{EX} cell responses to PD-1 blockade remains poorly explored. To identify such mechanisms, we took advantage of the murine LCMV model and performed an unprecedented pseudo-temporal framework analysis of T_{EX} cell responses to PD-1 therapy, capturing the precise cellular, functional, and transcriptional changes occurring in T_{EX} subsets across the whole duration of the therapy. This time-course analysis revealed that T_{EX} cell responses to PD-1 blockade decline in three temporally regulated phases including a transient regain of cytotoxic potential followed by a single proliferative wave initiated at the T_{EX} progenitor (T_{EX}^{prog}) stage that perpetrates in downstream subsets, resulting in a temporal numerical burst of "effector-like" T_{EX} intermediate (T_{EX}^{int}) cells. Importantly, we uncovered an acquired cell-intrinsic inability of T_{EX}^{prog} cells at sustaining this proliferative response that restricts the durability of T_{EX} responses to PD-1 therapy. Using longitudinal scRNAseq profiling of T_{EX} cells isolated across the duration PD-1 therapy, we uncovered the transcriptional changes underlying T_{EX}^{prog} cell acquired proliferative impairment. This analysis pointed-out dynamic activities of various pathways and transcription factors in T_{EX}^{prog} cells across PD-1 therapy with potential incidence on the biology of this key T_{EX} subset. Together, this work uncovers a direct incidence of PD-1 therapy on T_{EX}^{prog} cell biology and identifies potential causative pathways for the arrested reinvigoration of these cells that might represent intersting therapeutic targets.

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1974 – P2.06.10

Type 1 interferons and Foxo1 down-regulation play a key role in age-related T-cell exhaustion in mice

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Purpose: Aging of an organism is progressive and cumulative, leading to alterations in the functions of multiple cells, tissues and organs, including the immune system. Indeed, age-related alterations in the immune system make older adults more susceptible to infectious diseases and tumors, resulting in increased morbidity and mortality. In addition, the efficacy of vaccination is considerably reduced in the elderly population, limiting preventive prophylaxis. Foxo family transcription factors are critically involved in multiple processes, such as metabolism, quiescence, cell survival and cell differentiation. Although continuous, high activity of Foxo transcription factors extends the lifespan of some species, the involvement of Foxo proteins in mammalian aging remains to be determined. The aim of this project is to study Foxo1 involvement in T-cell aging.
Methods: Flow cytometry – transcriptomic analysis – adoptive transfer – cell culture.
Results: We observed that Foxo1 expression is down-regulated with age in mouse T cells leading to a rewiring of their transcriptional signature. Indeed, transcriptome analysis shows a significant overlap between the transcriptional signatures of naive CD4 T cells from young adult mice deficient for Foxo1 expression in T cells and old wild-type mice. Foxo1 down-regulation in T cells may contribute to several defects of the T-cell compartment with age including disruption of naive T-cell homeostasis leading to increased numbers of memory T cells and increased exhaustion of aged T cells characterized by PD1, TIGIT and TOX overexpression. Using adoptive transfer experiments, we show that the age-dependent down-regulation of Foxo1 in T cells is mediated by T-cell-extrinsic cues. In particular, we showed that type 1 interferons induce Foxo1 down-regulation *in vitro* and that this involves the PI3K/AKT signaling pathway. We confirmed these results *in vivo* as the T-cell compartment of old mice deficient for the expression of the type 1 interferon receptor appears to be less affected by aging than that of old wild-type mice.
Conclusion: Taken together, our data suggest that type 1 interferon-induced Foxo1 down-regulation is likely to contribute significantly to T-cell dysfunction in aged mice.

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1986 – P2.06.11

The functional effects of PMN-MDSC on PD-1+ and PD-1- effector memory CD4+ T cells under PD-1 immune checkpoint blockade

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Purpose: In the case of chronic inflammation such as cancer, prolonged inflammatory signals and over expression of growth factors stimulate the bone marrow to meet the increased demand for myeloid cells. This results in an emergency myelopoiesis in which the immature myeloid cells fail to differentiate into mature cells before their egress from the bone marrow. In recent years, it has become evident that the abnormal accumulation and function of the immature myeloid cells are important facets of the cancer. MDSC are composed of immature myeloid cells at different stages of myelopoiesis and are identified with certain myeloid lineage markers. As common properties of MDSC, they have low-density (<1.077 g/mL) and display HLA-DR$^{low/-}$ immunophenotype. MDSC are further categorized into two major groups as monocytic MDSC (M-MDSC) and granulocytic/polymorphonuclear MDSCs (PMN-MDSC). This study aims to analyze the suppressive effects of PMN-MDSC on PD-1$^+$ and PD-1$^-$ effector memory CD4$^+$ T cells in the presence of PD-1 blockade.
Methods: Peripheral blood samples of 8 colorectal cancer, 2 melanoma patients, and 6 healthy donors were collected. Peripheral blood samples were layered over 1.077 g/mL Histopaque solution and PBMC were collected. Cells were labelled with anti-human -CD45, -CD11b, -CD33, -CD14, -CD66b, -PD-L1, -HLA-DR, -CD3, -CD4, -CD8, -PD-1, -CCR7, -CD45RA, and -CD45RO monoclonal antibodies.
Results: The frequency of total and PD-L1-expressing PMN-MDSCs was increased in the patients. Amongst the central memory (T_{CM}), effector memory (T_{EM}), terminally-differentiated effector memory (T_{EMRA}), and naïve (T_{Naive}) CD4$^+$ T cell populations, T_{EM} was the most prominent subtype in which PD-1 expression (median 35%, min 4.4% - max 54.4%) was detected. Next, CD4$^+$CD45RO$^+$CCR7$^-$PD-1$^-$ and CD4$^+$CD45RO$^+$CCR7$^-$PD-1$^+$ T cells were purified by FACS. CD11b$^+$CD33^{dim}HLA-DR$^{-/low}$CD66b$^+$ PMN-MDSC were purified with MACS followed by FACS. CFSE-labelled autologous T cells were stimulated with CD3/CD28 beads and co-cultured with PMN-MDSCs in the presence or the absence of anti-PD-1 mAb. After 72 hours of incubation, PMN-MDSC suppressed proliferation of both PD-1$^+$ and PD-1$^-$ T_{EM} CD4$^+$ T cells.
Conclusion: Although PD-1$^+$ T_{EM} CD4$^+$ T cells were more prone to immune suppression by PMN-MDSC, PD-1 blockade recovered T cell proliferation in both groups to certain extent.

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2246 – P2.06.13

The impact of metformin on circulating natural killer and natural killer T cell activation in prediabetes

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Introduction & aim of the study: The function of natural killer and natural killer T cells is altered in people living with obesity and prediabetes. The role of these immune cells in the development of impaired glucose tolerance is not yet fully understood, especially in individuals who are living with prediabetes and are on glucose lowering therapy. This study aimed at investigating the frequency and activation status of circulating natural killer and natural killer T cells as well as assessing the effect of high-fat diet feeding and metformin monotherapy on NK and NKT cells using a murine model of diet-induced obesity.
Methods: Whole blood samples were drawn from the lateral tail vein of male C57BL/6 mice (n=24). The animals were randomised into three groups (n=8/group), these included an LFD group (n=8), HFD group (n=8) and HFD+Met group (n=8). All mice underwent an oral glucose tolerance test (OGTT) following the eight-week diet phase, to detect the onset of prediabetes. The animal body weights, lipid profiles and haematological parameters were measured at experimental weeks, 8 and 13. The levels of circulating NK and NKT cells were determined using flow cytometry.
Results: NK and NKT cell levels were significantly elevated in both the HFD and HFD+Met groups in comparison to the LFD group ($p<0.05$). The expression of CD49b was significantly increased in the HFD group 10.96[7.51-46.08] and HFD+Met 10.70[7.43-12.69] when compared to the control group 0.59[0.02-1.50], ($p=0.0004$). Similarly, the levels of NKp46 positive cells were increased in the HFD group (28.79 ± 6.23) and HFD+Met group (25.85 ± 5.87) in comparison to the control group (8.59 ± 8.41), ($p=0.0002$). Interestingly, the levels of NK1.1 positive cells, were significantly increased following HFD-feeding 11.28[8.72-15.36] and remained persistently elevated after short-term treatment with metformin 12.15[10.94-14.25] in comparison to the control group 2.43[0.29-6.10], $p=0.0032$. Furthermore, NK1.1 levels correlated with fasting plasma glucose concentrations ($r=-0.7233$; $p=0.0426$).
Conclusionù: We observed significant alterations in circulating NK and NKT cell levels following HFD-feeding, suggesting an association between immune dysregulation and metabolic dysfunction. Furthermore, metformin treatment demonstrated promising immunoregulatory effects by its ability to mitigate NK and NKT cell activation.

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P2.07 IMMUNE MEMORY DEVELOPMENT

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288 – P2.07.01

Tbet+CD11c+ B cells: Professional antigen-presenting cells in untreated rheumatoid arthritis

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Purpose: Among mature B cells, subsets of $CD21^{-/low}$ memory B cells (MBCs), including $CD27^{-}IgD^{-}$ (double negative) and $Tbet^{+}CD11c^{+}$ cells, are expanded in a range of chronic inflammatory diseases. In rheumatoid arthritis (RA), $CD21^{-/low}$ MBCs correlate with joint destruction, however whether these cells are $Tbet^{+}CD11c^{+}$, and their function in RA, is unknown. Here, we investigate $CD21^{-/low}$ $Tbet^{+}CD11c^{+}$ MBCs and their association with disease in untreated RA (uRA) patients.
Methods: We (i) combine clinical observations with extensive flow cytometry and in-depth single-cell (sc) RNA and V(D)J sequencing (seq) of peripheral blood MBCs from uRA patients; (ii) compare the transcriptome of peripheral blood Tbet+CD11c+ MBCs to that of synovial B cells; (iii) co-culture $Tbet^{+}CD11c^{+}$ B cells with T cells in vitro.
Results: We find $CD21^{-/low}$ $Tbet^{+}CD11c^{+}$ MBCs are significantly increased in peripheral blood and correlate with joint destruction in uRA. These $Tbet^{+}CD11c^{+}$ MBCs show evidence of clonal expansion and express somatically mutated V-genes. From differential gene expression and functional enrichment analyses, we identify over 150 significantly upregulated genes associated with antigen presentation functions, signatures that are mirrored in $Tbet^{+}CD11c^{+}$ B cells from the inflamed synovium of established RA. In vitro co-culture of $Tbet^{+}CD11c^{+}$ B cells with T cells, polarize $CD4^{+}$ T cells to RORgT-expressing Th17 cells.
Conclusion: Our findings demonstrate that peripheral blood $Tbet^{+}CD11c^{+}$ MBCs correlate with joint destruction in uRA, that they are poised for antigen presentation in the RA synovium and drive polarization to Th17 cells, thereby potentially contributing to ectopic lympho-neogenesis and bone destruction in RA.

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834 – P2.07.03

Diversity and Survival of Circulating and Bone Marrow-resident Memory T Cells

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Memory T cells circulating in the blood and those residing in tissues, can be detected for decades after apparent clearance of their cognate antigen. We have shown earlier that memory T cells residing in the bone marrow are essential to maintain long-term memory to systemic antigens. Obviously, circulating memory T cells (T_{CIRCM}) and bone marrow-resident memory T cells (T_{BM}) have different lifestyles. Here we define their gene expression profiles and survival mechanisms.
We have analyzed the single cell transcriptomes of T_{CIRCM} and T_{BM} and their antigen receptor repertoires, from three human donors. Their different repertoires define T_{CIRCM} and T_{BM} as separate compartments, including CD69 negative T_{BM} as truly resident cells. Gene expression profiles, not unexpectedly, indicate T_{CIRCM} as mobile cells, while T_{BM} are expressing genes of quiescent cells, encoding mRNA-binding proteins that prevent the translation of bound mRNAs, in particular those with ARE-motifs, e.g. TNF-family genes.
In addition, for murine T_{BM} we show, by blocking the integrin-mediated binding of T_{BM} to the stromal cells, or the resulting activation of the PI3K-signaling pathway, that maintenance of CD69+ T_{BM} is dependent on their contact to stromal cells, similar to the maintenance of bone-marrow-resident memory plasma cells. In contrast, survival of human T_{CIRCM} (*ex vivo*) is dependent on a distinct serum component, other than interleukin-7 or -15, activating a particular G-protein coupled receptor and transducing signals apparently through AKT, bypassing PI3K, but addressing the same downstream signaling pathway.
Dependency on a fluctuating serum component versus a stable stromal cell connection may thus contribute to the differences in persistence of T_{CIRCM} and T_{BM} over time. Beyond that, persistence may also depend on the individual competence of the memory T cell, i.e. its gene expression profile. In summary, our analysis confirms the compartmentalization of T cell memory, and defines different survival mechanisms for T_{CIRCM} and T_{BM}.

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835 – P2.07.04

Specificity in encoding and phenotype of innate immune memory

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Purpose: Innate immune cells build memory of prior experiences in a process termed "trained immunity," where "trained" cells respond stronger and faster to restimulation compared to non-experienced cells.[1] But which aspects of this prior experience are remembered, and which are forgotten? Here, we interrogate **specificity in the encoding of cellular memory** in macrophages across three key axes: the single cell, the individual donor, and the inducing stimulus.
Methods: Monocyte-derived macrophages were obtained from the apheresis product of healthy donors. Inflammatory stimulus was added to culture for 24 hours, followed by 5 days of rest. Memory of this prior exposure in the epigenome was evaluated by bulk ATAC sequencing. The functional effects of this memory were evaluated over time at single cell resolution with RNA fluorescence in-situ hybridization (smFISH)[2] of *TNF, IL6, IL1β,* and *CXCL10*, and pH-rodo labelled *E. Coli* was used to assess phagocytosis.
Results:

- ***Any single cell can build memory:*** Within hMDMs, there does not appear to exist a privileged subpopulation of cells that build memory, suggesting a lack of single-cell specificity in memory encoding.
- ***Memory retention is dependent on initial state:*** We can predict whether an individual donor's macrophages will show training based on the untrained (baseline) state of the population – cells with a low baseline inflammatory response are best able to be trained ex vivo.
- ***Different stimuli build distinct memories*****:** hMDMs trained with different inflammatory stimuli differ in functional capabilities, chromatin accessibility, and gene expression patterns across time, suggesting that cells specifically remember what was previously experienced and retain that information over several days.

Conclusions: We propose that cellular memory (such as that displayed by trained macrophages) allows for context-adjusted and time-adjusted responses to stimulation, as cells 'learn' appropriate responses based on prior experiences. As we continue efforts to profile cellular heterogeneity, map cell fate and function, and engineer cells to perform specific tasks, we require a stronger understanding of how cells remember their history.

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1. Novakovic, B. et al. *Cell* (2016) (2) Niu, Z. et al. *Biorxiv.* 2024.01.31.578123 (2024)

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842 – P2.07.05

Investigating the impact of Staphylococcus aureus exposure on innate immune memory

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Purpose: Although once considered to be an exclusive feature of the adaptive arm of the immune system, evidence suggests that innate immune cells develop immunologic memory-like features in response to specific stimuli in a process known as "trained immunity". Given its duality as a commensal and a pathogen, *Staphylococcus aureus* antibodies are found almost ubiquitously in the general population. It's possible this exposure stimulates innate immune training which could greatly contribute to host defence against subsequent insult and have wide-reaching potential in disease treatment.
Methods: To investigate the impact of *S. aureus* exposure on innate training we established a novel model of long-term persistent colonisation whereby germ-free mice were mono-colonised with *S. aureus*. Bone marrow-derived macrophages (BMDMs) were generated and challenged in vitro with LPS, *S. aureus*, or *Klebsiella pneumoniae*. In a second model, conventional mice received recurrent intraperitoneal *S. aureus* injections on Day 0, 7 and 14. On Day 35 mice were sacrificed and the immune response was investigated.
Results: BMDMs from mono-colonised mice released elevated levels of IL-6 and IL-10 in comparison to germ-free controls. Additionally, bone marrow extracellular fluid (ECF) gathered from mono-colonised mice expressed more IL-1β than germ-free mice when measured via ELISA.
BMDMs from previously-infected *S. aureus* mice exhibited elevated IL-6 and IL-10 levels compared to naïve counterparts. Furthermore, ECF IL-1β levels were higher in previously-infected *S. aureus* mice when compared to their naïve controls.
Flow cytometric analysis on bone marrow progenitor cells of mono-colonised and previously-infected mice revealed increases in the levels of long-term haematopoietic stem cells and multipotent progenitor 3 cells compared to germ-free and naïve mice respectively.
Conclusion: Overall, both commensal or pathogenic *S. aureus* exposure impacts myelopoiesis and induces innate immune training which is protective against *S. aureus* or heterologous insults such as LPS or *K. pneumoniae*. The development of future efficacious vaccines and therapeutics against *S. aureus* are contingent on our understanding of this *S. aureus*-induced innate immune training.

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1307 – P2.07.07

Examining B cell maturation and antibody diversity in HIV-1 vaccine response: A multimerization and high-throughput sequencing study in the rhesus macaque model

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Purpose: Human Immunodeficiency Virus 1 (HIV-1) continues to be a significant global health challenge, affecting approximately 39 million people worldwide. Despite advances in treatment, an effective HIV-1 vaccine remains elusive. This gap highlights the need for in-depth understanding of immune mechanisms, particularly B-cell maturation, which is crucial for generating effective immune responses. Our study addresses this gap by examining the impact of antigen multimerization on vaccine-elicited B cell maturation in *rhesus macaques* (RM). Focusing on key immunological processes such as the IgM to IgG class switch, clonality, and somatic hypermutation (SHM), we aim to uncover insights that could guide the development of more efficacious HIV-1 vaccines.
Methods: We employ two distinct yet complementary high-throughput sequencing approaches. Firstly, we leverage the 10X Genomics platform (10X) to sequence tetramer-sorted Envelope (Env)-specific B cells. High throughput processes enable the expression and characterization of paired heavy (H) and light (L) chains as recombinant antibodies, linking B cell receptor (BCR) sequence features to antibody functionality. Secondly, we utilize Next-Generation Sequencing (NGS) to analyze the immunoglobulin H (IgH) locus, capturing the broad spectrum of BCR diversification and evolution. This involves isolating B cells from peripheral blood mononuclear cells of immunized RMs, followed by culture stimulation to induce IgH/L locus transcription. Post-stimulation, the IgH locus is amplified using 5'RACE PCR and prepared for in-depth Illumina sequencing, which allows for detailed insights into the BCR repertoire.
Results: Preliminary analysis indicates differences in BCR clonality and SHM correlated with the level of antigen multimerization. The application of 10X has enabled detailed characterization of Env-specific B cell maturation processes. Concurrently, NGS has facilitated a broad analysis of the BCR repertoire evolution. This dual approach allows for an in-depth examination of the effects of antigen presentation on B cell responses following HIV-1 vaccination.
Conclusion: Our research advances understanding of B cell responses to HIV-1 vaccine modalities in RM, focusing on antigen multimerization effects. Utilizing 10X and NGS, we dissect key B cell maturation aspects crucial for HIV-1 vaccine development. This study informs future vaccine design, enhancing our ability to tackle HIV-1 and other pathogenic challenges.

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1321 – P2.07.08

Training for disaster: Macrophages, obesity, and the potential of mesenchymal stromal cells

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Purpose: In recent years, innate training has drawn increasing attention as both a potential beneficial and detrimental influence in health and disease. Training stimuli associated with obesity, like saturated free fatty acids (FFAs), have been associated with increased disease severity and higher mortality in pre-clinical models due to elevated expression of pro-inflammatory cytokines by trained macrophages. Mesenchymal stromal cells (MSCs) are known for their anti-inflammatory properties. In our study, we aimed to determine if palmitate trains macrophages, if this training is associated with epigenetic and metabolic changes, if MSCs are able to block the training by the saturated FFA palmitate, the mechanism by which this is accomplished, and if MSCs are able to suppress *in vivo*-trained macrophages to the same degree as untrained macrophages.
Methods: For the training experiments murine haematopoietic stem and progenitor cells and human peripheral blood monocytes were exposed to 0.3 mM palmitate for 24 hr, washed and differentiated into macrophages over 7 days. After this rest period, the macrophages were stimulated with 100 ng/mL LPS for 24 hr, supernatants were collected and analysed for concentrations of TNFα and IL-6. For epigenetic changes, the methyltransferase inhibitor methylthioadenosine was added 30 minutes before palmitate and remained in the well for the training period. Metabolic changes were observed by adding 2-DG in low glucose medium, oligomycin, CB839, or etomoxir during stimulation with LPS. To investigate if MSCs are able to block palmitate-mediated training and further elucidate potential associated pathways, MSCs were added in a transwell during the training period, either untreated, pre-treated with the COX-2 inhibitor NS-398, or in the presence of an IL-1RN neutralising antibody. Finally, to investigate *in vivo*-trained macrophages, blood from human patients with obesity (PWO) and healthy controls was collected.
Results: Palmitate-trained macrophages from both mouse and human sources responded to secondary LPS stimulation with increased TNFα and IL-6 expression. Inhibition of methyltransferases and co-incubation with MSCs in transwells inhibited training. Blocking of the COX-2 pathway in MSCs restored macrophage training for TNFα, while neutralisation of IL-1RN restored macrophage IL-6 training, implying different training pathways. Interestingly, MSCs also affected the training-associated changes in macrophage metabolism.

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1408 – P2.07.09

Dietary supplementation with b-glucan induces trained immunity, correcting immune-metabolic perturbations and enhancing anti-tumour function in dietary induced obesity

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Background: Innate immune cells can exhibit memory-like features when exposed to stimuli such as yeast β-glucans. This phenomenon is termed "trained immunity", defined by bone marrow myelopoiesis and enhanced production of cytokines following secondary stimulation. Recently, it has been shown that induction of trained immunity by IP administered β-glucans supports anti-tumor function. Little is known about the immuno-metabolic consequences of **dietary** β-glucans (WGP). High fat diets (HFDs) drive myelopoiesis and enhance inflammatory functions of myeloid cells. This inflammatory phenotype, induced by HFD, is pathogenic and promotes the development of metabolic disease. We hypothesized that dietary WGP could reverse the metabolic defects caused by HFD by inducing beneficial trained immunity.
Results/Methods: Dietary supplementation with WGP increases myelopoiesis, as evidenced by increased MPP3 population in bone marrow. In BMDMs, WGP enhances TNF production in response to LPS stimulation and heightens phagocytic ability. BMDMs also show altered epigenetic profiles, with heightened global H3K27 acetylation, and altered metabolic function, with heightened OXPHOS and glycolysis. Given the heightened metabolism and increased cytokine production, we hypothesized that WGP may protect against the accelerated tumor growth often seen in mouse models of HFD. In MC38 tumor model, tumor growth was inhibited in WGP fed mice. This effect is likely due to heightened mitochondrial ROS (mitoROS) production and heightened TNF and IFNg production in tumor macrophages and NK cells. Feeding HFD and supplementing a HFD with WGP (HFD+WGP) both show expansion of myeloid progenitor precursors. HFD+WGP feeding attenuated the rates of glycolysis and OXPHOS in unstimulated BMDMs. In MC38 tumor model, HFD mice show accelerated tumor growth, potentially due to decreased NK and CD8 T cell function. Mice fed HFD+WGP show increased tumor clearance, with heightened TNF and IFNg production, which are important for anti-tumor immunity. In HFD tumors, macrophages have lowered mitoROS production, HFD+WGP tumor macrophages and NK cells rescue mitoROS levels with a return to SFD mitoROS levels.
Conclusions: Feeding WGP induces trained immunity features, altering hematopoiesis and immune function. Dietary WGP is a simple, cost-effective means to reverse the immunometabolic defects of obesity. This may have implications for treatment of metabolic diseases such as cancer.

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1528 – P2.07.11

EBI2 expression is a ubiquitous feature of murine and human tissue-resident memory T cells and functionally segregates them.

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Inflammatory skin diseases like psoriasis, atopic dermatitis (AD) and allergic contact dermatitis (ACD) are characterized by persistent site-specific recurring lesions. Tissue-resident memory T (T_{RM}) cells remain sessile in those skin lesions and have been linked to the recurring chronic pathology. Epstein-Barr Virus-induced gene 2 (EBI2 or GPR183) mediates chemotaxis towards its ligand 7α,25-dihydroxychlesterol (7α,25-OHC). CH25H and CYP7B1 are the enzymes that synthesize 7α,25-OHC. A number of inflammatory disease pathologies are associated with the EBI2-oxysterol-axis, such as of rheumatoid arthritis, MS, COPD and IBD. EBI2 has not been described in the context of T_{RM} cells as of today.
Using an experimental murine ACD model, contact hypersensitivity (CHS), and a public human transcriptome bulk-RNAseq dataset, we compared mRNA expression of EBI2-oxysterol-axis-genes in lesional vs. non-lesional skin. Utilizing high-parameter flow cytometry and clustering algorithms, we analyzed EBI2 expression of T_{RM} cells in lesional vs. non-lesional skin of biopsies from murine CHS ears and psoriasis patients. We also screened different T_{RM} cells of different human organs like the lung and the colon for EBI2 expression.
Gene expression of the EBI2-oxysterol-axis-genes was upregulated in murine (CHS) and in human (psoriasis and AD) lesional skin, compared to non-lesional skin. While only up to 40% of circulating $CD8^+$ T cells express EBI2, a striking 80 (human) to 95% (mouse) of skin $CD8^+$ T_{RM} cells are $EBI2^+$. Murine $CD8^+$ T_{RM} cells upregulate EBI2 expression during differentiation from effector cells. Lung and colon T_{RM} cells also express EBI2, similar to skin T_{RM} cells.
T_{RM} cells are known to reside in ex-lesional skin and mediate chronic flare ups. We find those cells expressing EBI2 in mouse and human skin. The upregulation of EBI2 expression of skin $CD8^+$ T_{RM} cells, compared to circulating $CD8^+$ T cells, in conjunction with the upregulation of the expression of EBI2-oxysterol-axis-genes in lesional murine and human skin, is a strong indicator that the EBI2-oxysterol-axis is involved in the pathology of inflammatory skin diseases and implicates a functional role for EBI2 on T_{RM} cells that is yet to be uncovered. High EBI2 expression by lung and colon T_{RM} cells suggests a similar role in those organs.

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1531 – P2.07.12

Exploring MAL signalling as a mechanistic mediator of IFNγ-induced trained immunity

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Background: Interferon gamma (IFNg) has been shown to play an important role in the induction of innate immune training, a memory-like state in innate immune cells involving metabolic and epigenetic reprogramming that results in heightened responses to infection. The mechanisms that drive this phenotype remains unclear. *TIRAP* is a gene that codes for MAL, an adaptor protein involved in multiple inflammatory signalling pathways. The S180L single nucleotide polymorphism (SNP), within *TIRAP* decreases the affinity of MAL for its receptors, thereby decreasing cellular signalling. This common SNP is reported to affect susceptibility and response to multiple infections including tuberculosis and sepsis. MAL facilitates signalling through the IFNg receptor. We therefore hypothesise that IFNg-induced innate immune training is affected by the presence/absence of the S180L SNP in *TIRAP*.
Methods: DNA was extracted from saliva samples collected from healthy donors (n=103) and genotyped for the presence of the S180L SNP using PCR allelic discrimination. Innate immune training assays were carried out on a small cohort of donors with either the presence of the SNP on both alleles (LL; n=3) a single allele (SL; n=7) or those who lack the presence of the SNP (SS; n=7). Blood samples were collected, and monocytes enriched from PBMCs, trained with IFNg for 24hrs, washed and allowed to return to homeostasis and rest. On day 6, monocyte-derived macrophages (MDMs) were challenged with irradiated *M.tb* for a further 24hrs. Activation and co-stimulatory marker expression was analysed using flow cytometry while cytokine production was measured by ELISA.
Results: Human MDMs show significantly increased TNFa, IL-1b, IL-6 and IL-10 production and CD40, CD14 and HLA-DR expression in response to *M.tb* challenge post IFNg training compared to untrained cells. SL and LL individuals produce significantly lower TNF*a* following IFNg training compared to SS individuals and similar trends are noted with regards to IL-1b and IL-10 production. However, no changes in IL-6 production or CD40, CD14 and HLA-DR expression were noted between genotypes post IFNg training.
Conclusion: Although MAL has a role in inflammatory cytokine production post IFNg training, MAL signalling is likely not the primary mechanism involved in IFNg-induced trained immunity.

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2081 – P2.07.14

Effect of immunosuppressive regimen on cellular and humoral immune response to SARS-CoV-2 vaccination in anti-CD20 treated hematological patients and in solid organ transplant recipients

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Purpose: Immunocompromised patients are a fragile category of subjects, particularly exposed to infections and characterized by an impaired ability to respond to vaccination. An extensive knowledge on the immune response status of these subjects in terms of specific antibodies production and T cells activation is fundamental to improve the immunization strategy.
Methods: We monitored humoral (IgG serum levels) and cellular (antigen-specific T cells by multiparametric flow cytometry) memory responses after mRNA SARS-CoV-2 vaccination in lung transplanted cystic fibrosis patients (CFT) or not transplanted (CF), in kidney transplant recipients (KT) and in hematological patients in course of biologic therapy with anti-CD20 monoclonal antibodies or 3-24 months after the end of the treatment.
Results: Immunocompromised transplanted patients displayed a weak cellular and humoral memory immune response to SARS-CoV-2 mRNA vaccination and in particular the therapy regimen including antimetabolites, mainly affecting the humoral compartment, induced a lower humoral and cellular response also after booster dose. As expected, SARS-CoV-2 specific humoral immune response is absent in hematological patients under anti-CD20 treatment, whereas is evident in hematological patients vaccinated after therapy termination. In particular patients treated also with bendamustine, an antimetabolite, showed lower levels of SARS-CoV-2 specific Ig. Both cohorts of hematological patients, vaccinated during of after the anti-CD20 treatment, showed a robust SARS-CoV-2 specific T cells response, not particularly influenced by bendamustine treatment.
Conclusion: These results suggested that immunocompromised patients, in particular in case of antimetabolite treatment, need adequate interventions to improve humoral and cellular immune response to mRNA SARS-CoV-2 vaccine such as additional jab or modulation of immunosuppressive therapy. Of note, these findings could open the way to further investigation on induction and/or maintenance of memory immune response in immunocompromised patients in response also other vaccination different from SARS-CoV-2 to improve immunization strategy.

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2132 – P2.07.15

Killing IgE+ plasma cells: The role of IgE B cell receptor in human IgE+ plasma cell survival

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Allergen-specific IgE antibodies, secreted by plasma cells (PCs), play a fundamental role in the severity of allergic disease, including asthma. Analysis of IgE^+ B cell differentiation into PCs indicate that class switching is followed by a dynamic up-regulation of surface expression of IgE B cell receptor (mIgE). Although mouse IgE^+ PCs express surface mIg 4-fold higher than $IgG1^+$ PCs, the crosslinking of the BCR on mouse PCs leads to their preferential killing in a Syk, BLNK and PLCγ2 dependent manner. However, the human mIgE exhibits unique characteristics, differing not only from those of other antibody classes but also from those of mouse mIgE. Namely, humans express a short and a long form of membrane IgE: $mIgE_S$ and $mIgE_L$, which differ in their capacity to signal and in their expression during PC differentiation; $mIgE_S$, a homologue of the mouse mIgE, is upregulated, whereas $mIgE_L$ is downregulated in IgE^+PCs.
To investigate the functional significance of this differential expression we generated IgE^+ cells from human tonsil B cells. We then evaluated the killing capacity of anti-IgE antibodies that target different regions of mIgE, and characterised the molecular mechanisms involved by combining CRISPR/Cas9 gene targeting with inhibitors against various BCR signalling components.
We observed that IgE^+PCs were highly apoptotic when either $mIgE_L$ or both $mIgE_S$ and $mIgE_L$ where crosslinked. Also, IgE^+ PCs were killed at a higher rate than $IgG1^+$PCs after BCR crosslinking with anti-**λ** and anti-**κ**. Inhibition of Syk and intracellular Ca^{2+} signalling rescued the killing of both IgE^+ and $IgG1^+$PCs, whereas the inhibition of PI3K p110δ enhanced the apoptosis of IgE^+PCs by modulating the expression of BIM, a pro-apoptotic factor. Most importantly, we discovered that IgE^+ PCs, compared to $IgG1^+$ PCs, express significantly higher levels of PTEN, a phosphatase that negatively regulates PI3K signaling. CRISPER/Cas9 knockout of PTEN, as well as that of BIM, was able to rescue the killing of IgE^+PC but not that of $IgG1^+$PCs.
Overall, here we show that Syk and intracellular Ca^{2+} are required for the BCR killing of both IgE^+ and $IgG1^+$PCs, whereas modulation of PI3K signalling plays an important role in the survival of IgE^+PCs only.

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P2.08 IMMUNE REGULATION IN CANCER

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98 – P2.08.01

Stiffness regulates dendritic cell and macrophage subtype development and increased stiffness induces a tumor associated macrophage phenotype in cancer co-cultures

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The mechanical properties of tissues including stiffness change throughout our lives, during both healthy development as well as accompanying chronic diseases like cancer. The impact of changes to stiffness during cancer progression specifically on leukocytes remains unknown. To address this, I analyzed myeloid phenotypes resulting from mono- and cancer co-cultures of primary murine and human myeloid cells on two- and three-dimensional hydrogels with varying stiffnesses. On soft hydrogels, conventional DCs (cDCs) developed, whereas on stiff hydrogels plasmacytoid DCs (pDCs) developed. Cell populations expressing macrophage markers CD14, Ly6C, and CD16 also increased on stiff hydrogels. In cancer co-cultures, CD86+ populations decreased at higher stiffnesses across six different cancer models. High stiffness also led to increased VEGFA, MMP, and CD206 expression; 'M2' markers expressed by tumour-associated macrophages (TAMs). Indeed, the majority of CD11c+ cells expressed CD206 across human cancer models with high stiffness. Targeting the PI3K-Akt pathway led to a decrease in CD206+ cells in murine cultures only, while human CD86+ cells increased. An increased stiffness in cancer could thus lead to the dysregulation of infiltrating myeloid cells and shift their phenotypes towards a M2-like TAM phenotype actively enabling tumor progression. Additionally, stiffness-dependent signaling appears species-dependent, which might contribute to the high failure rate of clinical trials.

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152 – P2.08.02

RNAseq analyses uncover the transcriptional signature of NK cells and reveal S1P5 and CXCR4 as new targets to modulate tumor cell killing

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Natural Killer (NK) cells are innate lymphocytes that can kill stressed cells without antigen-restriction. Malignant tumor cells often down-regulate MHC-I molecules to escape killing by conventional CD8+ T cells. NK cell activity is thus critical in such instances to build a robust anti-tumoral immune response. Circulating NK cells are highly heterogeneous and present different profiles in cytokine response, cytotoxicity and tumor infiltration. Here, we set out to identify new intrinsic modulators of NK cell activation. We challenged cytokine-treated and untreated human primary blood-borne NK cells with the HCT116 human colon cancer cell line and used detection of LAMP1 (CD107a) in the plasma membrane as a marker of rapid NK cell degranulation and thus, engagement. RNA sequencing of these cells produced a transcriptomic profile restricted to LAMP1^{hi} NK cells which we refer to as "Early-bird signature". *CXCR4* and *S1PR5* stood out as top down-regulated genes in the rapid NK cell responders. Using compounds which modulate activity of CXCR4 and S1P5, we confirmed that both proteins play a role not only in NK cell, but also in CD8+ T cell cytotoxicity. Along with S1P5, other members of the Shingosine-1-Phosophate (S1P) signaling pathway are demonstrated to be involved in NK and CD8+ T cell activity. Interestingly, ligands of both CXCR4 and S1P5 are enriched in the TME of some tumors and act as chemoattractants to facilitate lymphocyte infiltration. The co-regulation of CXCR4 and S1P5 will be examined. Together, these data suggest that specifically targeting CXCR4 and S1P5 activity in the TME could be a good strategy to unleash full cytotoxic potential of cytotoxic effector cells in the tumor.

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201 – P2.08.03

KIR2DL5 in bladder cancer susceptibility and treatment response

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Purpose/Introduction: KIR2DL5 is an inhibitory human killer-cell immunoglobulin-like receptor (KIR) that has gain interest recently as a cancer immunotherapy target since poliovirus receptor (PVR, CD155) has been described as its ligand. The biology and therapeutic potential of the KIR2DL5/PVR interaction are largely unknown. It has been shown that KIR2DL5/PVR interaction inhibits synapse formation and its blockade robustly augmented the NK cytotoxicity against PVR+ human tumors. Data from our groups involved KIR2DL5 with bladder cancer susceptibility and cancer progression.
Methods: This work explores the frequency of KIR2DL5 in three independent series of patients with bladder cancer, one retrospective (n=79) and two prospective series 2013-15 (n=59) and 2019-22 (n=191) as well as in other solid (n=196) and hematologic (n=323) cancers and healthy donors (n=615). The impact that the presence of KIR2DL5 has on the response to BCG (non-infiltrating cancers) or chemotherapy (infiltrating cancers) treatments is analyzed.
Results: The frequency of KIR2DL5 was increased in the 3 bladder cancer series (68.4%, 65.9%, and 63.9%, $p<0.001$) compared to other solid (56.6%) and hematological (51.1%) cancers or healthy donors (51.5%). A total of 152 patients were treated with BCG (46.2%). KIR2DL5 was associated with better overall survival (OS) in patients with non-infiltrating tumors treated with BCG (mean OS of 7.7±0.4 vs. 6.3±0.55 years, $p<0.05$), but not in patients with infiltrating tumors treated with chemotherapy (mean OS of 56.4±0.35 vs. 58.5±0.54 years). KIR2DL5 did not significantly impacted OS of patients with other solid or hematological cancer.
Conclusion: KIR2DL5 was significantly associated with higher susceptibility to bladder cancer. Unexpectedly, KIR2DL5 was associated with better survival in patients treated with BCG. Our data suggest that it would be advisable to investigate whether intraurethral stimulation with BCG favors more potent antitumor NK cell responses, and therefore if the use of immunotherapies against KIR2DL5/PVR interaction could be useful.

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228 – P2.08.04

Modulation of ILC3 metabolism as a potential therapeutic target for colitis-dependent colorectal cancer

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Colorectal cancer (CRC) is a leading cause of death. Patients with inflammatory bowel disease (IBD) are more likely to develop CRC, although the factors that mediate the IBD-CRC transition remain unclear. Innate type 3 lymphocytes (ILC3s) are tightly involved in IBD pathogenesis and may be related to the transition from IBD to CRC. Moreover, the hypoxia-inducible transcription factor (HIF-1α) favors tumor growth in hypoxic environments and regulates ILC3 function.
To elucidate the function of HIF-1α in ILC3s during the transition from IBD to CRC, mice with HIF-1α deletion in RORγt^{+} cells (HIF-1α$^{\Delta Rorc}$; CRE) were analysed. Additionally, RAG-1KO HIF-1α$^{\Delta Rorc}$ mice (CRE), which produce no mature B or T cells, were used to ascertain HIF-1α's specific role in ILC3s. Both models were subjected to chemical treatments with 3% DSS that induced colitis or with the carcinogen AOM followed by 2.5% DSS as a CRC model.
In both colitis and CRC models, CRE mice exhibited more severe tissue damage and significantly lower survival than their controls, throughout the treatment. Furthermore, in the colitis model, CRE mice presented a greater number of ILC3 NCR^{+} lymphocytes.
Results reveal the protective role of the endogenous factor HIF-1α in ILC3 cells during the development of the colitis-associated CRC. Additionally, the higher number of ILC3 NCR^{+} lymphocytes in CRE mice suggests enhanced cell proliferation in the absence of the HIF-1α factor. However, further research is needed to delve into the mechanism through which ILC3s, without HIF-1α, affect CRC development and susceptibility.

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371 – P2.08.05

Multiparametric lymphocyte characterization in ovarian cancer: Identifying possible targets for immunotherapy.

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Purpose: Ovarian cancer (OC) is the deadliest gynecological malignancy, characterized by accumulation of fluid termed ascites at an advanced stage. Considering that OC-ascites comprises tumor cells, tumor cell spheroids, innate and adaptive immune cells, and its accessibility, ascites provides an excellent source to investigate the impact of tumor microenvironment on anti-tumor immune response. It is known that OC-ascites influences the differentiation and activation state of several lymphatic subpopulations. Published data suggest alterations in innate and adaptive lymphocyte populations, which contribute to tumor progression. The limitations of these studies is the focus on only one lymphatic subpopulation. The goal of this comprehensive study is to identify alterations in composition of the innate and adaptive lymphocyte populations by comparing OC-ascites to patient peripheral blood (PB) and PB from healthy donors (HD).
Methods: To identify alterations in the lymphocyte composition caused by OC, PB from OC patients and healthy donors are compared. Further, alterations in the lymphocyte composition caused by OC-ascites were analyzed by comparing PB and ascites samples from the same OC patients. For this, PBMCs from healthy donors, PBMCs, and ascites from patients with short and long survival in late stages of OC are analyzed. To identify possible targets within lymphocyte subpopulations in PB and ascites from OC patients for immunotherapy spectral flow cytometric analysis of PBMC and ascites lymphocytes was used. Two 37 marker panel to identify several subpopulations, including several CD4, CD8 and NK subpopulations, but also their differentiation, activation and cytokine profile were used.
Results: Comparing lymphocyte clusters from HD, OC blood and ascites samples revealed distinct expression patterns and differences between the three groups. Samples from ascites showed increased CD8 and NK cell cluster and decreased CD4 cluster, whereas PB from HD and OC patients differed in the abundance of CD8 and CD4 Treg cluster. Further, each group showed clear distinct patterns in their differentiation and activation state.
Conclusion: We believe that the obtained knowledge will give hints for the development of new therapy strategies employing specific drugs to reverse the OC-caused alterations in the lymphocyte clusters, their activation, differentiation, exhaustion, or functional state.

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379 – P2.08.06

SH2D2A is an indicator of favourable prognosis in bladder cancer and is enriched in activated Treg cells

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The growing importance of RNA sequencing (RNA-seq) technologies has brought about an expansion in the quality and accessibility of the method and its data output. The conventional approach that has defined much of life sciences begins with measurable phenomena, natural or otherwise, and proceeds, by identification of the mutations or aberrations that produce it, to a gene's function. While this approach has produced a great body of scientific progress, its Achille's heel is its reliance on measurable phenomena. With the expanding availability of RNA-seq and other such high dimensional data it is now possible to consider a complementary approach working in the opposite direction *i.e.*, from transcriptomics up towards protein function. This approach may prove fruitful in producing information on the function of cytosolic-bound proteins such as adaptor proteins.
To test the validity of this method, we made use of several public datasets to interrogate the cancer-specific role of the adaptor protein SH2D2A: a protein enriched in T and NK cells, and a known interactor of the kinase LCK, whose function remains uncertain. We found that SH2D2A is a favourable marker for prognosis in urothelial bladder cancer (BLCA). Digging further, we identified a population of $SH2D2A^{+}$ $FOXP3^{+}$ $IL2RA^{hi}$ activated Tregs as the main expressors of SH2D2A in BLCA. This suggests that the expression of SH2D2A in these Tregs contributes to a beneficial prognostic effect. Further comprehension of SH2D2A's function in these cells holds the potential for advancing treatment in BLCA.

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414 – P2.08.07

Study of the interaction of tumor exosomes with neutrophils. Implication in the induction of NETosis

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Tumour exosomes induce changes in immune system cells, promoting a state of tolerance that facilitates tumour progression. In neutrophils, exosomes from different tumours induce the release of extracellular traps (NETs), which contribute to pre-metastatic niche formation and tumour dissemination. However, little is known about the adhesion molecules and receptors that mediate the binding and uptake of these exosomes and the pathways and type of NETosis they induce. Our group has described, among others, that the tetraspanin CD9 is involved in the uptake of colocarcinoma exosomes by mesothelial cells by modulating the activity of several adhesion molecules and metalloproteases. In this work, we investigate the ability of exosomes from the colocarcinoma cell line Colo320 to induce NETosis in neutrophils from the promyelocytic line HL-60 and the role of tetraspanin CD9 in tumour exosome-neutrophil interaction.
Exosomes were isolated from the Colo320 or the CD9-overexpressing Colo320 colocarcinoma cell lines using tangential flow filtration and size exclusion chromatography. Exosomes were incubated with neutrophils derived from the retinoic acid treated human promyelocytic HL-60 cell line. As controls, neutrophilic HL60 cells were exposed to LPS or PMA for NET induction. NET production was analysed by fluorescence microscopy and flow cytometry using DAPI, MPO and citrullinated H3 as NETosis markers.
Our results indicate that tumour exosomes derived from colocarcinoma cells have the ability to induce NETosis in neutrophilic HL60 cells by inducing H3 citrullination and the release of DNA and MPO at early time points. In addition, CD9 expression on tumour exosomes greatly increased NET formation, shortened the time required to detect NETosis and increased the number of cells undergoing NETosis.
Expression of the tetraspanin CD9 in tumour-derived exosomes promotes the binding/uptake of these microvesicles by neutrophils, resulting in an increase in the formation of NETs that are detected at early time points.

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428 – P2.08.08

Mitochondria transfer, a potential mechanism of CD4+ T cell exhaustion in oral cancer

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Purpose: Oral squamous cell carcinoma (OSCC) is the most frequent type of oral cancer, and its tumor microenvironment (TME) induces an exhausted phenotype and metabolic reprogramming in CD4+T- lymphocytes. The mitochondria are the main metabolic organelle and in recent years it has been shown that several cells have the capacity to transfer mitochondria, including cancer cells. Moreover, we identified several mitochondrial proteins in secretomes from biopsies of patients with OSCC. However, it has not been evaluated whether mitochondria transfer from OSCC cells into CD4+T-lymphocytes promotes an exhausted phenotype in the T cells. The aim of this work was to analyze the exhausted phenotype with metabolic changes in CD4+T-lymphocytes after artificial transfer of mitochondria obtained from the oral cancer cell line HSC-3.
Methods:
MitoTracker-labeled mitochondria from oral cancer tumor cells were Mitocepted into CD4+T- lymphocytes. Then, surface molecule expression, proliferation and cytokine secretion mediated by tumor mitochondrial transfer were analyzed by flow cytometry. In addition, Mitocepted CD4+T- lymphocytes were evaluated by proteomic and metabolomic analysis, determination of superoxide production, glucose uptake and lactate production.
Results:
TCD4+ lymphocytes that acquired malignant mitochondria had increased expression of TIGIT, CTLA4, PD-1, PLD-1 and LAG3, compared to the control group. In addition, the mitocepted lymphocytes exhibited a significant decrease in proliferation compared to the control. For cytokine analysis, a significant decrease was observed in the mitocepted group for IFN-gamma, TNF-alpha, IL-10, and IL-4 secretion, compared to control. Metabolomic and proteomic analysis showed a reduction in the pyruvate dehydrogenase cofactor called Vitamin B1 and an increase in hypoxia, glycolysis, and mitochondrial superoxide production in the mitocepted group versus the control. In fact, mitocepted lymphocytes with malignant mitochondria uptake more glucose, produce more reactive oxygen species and lactate than non-mitocepted cells and cells mitocepted with autologous mitochondria from the same T-cells.
Conclusion: The acquisition of isolated mitochondria from HSC-3 cancer cells by CD4+T-lymphocyte induces mitochondrial oxidative stress in the recipient cell and a possible reduction of the Krebs cycle, mediated by low thiamine. This effect promotes a salvage glycolytic metabolism, an exhausted phenotype and a dysfunctional $CD4^{+}$T-cell, affecting the anti-tumor response.

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460 – P2.08.09

Differential regulation of the chemokine CCL21 in the antitumor immune response in non-small cell lung cancer.

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Lung cancer is one of the leading causes of death worldwide. The use of therapies aimed at enhancing the antitumor immune response, such as anti-PD-1 antibodies, has been a milestone in the treatment of this pathology. However, the effectiveness of this therapy is still limited. Therefore, the study of the mechanisms that mediate the antitumor effect of these treatments is of great relevance. Previous studies from our group demonstrate that anti-PD-1 antibodies induce an increase in CCL21 production in the tumor microenvironment of non-small cell lung cancer (NSCLC), as well as the formation of tertiary lymphoid structures (TLS) within the tumor, which correlate with the response to treatment.
Using a murine model of NSCLC, we have analyzed the possible source of tumor CCL21 by immunohistochemistry techniques in tumor samples, and by PCR in different cell types *in vitro*. Likewise, we have studied serum CCL21 levels in these mice using ELISA, to determine if they can be useful in monitoring tumor evolution and response to immunotherapy.
We have found two patterns of CCL21 production in the tumor, one associated with TLS and another associated with the tumor stroma. Likewise, we have determined that tumor cells, reticular fibroblastic cells, and endothelial cells are capable of expressing CCL21 mRNA in response to proinflammatory cytokines *in vitro*. Finally, we have observed an increase in serum CCL21 concentration in parallel with tumor progression, although it is not related to the size of the tumor and its progression is independent of treatment with anti-PD-1 antibodies.
The data obtained reflect that tumor CCL21 can originate locally in various cellular compartments, and point to a differential regulation of CCL21 detected systemically. The detailed characterization of the regulatory mechanisms of CCL21 will provide relevant information about the antitumor immune response and the response to immunotherapy in NSCLC.

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625 – P2.08.10

Exploiting the cGAS/STING pathway to revert the immunosuppressive environment of multiple myeloma and boost Natural Killer cell-mediated immunosurveillance

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Purpose: Multiple myeloma (MM), a bone marrow-resident hematological malignancy of plasma cells, is characterized by an immunosuppressive microenvironment, which causes a progressive dysfunction of immune cell populations, such as NK cells, implicated in MM surveillance. The main subject of this study is to reverse the tumor-mediated immune paralysis potentiating NK cell-mediated functions against MM cells. The GAS/STING pathway that leads to type I IFNs production is currently being explored as potential immunotherapeutic target in cancer. Since IFNs prime strong effector activity in NK cells, the GAS-STING pathway is an attractive candidate to our aim. We propose to improve this pathway directly in cancer cells by using the synthetic STING agonist diABZI.
Results and Methods: Reduced survival rate of MM patients is significantly correlated to a low STING expression in MM cells and we also found through Real Time PCR that STING levels were negatively regulated by bone marrow plasmas of MM patients, thus it is crucial to find strategies to reinforce the cGAS/STING pathway. Our results showed that diABZI induced the production of IFNs, IFN λ among all, and the activation of *interferon-stimulated genes* (ISGs) in MM cells. Notably, treating MM cells with recombinant IFN λ led to a strong inhibition of their proliferation. Since combinatorial therapy is the best approach for MM treatment, diABZI was used in combination with the chemotherapeutic agent bortezomib and the monoclonal antibody Daratumumab, which targets the ISG CD38. The combined use of diABZI and bortezomib remarkably induced MM cellular senescence performing β-galactosidase assay and an immunostimulatory Senescence-Associated-Secretory-Phenotype (SASP). Moreover, we observed through Real time PCR and flow cytometry that diABZI significantly upregulated CD38 expression in MM cells and that increased NK cell mediated-*ADCC* triggered by Daratumumab.
Conclusion: In sum, these results provide the rationale for the combined use of STING agonist, monoclonal antibodies and chemotherapeutic agents as new strategy to potentiate MM immunosurveillance and overcome tumor immune evasion.

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626 – P2.08.11

RNA Polymerase I Inhibition and activation of Nucleolar Stress Response in the anticancer activity of Natural Killer cells against Multiple Myeloma

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Purpose: RNA polymerase I (Pol-I)-mediated transcription of the ribosomal RNA (rRNA) genes (rDNA) is rate-limiting for ribosome biogenesis, a process that is significantly elevated in rapidly dividing tumor cells. Perturbation of rDNA transcription is known to elicit Nucleolar Stress Response (NSR), which is heightened in cancer cells and offers novel opportunities for therapeutic targeting of human malignancies. To date little information has been provided about the role of the NSR and regulation of anticancer immune response.
We investigated the regulation of NSR in the tumor model Multiple Myeloma (MM) and its role in the Natural Killer (NK) cell response against this tumor.
Methods: Induction of NSR was triggered in MM cells using two "first in class" Pol-I inhibitors (Pol-Ii), CX-5461 and BMH-21 (nanomolar range). SKO-007(J3) and RPMI-8226 MM human cell lines have been treated with Pol-I inhibitors (at different concentrations/time) to evaluate by flow-cytometry and qReal-Time PCR, the expression levels of ligands for NK cell-activating and inhibitory receptors (MICA/B, ULBPs, PVR/CD155, Nectin-2, NCR-ligands), HLA-ABC, HLA-E, and CD38 (the molecular target of the therapeutic mAb Daratumumab). To investigate the functional consequences of Pol-Ii regulated changes of NK cell-activating ligands, HLA and CD38 expression, we analyzed by flow cytometry the upregulation of the lysosomal marker CD107a on NK cells of PBMCs from healthy donors, upon interaction with SKO-007(J3) cells (degranulation assay).
Results: We observed that the two drugs differently regulated the expression of NK cell activating ligands, HLA and CD38 in MM cells. Interestingly, while BMH-21 increased degranulation activity of NK cells against MM cells and IFN-γ/TNF-α levels, CX-5461 did not elicit the same effect. This correlated with a higher upregulation of DNA Damage Response (DDR) and senescence by CX-5461 as compared to BMH-21. RNA-seq analysis showed a significant enrichment of genes related to MHC presentation only in CX-5461 treated cells, and we confirmed higher levels of HLA-ABC and HLA-E by FACS analysis. Interestingly, inhibition of the ATR kinase reduced upregulation of HLA-E and increased degranulation activity when used in combination with Pol-Ii.
Conclusions: Inhibition of Pol-I and induction of NSR in MM, enhances NK cell-mediated response against this tumor.

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632 – P2.08.12

Dendritic cell reprogramming through the tumor secretome affects anti-tumor immunity in PDAC

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Purpose: Antigen presenting cells, such as dendritic cells (DC) are crucial to initiate antigen-specific adaptive immune responses making them highly relevant in the context of anti-tumor immunity. DCs differentiate from hematopoietic stem cells and belong to the myeloid lineage. However, their ability to present antigens is reduced in many cancers, leading to ineffective anti-tumor immunity. Based on these observations we hypothesized that cold tumors, such as aggressive solid cancers like pancreatic ductal adenocarcinoma (PDAC) and ovarian cancer might be particularly affected by this response contributing to the ineffective priming of adaptive immune cells.
Methods: To develop a robust mid-throughput screening platform to assess the activation status of primary DC from murine and human stem cells, we have optimized murine classical DC culture systems and phenotyped the resulting immature DC by flow cytometry using a detailed myeloid cell surface marker panel to differentiate between various DC lineages and activation states. Immature DC were exposed to tumor cell line conditioned media from aggressive and remissive PDAC tumors to assess cellular activation/suppression. In parallel, we analyzed DC activation *in vivo* focusing on the expression of DC activation and inactivation markers.
Results: We observed DC inactivation profiles in both *in vitro* and *in vivo* settings, suggesting that tumor secreted molecules directly contribute to DC suppression. We now aim to identify the molecular mechanisms that result in DC suppression and profile a number of different tumor types to identify common and specific mechanisms of DC suppression/exhaustion.
Conclusion: Our preliminary data suggest that immune suppressive effects within the tumor microenvironment affect DC suppression and exhaustion and might represent suitable targets for solid tumor immunotherapy.

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638 – P2.08.13

Investigating the expression of soluble immunomodulatory mediators in risk-stratified pancreatic cystic fluid and its effect on immune cell migration and T cell function.

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Pancreatic cystic lesions (PCLs) are fluid-filled structures found within or on the surface of the pancreas. While many PCLs are benign, others such as intraductal papillary mucinous neoplasms can undergo malignant transformation and are regarded as precursor lesions of pancreatic cancer (PC). Current guidelines used to stratify PCLs as low- or high-risk of PC progression remain highly contended. Chemokines guide the migration of immune cells into tissues. However, the desmoplastic microenvironment in PC alters the dense extracellular matrix and adhesive capacity causing exclusion of anti-tumour effector immune cells. Furthermore, dysregulated chemokine release contributes to poor immunotherapy responses in immune-excluded pancreatic tumours. The influence of PCL-secreted chemokines and growth factors on PC initiation, promotion and progression remains unstudied.
Custom flow-based multiplex ELISAs were employed to screen pancreatic cystic fluid (PCF) and patient-matched sera for a range of relevant chemokines and growth factors that are associated with the promotion and progression of cancer. We assessed this panel of analytes in PCF from 18 patients. Pseudocysts, largely pancreatitis-derived non-neoplastic lesions with no risk of malignant transformation, were used as controls. Experimental findings were correlated with standard recorded clinical parameters. Functional chemotaxis assays were performed to elucidate key chemokine pathways that drive immune cell migration within the soluble microenvironments of PCLs and surrounding tissues. Flow cytometric analysis was carried out to investigate the capacity of risk-stratified PCF to impair T cell activation, anti-tumour functions and to assess if risk-stratified PCF induces a phenotypic switch in T cells towards a pro-tumour phenotype.
Twenty-five soluble immunomodulatory mediators were detected across all samples at varying concentrations, dependent upon sample type and risk status. Immune cell migration was assessed using Boyden chamber chemotaxis assays. Our results indicate that the migration of effector immune cells towards high-risk PCF is dysregulated compared to low-risk PCF.
Our preliminary data identifies immune targets within PCF and sera that may contribute to PC development and elucidates potential immune pathways that could be targeted to enhance immune cell infiltration into tumour tissue. These immune parameters could potentially be incorporated into stratification guidelines to more accurately identify high-risk patients for earlier diagnosis and treatment.

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701 – P2.08.14

Characterizing the Impact of Lymphocyte-Derived Extracellular Vesicles on T Cell Function in Hematologic Malignancies

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Purpose: Extracellular vesicles (EVs) are small, membrane-derived particles that have been identified as key players in cell-to-cell communication and were shown to influence immune responses, blood clotting, and the spread of cancer. In hematologic malignancies, tumor-derived EVs can significantly inhibit immune functions, impacting the effectiveness of immunotherapies, including advanced T cell-based treatments like CAR T cell therapies. The aim of this study is to optimize isolation and characterization processes of lymphocyte EVs from patients with hematologic malignancies and to determine the potential inhibitory properties of the isolated EVs on T cell function, which is critical for understanding and enhancing the efficacy of T cell immunotherapeutic products.
Methods: EVs were isolated from blood of healthy donors and patients with hematologic malignancies using high-speed centrifugation followed by purification with tangential flow filtration. The isolated vesicles were stained with a variety of reagents and fluorescently labeled antibodies, including carboxyfluorescein succinimidyl ester (CFSE), CellMask Deep Red^{TM}, CD9, CD63, and CD83 antibodies to assess their efficiency in staining lymphocyte vesicles. Preliminary measurements were obtained using a BD FACS Canto I cytometer, and the origin and subsets of the vesicles were determined by measuring parent cell markers for lymphocyte subpopulations (CD19, CD3, CD4, CD8). Our further research will focus on determining the exhaustion and activation signals of EVs on immune cell effector function, with an emphasis on identifying potential markers of exhaustion and activation.
Results: The staining techniques using CFSE and CellMask Deep Red^{TM} were shown to effectively stain the majority of the isolated lymphocyte vesicles, staining 98 % and 72 % of isolated lymphocyte vesicles, respectively. These methods proved to be significantly more effective than staining with CD9, CD63, and CD83 antibodies, which only labeled 46 % of the vesicles. The preliminary results suggest a significant role of EVs in modulating immune responses, highlighting their potential in predicting and monitoring the inhibitory effects on immune cells.
Conclusion: EVs show promising potential for predicting and monitoring immunotherapy progression. However, the standardization of EV handling procedures and the identification of precise vesicle biomarkers are crucial for better understanding their involvement in hematologic malignancies and immune system responses.

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786 – P2.08.16

An organoid-based model to study innate lymphoid cells in colorectal cancer.

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Purpose: Innate lymphoid cells (ILCs) emerged as central innate immune mediators during gastrointestinal homeostasis, inflammation, and tumorigenesis. The role of ILCs in cancer is still controversial since they have been associated with either pro- and antitumor activities. The aim of this study was to perform a comprehensive evaluation of phenotype and functions of natural killer cells (NK) and ILC subsets in the context of colorectal cancer (CRC) focusing on the direct impact of tumor on these lymphocytes.
Methods: We developed an 3D intestinal organoid model to use in *in vivo* and *in vitro* approaches. We set out a mouse model of CRC based on orthotopic transplantation of tumour organoids in an immunocompetent setting and to provide an in-depth characterization of tumor infiltrating ILCs we employed an 18-color flow cytometry panel. Moreover, to study tumor-ILC interaction, we performed RNAseq analysis to profile gene expression and flow-cytometric analysis of evaluate the degranulation ability and cytokine production of NK/ILC upon co-culture with tumor organoids.
Results: In the mouse model of CRC, we observed that healthy intestinal lamina propria was populated by ILC1, ILC2 and ILC3 while innate lymphocytes infiltrating colon tumours consisted mainly of NK and ILC1-like cells, indicating a key role for these subsets in controlling cancer progression. In parallel, *in vitro* tumor organoid-NK cell coculture system showed that cancer-derived soluble factors can inhibit target cell-induced degranulation and IFN-γ production by NK cells. These functional consequences are associated with changes of NK cell gene expression program. Indeed, RNAseq analysis demonstrated that tumor intestinal organoids can affect gene pathways controlling leukocyte proliferation, metabolism, and cell-cell-adhesion in NK cells.
Conclusion: Taken together, these results suggest a major role of NK and ILC1-like cells in innate immune response against CRC as well as the direct effects of inhibitory molecules produced by cancer cells on NK cells possibly leading to tumor escape.

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969 – P2.08.17

A prototype of biocompatible solid lipid nanoparticles targeting CD19 antigen

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Purpose: The aim of this investigation was to generate a preclinical model of biocompatible solid lipid nanoparticles (SLNs) targeted with an antibody against CD19, with the perspective of immunomodulate the immune system.
Methods: SLNs were developed through microemulsion, functionalized with variable proportions of anti-CD19 using avidin-streptavidin (Av:Ab) and dialysis systems and conjugated with FITC. Parameters including Z-average size (nm), polydispersity index, and Z-potential (mV) were assessed. Once generated, SLNs were co-cultivated for 1 up-to 24 hours with $CD19^-$ Jurkat cell line, $CD19^+$ NALM-6, as well as total blood and peripheral mononuclear cells to determine optimal nanoparticle concentration, dynamics and specificity of capture/internalization. Trypan-blue was used to quench extracellular signal and differentiate between capture and internalization processes. Additionally, SLNs were covered with PEG to improve capture/internalization specificity. Experiments fixing the cellular membrane and blocking CD19 were also performed. All the analyses were conducted using Flow cytometry (Facs CantoII) and analysed based on median intensity fluorescence (MFI).
Results: Proportions of Av:Ab at 1:50 and 1:150 demonstrated the most promising results with stable z-average size, polydispersity index and z-potential. Concentrations above 2 mg/mL of SLNs were toxic, whereas concentrations below 0.1 mg/mL were not enough to be captured/internalized. Capture/internalization was directly proportional to the concentration of nanoparticles but inversely proportional to the amount of anti-CD19 loaded. Almost all nanoparticles were internalized after 10 minutes of incubation. When fixing cellular membrane, capture/internalization was even higher. Optimal specificity was not achieved as SLNs were captured and internalized by both $CD19^+$ and $CD19^-$ cells even when they were covered with PEG. However, it seemed that B lymphocytes exhibited higher MFI values. This PEG neither achieved capture/internalization of SLNs in total blood samples. Finally, when blocking CD19 target, a slight decrease in the MFI was observed in NALM6 but not in Jurkat cells.
Conclusion: Hydrophobic nature of SLNs could be the underlying cause of not achieving a specific capture/internalization by $CD19^+$ cells. The inclusion of PEG did not improve that specificity. Further experiments involving the charge and the size of SLNs, the type of transport or alternative covering materials are needed to succeed in the specificity.

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993 – P2.08.18

Repeated antigenic stimulation induces expression of surface 'Tumor necrosis factor Related Apoptosis Ligand' on human invariant Natural Killer T cells.

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Purpose: Invariant Natural Killer T (*i*NKT) cells are unique innate-like T cells that recognize lipid antigens, like α-galactosylceramide (αGalCer), presented on CD1d. Following antigenic stimulation, *i*NKT cells rapidly display effector functions, like the production of various cytokines and cytotoxicity. The 'Tumor necrosis factor Related Apoptosis Inducing Ligand' (TRAIL), via its binding to the death receptors (DRs) 4 and 5, preferentially induced apoptosis in cancerous or unhealthy cells. Although it was suggested that *i*NKT cells can induce cell death in tumour cells via TRAIL, conflicting data were reported.
Methods: Peripheral blood mononuclear cells (PBMCs) of healthy donors were isolated and *i*NKT cells were expanded *in vitro* with αGalCer. TRAIL expression on *i*NKT cells (live $CD3^+$ $6B11^+$ cells) was analysed over time with or without activation by flow cytometry.
Results: Human PBMC-derived *i*NKT cells did not express TRAIL *ex vivo* and the expression on expanded *i*NKT cell lines was weak. However, restimulation of expanded *i*NKT cell lines with αGalCer gradually induced surface TRAIL expression, peaking on day 2 after the restimulation and decreasing towards baseline by day 7. Preliminary data suggest that surface TRAIL is lost by shedding.
Conclusion: Human peripheral blood *i*NKT cells express surface TRAIL only after a second antigenic stimulation. These data will help to better understand the TRAIL-mediated cytotoxicity by *i*NKT cells in the tumour microenvironment.

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1001 – P2.08.19

Effect of local X-ray radiation doses on circulating immune populations in melanoma tumour-bearing mice by flow cytometry

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Purpose: Melanoma is a disease whose incidence is rising nowadays, causing more than 7000 deaths in 2023. However, radiation theraphy alone is rarely an option to treat early-stage melanomas, although it is able to activate an antitumor immune response. **To characterise the effects of X-ray radiation on circulating immune cells in melanoma tumor bearing-mice.**
Methods: 27 male C57BL/6 of 26 grams of weight were subcutaneously inoculated with 200.000 melanoma B16/F1 cells in both hind flanks. Before tumors reached a size of 0,5 cm, the right hind tumor was irradiated with a single dose of 4 Gy (n=9), 8 Gy (n=5), 12 Gy (fraccionated in two sessions; n=5), or no radiation (controls, n=8). Periferal blood (PB) samples were collected pre-irradiation, at 2 hours and day 7 post-irradiation (p.i). Whole blood was labeled with 27 antibodies distributed across two panels and acquired in a 16-channel Flow Cytometer. Tumor growth was measured daily using calipers.
Results: Compared with controls, 4 and 8 Gy irradiated mice showed no differences in any PB immune subpopulations. However, 12 Gy irradiated animals exhibited a decrease in the total leukocytes 2 hours p.i. This reduction was observed both in the myeloid and lymphoid compartments, particularly in granulocytes, monocytes, type 1 dendritic cells, NK cells, and T and B lymphocytes. However, at 7 days only the MHC-expressing neutrophils were decreased. Tumors growth was decreased only after 12 Gy.
Conclusion: Fraccionated irradiation with 12 Gy induced a reduction in PB leukocytes and was succesful in the treatment of the irradiated melanoma tumors by limiting their growth. The decrease of total leukocytes could indicate either a migration of the immune cells to the irradiated tissue, or an immune depletion due to radiation, resulting in fewer immune cells present in the peripheral blood. However, single doses of 4 Gy and 8 Gy have no effect in tumor growth and the circulating immune populations.

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1065 – P2.08.20

Blinatumomab and Tyrosine Kinase Inhibitors in B-acute leukemia: friends, or enemies?

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Introduction: Blinatumomab, a bi-specific T cell engager (CD3/CD19), is primarily used in the treatment of relapsed and refractory B-acute lymphoblastic leukemia (B-ALL) or as a salvage therapy before transplantation. It has been observed that relapses after blinatumomab treatment often manifest as extramedullary disease, indicating potential changes in cell adhesion specificity. Tyrosine kinase inhibitors (TKIs) are widely employed in the therapy of Ph+ leukemia, targeting the BCR-ABL oncogene and Src family kinases while also influencing leukemia cell adhesion. Recent clinical trials have successfully combined blinatumomab with TKIs for treating Ph+ B-ALL, although theoretically, the combination should not be effective due to TKIs inhibiting the essential SRC kinases involved in T cell activation.
Materials & Methods: In our study, we utilized lymphocytes from healthy controls (either freshly isolated or expanded in vitro) and B-ALL cell lines (RAJI, REH, ARH-77) as experimental models. These cells were subjected to treatment with blinatumomab and/or TKIs (dasatinib and ponatinib). Using flow cytometry, we evaluated: 1) the effectiveness of blinatumomab- and TKI-induced cytotoxicity, 2) alterations in the expression of cell adhesion molecules prompted by the treatment, and 3) the activation status of both effector T cells and target leukemia cells. Additionally, we used the Seahorse XFp device to assess changes in cellular metabolism in B-ALL cells following the treatment.
Results & Conclusion: In summary, we demonstrate that even though TKIs inhibit SRC kinases necessary for T cell activation, the lowest clinically relevant levels of TKIs do not impede the cytotoxic effect of blinatumomab. Moreover, TKIs have an impact on the levels of CD62L on the surface of B-ALL cells, indicating potential changes in cell adhesion specificity. Additionally, TKIs decreased B-ALL cell oxygen consumption, while not having a significant effect on glycolysis. Together, these findings provide valuable insights into the mechanisms underlying the effectiveness of combination therapy and emphasize the importance of further investigating the interplay between blinatumomab, TKIs, and cell adhesion molecules in the context of B-ALL treatment.

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1126 – P2.08.21

Comparative analysis of adjuvants and their synergistic effect for the activation of murine liver non-parenchymal cells

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Purpose: Non-parenchymal cells (NPCs) within the liver hold a critical role in inducing liver tolerance, making the liver a challenging environment for intrahepatic immunotherapeutic approaches and limiting the potency of intrahepatic anti-tumor responses. However, these immune cells can be activated by suitable adjuvants to initiate effector T-cell responses. The aim is to identify an adjuvant and an adjuvant combination that highly activate the immune cell population in the liver, together with encapsulated mRNA- encoded antigen, to enhance the immune response in a therapeutic anti-cancer vaccine approach.
Methods: Murine liver NPCs were isolated by liver perfusion and stimulated with different adjuvants and combinations overnight to test *in vitro* activation. Expression of surface activation markers and the secretion of pro-inflammatory cytokines were measured by flow cytometry and cytometric bead array (CBA), respectively. Thymidine incorporation assay assessed T-cell proliferation in a co-culture setting. For the *in vivo* study, C57BL/6J mice were injected i.v. with *ovalbumin* mRNA encapsulated in lipid nanoparticles together with adjuvants to test *in vivo* activation of NPCs. Liver and spleen were dissected and the single-cell suspensions were analyzed by flow cytometry. Serum and culture supernatant were assessed for the secretion of pro-inflammatory cytokines by CBA.
Results: *In vitro* screening of adjuvants revealed that co-stimulation with combined adjuvants induced the highest activation response in regard to expression of activation markers and cytokine secretion compared to individual adjuvants, suggesting a synergistic effect. The *in vitro* T-cell proliferation assay showed high proliferation of $CD8^+$ T-cells with all adjuvants alone and combined, while $CD4^+$ T-cells only showed high proliferation with the adjuvant R848, alone and in combination. *In vivo* testing showed high expression of activation markers and cytokine secretion in both NPCs and splenic dendritic cells with adjuvant R848 alone and combined.
Conclusion: We could identify that R848 alone and in combination incorporated together with an mRNA-encoded antigen in a nano-carrier delivery system, highly activated the immune cell population in the liver. Conducting *in vitro* and *in vivo* testing of adjuvants is essential to identify potent activators for liver NPCs in order to overcome liver tolerance and enabling an adequate intrahepatic immune response.

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1151 – P2.08.22

B cells as a target for immunotherapy in non-alcoholic steatohepatitis and subsequently for hepatocellular carcinoma

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Purpose: The purpose of this study is to investigate the role of adaptive immunity, specifically B cells, in the development of non-alcoholic steatohepatitis (NASH) and and its role as a driver of hepatocellular carcinoma (HCC). Furthermore, we aim to explore the gut-liver axis by profiling liver and intestinal B cells.
Method: The researchers employed various mouse models, including $JH^{-/-}$ mice lacking B cells and μMT mice lacking mature B cells but retaining IgA^{+} B cells in the lamina propria, which were fed a choline-deficient high-fat diet (CD-HFD) to induce NASH. Additionally, wild-type mice and μMT mice were treated with αCD20 to deplete B cells. We employed high-dimensional flow cytometry and single-cell RNA sequencing to identify subpopulation and gene expression profiles.
Results: The study found mice lacking mature B cells exhibited the absence of steatosis, liver inflammation and fibrosis when fed a CDHFD, whereas μMT mice resulted in hepatic steatosis, inflamed but did not showed signs of fibrosis. In addition, depletion of B cells by αCD20 treatment in wild type and μMT under CD-HFD, showed an abrogation of steatosis, inflammation and fibrosis. Our preliminary data from single-cell RNA sequencing revealed three subpopulations of intestinal B cells: naïve, activated, and germinal center B cells. Germinal center B cells were decreased in the curse of NASH and exhibited the highest differential gene expression compared to controls.
Conclusion: The findings suggest that B cells play a significant role in the development of NASH, liver, fibrosis, and potentially hepatocellular carcinoma (HCC). Additionally, the study provides insights into the gut-liver axis, highlighting changes in intestinal B cell composition and gene expression profiles during NASH progression. This research underscores the importance of adaptive immunity, particulary B cells, in NASH and NASH-to-HCC transition. Eventually this may lead to the indentification of potential therapeutic targets for the disease.

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European Journal of Immunology
Clinical-Basic-Translational

1152 – P2.08.23

B cell expansion during gut inflammation exacerbates colorectal cancer development

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Colorectal cancer (CRC) is the third most common cancer worldwide with a rapidly increasing incidence rate in individuals even under 50 years of age. Besides genetic factors, patients with long-standing intestinal inflammation have a significant higher risk to develop CRC. However, the precise mechanisms underlying tumour-promoting inflammatory processes in CRC are, so far, insufficiently characterized. Here, we provide evidence that B cells exert dual roles, either tumour promoting or supressing, depending on the degree of intestinal inflammation. B cells are the dominant cell type in the healing colon after DSS treatment, with an expansion of an IFN-induced B cell subset. In line with these data, B cell depletion accelerated recovery upon DSS driven colonic injury. We further demonstrate that B cell-deficient mice show significantly ameliorated intestinal inflammation upon DSS administration to be resistant to CRC development accompanied with less immune cell infiltrates into the colonic lamina propria as well as reduced production of pro-inflammatory cytokines. In particular, we reveal IL-10 secretion by B cells to actively contribute to tumour development in the intestine via polarization/activation of gut resident macrophages towards an M1 phenotype. Thus, targeting B cell specific mechanism in colorectal cancer might be a promising avenue to combat this still fatal disease.

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1216 – P2.08.24

Acquired resistance to trastuzumab and neratinib sensitizes HER2+ breast cancer cells to trastuzumab-dependent cell-mediated cytotoxicity

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Purpose: Acquired resistance to HER2-targeted therapies leads to therapeutic failure in breast cancer patients and there is limited information whether trastuzumab-dependent cell-mediated cytotoxicity (T-ADCC) can be seriously affected. This study is aimed to study the impact on T-ADCC of acquired resistance to FDA-approved HER2-targeted therapies trastuzumab and neratinib in breast cancer cells.
Methods: Two acquired HER2-targeted therapy resistant breast cancer cell lines (BCCLs) were studied: trastuzumab-resistant SKBR3 (SKBR3-Tras) and neratinib-resistant HCC1954 (HCC1954-Ner). SKBR3-Tras and HCC1954-Ner were generated after 6-month continuous exposure to 10µg/mL of trastuzumab and 150nM of neratinib, respectively. Their therapy-naive or parental (Par) counterparts were used as baseline controls. Flow cytometry assays were carried out to determine T-ADCC using healthy volunteer (HV)-derived peripheral blood mononuclear cells against BCCLs. Levels of membrane-bound proteins modulating T-ADCC in BCCLs were also checked by flow cytometry. The panel included HER2, HLA-A/B/C, HLA-G, HLA-E, E-cadherin, MIC-A/B, ULBP-2/5/6, PD-L1, ICAM-1 and CD95. Unpaired t-tests were performed to compare therapy-resistant BCCLs against Par counterparts ($p<0.05$ is significant).
Results: SKBR3-Tras and HCC1954-Ner were more sensitive to T-ADCC than their respective parental counterparts (SKBR3 [HV3: p=0.0009; HV4: p=0.0484], HCC1954 [HV3: p=0.0004; HV4: p=0.0450]). In relation to their respective parental counterparts, SKBR3-Tras increased HER2 levels (p=0.081) but HCC1954-Ner experienced a decrease (p=0.0354). For ICAM-1, SKBR3-Tras showed lower levels (p=0.0069) while HCC1954-Ner had higher levels (p=0.0010). CD95 and E-cadherin did not change in SKBR3-T however, their expression dropped in HCC1954-Ner (p=0.0215 and p=0.0070, respectively). HLA-A/B/C and ULBP-2/5/6 downregulation were detected in SKBR3-Tras (p=0.0142 and p=0.0168, respectively) and no alteration was observed in HCC1954-Ner. Unmodified levels of PD-L1, HLA-G, HLA-E and MIC-A/B were seen in both SKBR3-Tras and HCC1954-Par.
Conclusion: Resistance to HER2-targeted therapies changes the expression profile of ADCC modulators. Acquired resistance to HER2-targeted therapies may sensitise tumour cells to T-ADCC.

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1252 – P2.08.25

Microbial metabolites suppress the tumorigenic functions of IL-36 cytokines in colon cancer.

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The Interleukin (IL)-36 cytokines are a recently described subset of the IL-1 family of cytokines, comprising of three agonists, (IL-36α, IL-36β, and IL-36γ), an antagonist (IL-36Ra) and a receptor (IL-36R). Similar to all other IL-1 family members, IL-36 cytokines have complex dual functions in cancer, exhibiting both pro- and anti-tumorigenic functions. Metabolites generated by the microbiome have also been shown to have dual functions in colon cancer. For instance, butyrate is a short chain fatty acid which has been shown to have tumour suppressive activity, while deoxycholic acid (DCA) is a secondary bile acid with potential tumour promoting functions.

The aim of this study was to investigate if microbiome-encoded metabolites alter the tumour-promoting activity of IL-36 family members in colon cancer. Cell proliferation was measured by both resazurin reduction and BrDU incorporation assay. Cell migration was assessed by wound scratch assay. Gene expression was measured by qRT-PCR and signalling was assessed by Western Blot.

Stimulation of human (HT29) and murine (CT26) colon cancer cell lines with IL-36β and IL-36γ increased cellular proliferation, migration, and inflammation in vitro. Butyrate (1mM) alone had little effect on cell proliferation and migration but significantly suppressed the ability of IL-36β and IL-36γ to induce these tumorigenic processes. Similarly, DCA alone (25μM) slightly suppressed basal level cell proliferation and migration, while co-treatment of DCA with IL-36 agonists significantly suppressed IL-36-mediated cell proliferation and migration. Western blotting for intracellular signalling pathways known to play a role in IL-36-mediated cell proliferation was preformed, with co-treatment of cells with butyrate and IL-36γ resulting in decreased phosphorylation of RPS6, potentially implicating the AKT/mTORC1 pathway. Both butyrate and DCA modified IL-36-induced cytokine and chemokine production.

These studies have shown that the pro-tumorigenic functions of IL-36 cytokines are suppressed by microbiome-derived metabolites, suggesting that bidirectional interaction with the microbiota may impact on the activity of IL-36 family members.

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1257 – P2.08.26

Serial Monitoring of the Inflammatory Response in Resectable Lung Cancer.

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Purpose: Profiling the immune response of patients undergoing lung cancer resection, could give valuable insights into systemic inflammation and immunosuppression, triggered by surgery. Detection of circulating tumour cells can be seen 6 weeks post-surgery, alongside post-operative immunosuppressive state, in some cases up to 6 months. Dissemination and evasion of tumour cells, impaired cellular immunity and elevated cytokines, due to surgical stress, can make way to recurrence, metastasis and negatively impact immunotherapy response. Inflammatory indexes can be used as a prognostic tool to predict clinical outcomes. Systemic Inflammatory Response Index (SIRI), Neutrophils x Monocytes / Lymphocytes, is shown to predict poor outcomes, including post-operative complications (≥1.2) and for pre-treatment chemoradiotherapy (≥2) in NSCLC. Systemic Inflammatory Index (SII), Neutrophils x Platelets / Lymphocytes, (>730) and SIRI were evaluated on peri-operative patients. Cross-linked fibrin is linked with tumour cell angiogenesis and invasion. Elevated preoperative D-dimer (>500ng/mL) is associated with tumour stage, nodal involvement and is a prognostic indicator for recurrence and metastasis.
Methods: Full Blood Count and D-Dimer were taken at baseline (n=26), 2-4 days post operatively (n=26) and 6-9 weeks (n=14) post-operatively. SIRI and SII were calculated. PBMCs were isolated and stored for serial monitoring of T and B cell subsets.
Results: 69% and 50% had an elevated SIRI and SII at baseline, of those 46% and 38% had complications. 31% and 50% did not have an elevated SIRI and SII at baseline, of those 20% and 46% had complications. The higher the SIRI and SII at baseline, the more likely to have complications. 64% and 93% continued to have an elevated SIRI and SII after 6-9 weeks. 38% had an elevated D-Dimer pre-operatively. 100% had a D-dimer of >500ng/mL after 6-9 weeks. T and B cell subsets will also be presented.
Conclusions: In this cohort of patients, baseline elevated SIRI and SII, can correlate with post-operative complications. Inflammatory indexes and D-Dimer remain elevated 6-9 weeks post-operatively, showing on-going dysregulation of immune response. This has previously been shown to impact immunotherapy response, overall and drug free survival. Further studies will characterise these cells using flow cytometry.

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1265 – P2.08.27

Cyp27a1-driven metabolic control in macrophages regulates the tumor microenvironment

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Purpose: 27-hydroxycholesterol (27-HC) is the most abundant oxysterol, and it is metabolized via a cytochrome P450 oxidase, CYP27A1. Although 27-HC-mediated functional diversity of macrophages has been studied in breast cancer and atherosclerosis, how the overall macrophage metabolism is influenced by CYP27A1 is yet to be explored. In this study, we aimed to investigate the CYP27A1-mediated association between oxysterol metabolism and other metabolic pathways along with the immunometabolic crosstalk between macrophages and lymphoid cells during cancer.
Methods: We generated bone marrow-derived macrophages (BMDMs) from macrophage-specific Cyp27a1 knockout mice (Cyp27a1 fl/fl, Cx3cr1-Cre; Cyp27a1 MKO) to explore the cellular metabolism using liquid chromatography-mass spectrometry. To study the functional aspects of altered oxysterol metabolism in macrophages, we introduced two murine cancer lines (B16-F10 and MC38) in our Cyp27a1 MKO mice to investigate cancer growth by survival analysis and measuring tumour volume. Through flow cytometry, we analyzed the composition of immune cell populations in these cancer models.
Results: At basal condition, in BMDMs from Cyp27a1 MKO mice, along with bile acid metabolism, we observed a significant decrease of intracellular purine and pyrimidine molecules compared to BMDMs from littermates. Since increased purine and pyrimidine metabolism in tumour-associated macrophages was associated with an anti-tumorigenic effect, we hypothesized that Cyp27a1 MKO mice should have a better cancer prognosis. In alignment with our hypothesis, we found that in both MC38 and B16-F10 tumour models, the Cyp27a1 MKO group had a better survival rate with a significant decrease in tumour volume. To delineate the underlying mechanism, we analyzed the immune cell population in tumour tissue collected from both groups. Surprisingly, we found significantly decreased CD4+ and CD8+ T cell infiltration in tumour tissues of the Cyp27a1 MKO mice.
Conclusion: Through our study, we have highlighted the association between 27-HC metabolism with purine and pyrimidine metabolism in macrophages. On top of that, Cyp27a1 in macrophages undermines efficient anti-tumour immunity by a surprising effect in regulating the composition of the tumour microenvironment.

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1279 – P2.08.28

Effect of melanoma on circulating immune populations in a murine model by flow cytometry

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Purpose: Melanoma is a disease whose incidence is rising nowadays, causing more than 7000 deaths in 2023. Although a dysregulation in the immune system is implicated in the emergence of tumor cells, the immune alterations are still unknown at early stages of the desease. To evaluate changes induced by subcutaneous melanoma implantation in peripheral blood (PB) immune populations by flow cytometry.
Methods: 43 male C57BL/6J mice of 26 grams of weight were used. 21 mice were subcutaneously inoculated with 200,000 melanoma B16/F1 cells in both hind flanks, while the remaining wild-type animals (WT, n=22) are controls. When tumors reached a size of 0.5 cm, 100µl of PB were collected in EDTA tubes from both groups. PB was lysed and labeled according to our 2 novel cytometry panels with 14 markers each, that enable to visualize most leukocytes subsets including different types of myeloid and lymphoid cells. PB samples were acquired in a 16 channels flow cytometer.
Results: Compared with WT, tumour-bearing animals showed a decrease in total leukocytes at 10 day. Also myeloid cells were increased, although no differences were found in the granulocytic population, except for those expressing the MHC marker, which were also increased in the tumor group. In the monocyte compartment, Type M1 and M2 monocytes were also increased. In contrast, plasmacytoid dendritic cells and conventional mature type 1 dendritic cells were decreased. Tumour-bearing animals showed a decrease in all T cell subpopulations, regarding differentiation (Th1, Th2, Treg..) and activation states (naive, effector and central memory). A decrease was also observed in NKT and plasmatic B cells.
Conclusion: Moderate growth of subcutaneous B16/F1 melanoma tumours (no more than 0.5 cm) induced alterations in the studied PB immunological populations of both lymphoid and myeloid lineages. These alterations allow us to better understand the immune status in early stages of tumour progression, even in minority subpopulations such as MCH II monocytes or dendritic cells.

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1297 – P2.08.29

RANK pathway inhibition impairs immunosuppression in macrophages enhancing the anti-tumour response

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Luminal breast tumours generally exhibit low immune infiltration and are unresponsive to immunotherapy. RANK pathway inhibitors have emerged as putative therapeutic target in breast cancer, with pleiotropic effects on cancer and immune cells. In breast adenocarcinomas RANK is expressed by tumour cells and tumour-associated macrophages (TAMs). Previous results demonstrate that RANK signalling activation in tumour cells enhances stemness and immunosuppression, but whether and how RANK influences macrophage (MØ) phenotype and functionality is unknown. We hypothesize that RANK expression in TAMs may contribute to generate an immunosuppressive phenotype, promoting tumour progression and metastasis.
Here, results from the window of opportunity clinical trial D-BIOMARK (NCT03691311) demonstrate that RANK expression in the stroma, which is mainly located in TAMs, associates with highly proliferative luminal tumours. Transcriptional profiling evidences that preoperative single agent denosumab in luminal breast cancer leads to a decrease in immunosuppressive MØs and an increase in monocytes-related genes, together with enhanced activation of innate and adaptive immune pathways. Indeed, RANK expression increases during the differentiation from human and mouse monocytes to macrophages. These results indicate that denosumab interferes with MØ differentiation and their immunosuppressive role.
In preclinical mouse models of breast cancer with strong RANK expression in TAMs, we have found that RANK deletion exclusively in myeloid cells delays tumour onset and reduces lung metastasis. At single cell level, an enrichment of the transcription factors Stat1/Irf1, Ifn-driven genes and antigen presentation genes is found in RANK-null TAMs, resulting in Ifn activation in T cells and enhancing the anti-tumour immune response. Comparably, pharmacological inhibition of RANKL also leads to a proinflammatory TAM profile in line with the clinical data.
Altogether, our preclinical and clinical results demonstrate that RANK expression in myeloid cells favours breast cancer progression, and that RANKL inhibition impairs the immunosuppressive phenotype of TAMs, supporting the immunomodulatory potential of RANKL inhibitors in breast cancer.

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1313 – P2.08.30

ROR-1 defines a population of atypical B cells in peripheral blood phenotypically linked to malignant cells of chronic lymphocytic leukemia

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Chronic lymphocytic leukemia (CLL) is the most common leukemia in the Western world. It is a hematological malignancy characterized by clonal expansion of B cells in secondary lymphoid organs and their evasion to the peripheral blood (PB). It is a heterogeneous disease the origin of which is still unknown. However, there are several diagnostic markers which unify CLL cell phenotype among patients. One such marker is the receptor tyrosine kinase like orphan receptor 1 (ROR-1) which is one of key players in Wnt/planar cell polarity (PCP) signalling. In our previous study, we have shown that CLL cells exhibit increased expression of not only ROR-1 but multiple other components of the Wnt/PCP signalling (Kaucká et al., 2013). This indicates that ROR-1-mediated signalling might be contributing to malignant transformation in CLL. This hypothesis was further supported by almost complete absence of ROR-1+ B cells in PB of healthy donors (HD, 1-5% from total B cells) and their partial enrichment in individuals with condition preceding CLL, called monoclonal B lymphocytosis (MBL, 30-98% from total B cells).
Therefore, we aimed to describe ROR-1+ B cells and elucidate if a specific subpopulation of ROR-1+ B cells might give rise to CLL clone. Using spectral flow cytometry, we showed that ROR-1+ B cells of HDs are enriched mainly in the population of unswitched memory B cells but they also form clusters of cells phenotypically close to CLL clone. To inspect ROR-1+ B cell heterogeneity, we performed CyTOF experiment with 30-marker panel and we found a specific cluster of ROR-1(dim) cells lacking the expression of CD21 the absence of which has already been linked to atypical B cells with possible autoreactive potential. Furthermore, trajectory analysis of CyTOF data (performed using specialized tool, tviblindi) identified ROR-1(dim) CD21(low) population as the cluster interconnecting normal B cell development trajectory with the malignant clone. Finally, using scRNAseq combined with advanced data analysis, we elucidated that ROR-1+ population of interest displayed increased activity of multiple signalling pathways (NFκB, MAPK) and transcription factors (STAT3, JUN) supporting proliferation and survival of CLL cells.

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1339 – P2.08.31

Autoantibodies against AFP in liver diseases

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Purpose: Hepatocellular carcinoma (HCC) ranks as the second leading cause of cancer-related mortality and the sixth most prevalent cancer globally. Unfortunately, the majority of HCC cases are diagnosed at advanced stages, resulting in poorer survival outcomes. A surveillance program, primarily targeting at-risk individuals with cirrhosis, recommends regular abdominal ultrasound screenings every six months. The immune system plays a crucial role in cancer control, with tumor-associated antigens (TAA) emerging as essential proteins implicated in cancer transformation and progression to malignancy. Therefore, they hold significant promise as diagnostic and therapeutic targets. This study aims to investigate the presence of antibodies against the TAA human recombinant AFP (hrAFP) in HCC and other liver diseases, with the goal of assessing its potential as a biomarker for early diagnosis and monitoring of HCC patients.
Methods: We recruited diferent populations of patients to compare the levels of hrAFP and to evaluate their useful as a pilot study. We included 67 patients with HCC and cirrhosis (HCC-Cirr), 29 HCC without cirrhosis (HCC-NoCirr), 73 patients with cirrhosis without HCC (Cirr), 48 chronic hepatitis (CH), and 44 non-hepatic diseases serum blood donors. The anti-AFP antibodies were analyzed using a homemade ELISA assay employing recombinant human AFP. A group of positive sera was selected for epitope mapping to identify the epitope eliciting the highest reactivity.
Results: The presence of anti-AFP antibodies was assessed using rhAFP, with a cutoff established at 93.2% specificity compared to healthy donors. The presence of these autoantibodies was 83.6% in HCC-Cirr, and 6.9% in HCC-NoCirr, 75.3% in Cirr and 29.2% in HC. Epitope mapping was conducted with a group of positive samples, identifying a 20-amino acid peptide. The immunoassay of the identified peptide was performed using a cutoff with 92.3% specificity compared to healthy donors. The presence of autoantibodies against the peptide was 89.4% in HCC-Cirr, 0.0% in HCC-NoCirr, 87.1% in Cirr and 45.8% in HC. There were no statistically significant differences observed between the use of rhAFP and the identified peptide.
Conclusion: Anti-AFP autoantibodies are not a reliable biomarker for HCC diagnosis but can serve as a biomarker for liver cirrhosis.

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1389 – P2.08.32

Role of innate CD8 T-cells in the control of residual disease in chronic myeloid leukemia

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Purpose: Innate CD8 T-cells (ITC-CD8) are unconventional T-cells sharing features of both adaptative and innate immunity. Based on their NKG2A and panKIR2D and/or KIR3DL1DL2 surface expression profiles, ITC-CD8 can be divided into 4 subsets. The ITC-CD8-NKG2A+ subset exhibits production of IFN-γ in response to IL-12/IL-18 stimulation while ITC-CD8-KIR+ subsets display NK-like cytotoxicity through CD16, perforin or granzyme B expression. These characteristics suggest a potential role in protection against cancer, an hypothesis that we have been tested in a hematopoietic cancer, chronic myeloid leukemia (CML), in which a control by the immune system is supported by the appearance of CML-specific CD8 T-cells in patients in remission. Importantly, ITC-CD8, which are impacted numerically/functionally at CML diagnosis, are in part restored in patients in remission. Here, we hypothesized that ITC-CD8 subsets contribute to the control of residual disease following treatment discontinuation, leading to successful maintenance of sustained treatment-free remission (TFR). To this end, ITC-CD8 subsets were compared between patients with TFR and those who experienced disease relapse (DR).
Methods: Peripheral blood mononuclear cells (PBMC) from healthy donors (HD), CML-TFR and CML-DR patients were thawed and stained for analysis by flow cytometry *ex vivo*. Data were acquired on an Aurora spectral flow cytometer (Cytek Biosciences). Unmixing/compensations and statistical analysis were performed using SpectroFlo v3.0, and FlowJo10, GraphPad Prism 10 and OMIQ software applications, respectively.
Results: Numerical analysis highlights an imbalance in ITC-CD8 subset distribution in favour of ITC-CD8-panKIR2D+, known to include activating KIR receptors, from CML-TFR patients (*versus* HD and CML-DR patients). Transcription factor expression (Eomes/PLZF/T-bet) are impaired at diagnosis, and recovered in all ITC-CD8 subsets from both groups of patients. Functional analysis shows higher expression of CD69 in all the ITC-CD8 KIR+ subsets from CML-DR patients (*versus* HD) while perforin surface-expression frequencies of ITC-KIR+ subsets tend to increase in CML-TFR patients (*versus* CML-DR patients). In contrast, PD-1 expression is higher in all ITC-CD8-KIR+ subsets from CML-DR patients (*versus* CML-TFR patients).
Conclusion: Taken together, our results support the hypothesis that the ITC-CD8 compartment, especially its KIR+ subsets, contribute to the control of residual CML disease, and could represent a functional signature of TFR.

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1397 – P2.08.33

Immunomodulatory Effects of Repetitive Radiofrequency Ablation in Inoperable Pancreatic Adenocarcinoma

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Pancreatic ductal adenocarcinoma (PDAC) remains a formidable challenge in oncology, claiming the lives of half a million individuals annually worldwide. Despite advances in treatment, the dense tumor stroma and immune suppression characteristic of PDAC often hinder the effectiveness of therapeutic interventions. Radiofrequency ablation (RFA) has shown promise in triggering tumor-specific immune responses and disrupting the tumor microenvironment. In our study, we investigated the impact of repetitive RFA on immunological parameters in patients with inoperable PDAC. Peripheral blood samples were collected from PDAC patients subjected to three consecutive rounds of endoluminal RFA and compared to age-matched healthy controls. Our findings revealed that repetitive RFA led to a significant reduction in protumorigenic cytokines, including transforming growth factor-beta (TGF-β) and interleukin-17 (IL-17), as well as a decrease in protumorigenic CD4+CD39+ and naive CD45RA T cells. Notably, these immunomodulatory effects were observed only after repetitive RFA, highlighting the distinct impact of multiple treatments.This study represents a pioneering exploration into the immunological benefits of repetitive RFA in inoperable PDAC patients. While these findings underscore the potential of repetitive RFA in modulating the immune landscape of PDAC, it is important to note that the observed changes cannot be solely attributed to RFA due to the absence of a control group with alternative treatments. Further comprehensive investigations, involving larger cohorts, diverse treatment modalities, and intricate immune readouts, are essential to validate and enhance our understanding of the clinical advantages associated with repetitive RFA in the context of pancreatic cancer.

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1435 – P2.08.34

The Tyrosine Kinase Inhibitor Dasatinib impedes adaptive cell surveillance in peripheral lymph nodes

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Dasatinib is a tyrosine kinase inhibitor approved as first-line treatment for Philadelphia chromosome-positive leukemia including chronic myeloid leukemia and is increasingly used in other clinical settings. While effective and well tolerated, patients commonly present a diverse range of side effects, reflecting a wide spectrum of dasatinib-inhibited off-targets. Examining preclinical mouse models and human patients data, we have recently published that dasatinib induces a temporary contraction of spleen stroma, which leads to an expulsion of splenocytes. This explains the drug-induced transient lymphocytosis previously associated to a better response rate in patients. Whether dasatinib also affects the function of other secondary lymphoid organs, such as peripheral lymph nodes (PLNs), has remained elusive. Here, using mouse models we report that whereas dasatinib did not induce PLN contraction, intravital imaging uncovered a strong reduction in interstitial motility of both T and B cells. As dasatinib affects in vitro B cell but not T cell migration to lymphoid chemokines, we examined an indirect activity of dasatinib leading to reduced lymphocyte motility in vivo. Lymphocyte arrest following dasatinib treatment correlated closely with a local increase in PLN hypoxia, which was accompanied by a constriction of PLN blood vessels as assessed by intravital imaging. Accordingly, dasatinib-induced hypoxia was reversed by pharmacological inhibition of the contraction-promoting factor Rho-associated protein kinase. In sum, our work provides evidence that dasatinib-induced reduction of lymphocyte motility in PLNs is a consequence of drug-induced vasoconstriction and the subsequent decline in oxygenated blood supply, affecting adaptive immune cell function in these organs.

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1587 – P2.08.35

Dysfunctional γδ T cells are associated with aggressive squamous cell carcinoma in Epidermolysis bullosa

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Epidermolysis bullosa (EB) is an inherited skin disorder, characterized by mucocutaneous fragility and blister formation upon minimal trauma. Patients who suffer from the severe form, recessive dystrophic EB (RDEB), often develop highly aggressive cutaneous squamous cell carcinomas (SCC). SCC in RDEB patients has a higher morbidity and mortality compared to SCC in patients without RDEB, although they share similar driver mutations. The patho-mechanisms are still largely unknown and currently there is no effective therapy available. Therefore, we analyzed whether the aggressive nature of SCC in RDEB patients is associated with a dysfunction in tumor immune surveillance. Gamma Delta (γδ) T cells display anti-tumor functions and the presence of tumor infiltrating γδ T cells was the most significant favorable prognostic immune population among 39 human cancer types. We discovered that the fraction of IFN-g producing cells was reduced among circulating and tumor infiltrating gd T cells isolated from RDEB patients with SCC. Based on these results, we hypothesize that γδ T cells are dysfunctional, and that this dysfunction is mechanistically linked to the formation of SCC. Whereas γδ T cell function has been studied in various skin cancer entities, their role in regulating the growth of the uniquely aggressive SCC in RDEB patients has not been investigated. To fill this knowledge gap, we mechanistically dissect how the SCC tumor microenvironment modulates the function of γδ T cells in RDEB patients. We utilize our innovative and unique *in vitro* 3D organotypic skin and *in vivo* tumor xenografting models to elucidate the cellular communication between γδ T cells and tumor cells. This study will provide comprehensive and mechanistic insights on the anti-tumor function of γδ T cells in RDEB patients with SCC. Furthermore, our results will contribute to the development of effective immuno-therapies against the highly aggressive SCC in RDEB patients.

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1592 – P2.08.36

Role of SHP-2 and PD-1 in lymphoid and myeloid antitumor responses

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Immune cells infiltrate the tumoral mass and, due to antigenic and inflammatory stimuli, their functions can be altered. For example, T cells can reach a state of "exhaustion", characterized by expression of inhibitory receptors, such as programmed cell death protein 1 (PD-1). SH-2 domain-containing phosphatase (SHP)-2 is reported to be an important component of the inhibitory effects of PD-1 by interacting with it. Paradoxically, SHP-2 is best known as a positive regulator downstream of growth factor receptors and inhibitors targeting this phosphatase are currently under clinical evaluation to dampen cancer progression.
To date, the biological meaning of the interplay between SHP-2 and PD-1 and their downstream signalling remains an open question. Whereas their interaction is thought to be essential for T cell exhaustion, *in vivo* data from our lab indicate that Shp-2 is dispensable for PD-1 signaling in T cells and that its homologous phosphatase SHP-1 does not play a redundant function.
This brought us to:
1) generate mice lacking PD-1 in the T cells, which show an improved tumor control and of which we will further characterize the molecular mechanism;
2) evaluate whether the PD-1/SHP-2 axis might be important in other immune cells, such as macrophages, emerging mediators of the immune anticancer responses. In our hands, single deletion of these proteins in myeloid cells causes delayed tumor growth, indicating that these cells are key for establishing tumor suppression. Moreover, the lack of Shp-2 results in altered phenotype and signaling downstream of colony-stimulating factor 1 receptor.

We are currently investigating through *in vitro*, *in vivo*, and multi-omics approaches how PD-1 and Shp-2 diverge or converge into a tumor-supportive role of lymphoid and myeloid cells. Our results will help elucidate the role of PD-1 and SHP-2 in different aspects of the antitumor immune response by highlighting the potential benefits and risks for future anti-cancer therapies.

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1600 – P2.08.37

Analysis of infiltrating MC subsets in Colorectal Cancer by single-cell RNA-sequencing

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Purpose: Mast cells (MCs) are tissue resident cells that derive from bone marrow pluripotent cells and are mainly involved in IgE-mediated allergic disorders. They are frequently observed in different tumors including colorectal cancer, but their role is poorly understood. Two main subsets of MCs have been described so far in the intestine: mucosal MCs located near the epithelium and expressing the proteases Mcpt1 and Mcpt2 and connective tissue-like MCs that are present in intestinal submucosa and express Mcpt4-7. The aim of this study was to phenotypically and functionally characterize tumor-infiltrating intestinal MC subsets.
Methods: To this aim we used a conventional colitis-induced CRC mouse model (AOM/DSS). We first analyzed by flow cytometry MC frequency and cytokine production in both adenomas and adjacent tissue. To further investigate the role of tumor-infiltrating MCs we performed single cell RNA-sequencing analysis on ckit+/FceRI+ cells, isolated from intestinal adenomas.
Results: We found that MCs accumulate in tumor lesions and produce a higher amount of proinflammatory cytokines compared to MCs that reside in tumor-free adjacent tissue. Single cell analysis demonstrated that tumor infiltrating MCs form different clusters based on differentially expressed genes (DEGs). A first cluster expresses both mucosal and connective protease transcripts and is enriched in pathways related to cell adhesion, migration, and proliferation, suggesting that it represents a MC precursor population. A second cluster shows a selective mucosal phenotype and displays cell activation and cytokine production signatures. The last cluster displays a connective-tissue phenotype and shows a clear signature of MC degranulation and activation expressing the highest levels of genes involved in histamine and prostaglandin biosynthesis and in the angiogenesis pathway.
Conclusion: All together our results shed light in the functional heterogeneity of tumor-infiltrating MCs, revealing the existence of distinct subsets that may play different roles in tumor progression. However, further studies are needed to explore the transcriptional differences between them in order to better discriminate their developmental trajectories. To this purpose, we are currently repeating a single cell RNA-seq on MCs deriving from tumors as well as from adjacent tissue.

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1727 – P2.08.38

Intra-tumour mature tertiary lymphoid structures (TLSs) predict better prognosis and response to immunotherapy in clear cell renal cell cancer

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Purpose: Tertiary lymphoid structures (TLSs) are structured clusters of immune cells that arise in response to pathological conditions, such as chronic inflammation and tumours. TLSs normally consist of B-cell follicles with a germinal centre enclosed by T-cell zones and dendritic cells. The increasing interest in TLSs in the tumour microenvironment (TME) is owing to their prognostic significance and their potential role as mediators of anti-tumour immunity. This study comprehensively evaluated the predictive value of TLSs for prognosis and response to immunotherapy in patients with clear cell renal cell cancer (ccRCC).
Methods: We collected long-term follow-up records from 395 patients with ccRCC who received surgical excision and 230 patients who underwent combination therapy with Tyrosine Kinase Inhibitors (TKIs) and immune checkpoint inhibitors (ICIs), from multiple cohorts. The relative location to the tumour and the maturation status of TLSs were examined by H&E staining and immunohistochemistry. The immune cell composition of TLSs and infiltration into the surrounding TME was assessed using 12-marker multispectral fluorescence. The prognostic implications of TLSs were assessed using log-rank tests, Cox proportional hazards models and scoring nomograms. The molecular mechanism of TLS function was explored by analysing spatial transcriptomic data.
Results: TLSs, particularly intra-tumour TLSs and secondary follicle-like TLSs, showed a strong correlation with longer survival of ccRCC patients and better objective response to anti-PD-1/PD-L1 immunotherapy. In ccRCC samples harbouring peri-tumour TLSs and enriched in primary follicle-like TLSs, the proportion of tumour-associated macrophages and Treg infiltration in the peri-tumour regions increased significantly, indicating a suppressive TME. Spatial transcriptome annotation revealed an abundance of mature plasma cells within mature TLSs producing IgA and IgG, and stem T cells outnumbering exhausted T cells. An immunologically active zone composed of IgG^+ PCs in close proximity to $CXCL13^+$ T cells and $SPP1^+$ macrophages was also observed in ccRCC TLSs.
Conclusion: This work has provided the first insights into how the localisation and maturation heterogeneity of TLSs affect the immune status and responses of ccRCC and suggested an easy-to-use, cost-effective tool for predicting the prognosis of ccRCC patients in clinical application.

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1801 – P2.08.39

A tale of two species: Unveiling the involvement of tissue type 3 dendritic cells with intratumoral T cell behavior

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Conventional dendritic cells (cDCs) play a pivotal role in activating adaptive immune responses. They include cDC1s and cDC2s, with cDC2 represented by two major populations: DC2s and DC3s. DC3s have significant implications in chronic conditions such as lupus, psoriasis, and severe COVID-19.
We investigated the potential role of DC3s in lung cancer by analyzing publicly available sc-RNAseq datasets of non-small cell lung cancer (NSCLC). We observed an enrichment of DC3s with a potential immunosuppressive phenotype in advanced stages. This was accompanied by a reduction in the frequency of DC2 and cDC1 subsets. By conducting crossspecies sc-RNAseq analysis of human and mouse lung cells, we identified tissue murine DC3s and established a flow cytometry-based method for cDC1s, DC2s, and DC3s distinction. We confirmed DC3 enrichment in an ICB-resistant adenocarcinoma mouse model, mirroring findings in advanced human lung adenocarcinoma.
Using a multiplexing analysis, we spatially mapped immune components in mouse adenocarcinomas. This revealed that DC2s were located outside the tumor, while DC3s were positioned inside the tumor near CD4+ T cells, including Tregs. Similar proximity between cDC2s and CD4+ T cells was confirmed in human tumors. DC3s constituted the primary subpopulation among cDC2s in NSCLC. Furthermore, an analysis of whole-slide sections demonstrated remarkable heterogeneity in T cell and DC distribution within the same tumor. Some regions were enriched in DC3s and CD4+ T cells, while others in CD8+ T cells but lacked DC2s.
Our investigation is still ongoing to obtain functional insights through CITE-seq and single-cell spatial transcriptomic analyses on adenocarcinoma samples. Given that ICB therapy relies on reactivating CD8+ T cells, confirming that DC3- and CD4+ T cell infiltration is preferred in advanced stages could provide valuable insights into ICB treatment outcomes.

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1864 – P2.08.40

Neutrophil subset investigation in stage III melanoma patients undergoing anti-PD-1 immunotherapy or anti-BRAF/MEK target therapy

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Background: Melanoma displays a rising incidence and the mortality associated with the metastatic form remains high. Monoclonal antibodies (mAbs) that block programmed death (PD-1) and PD-Ligand 1 (PD-L1) network as well as small molecules targeting BRAF/MEK axis have revolutioned the therapeutic approaches to melanoma patients. PD-L1 is expressed on several immune cells and can be also expressed on human neutrophils. In addition to normal density neutrophils (NDNs), a population of low density neutrophils (LDNs) increases in certain cancer patients and correlates with cancer progression. The role of NDNs and LDNs in melanoma patients is largely unknown.
Methods: 59 stage III melanoma patients (MPs) candidates to anti PD-1 therapy or to anti-BRAF/MEK therapy were prospectively recruited. 28 Healthy controls (HCs), sex- and age- matched, were also recruited. NDNs and LDNs were isolated from peripheral blood, before and during the therapy, to evaluate their activation status and PD-L1 expression by flow cytometry. Plasma concentrations of cfDNA, Citrullinated Histone H3 (CitH3) and MPO-DNA complexes were measured as Neutrophils Extracellular Traps (NETs) biomarkers. Moreover, matrix metalloproteinase-9 (MMP-9) and CXCL8/IL-8 plasmatic levels were evaluated as neutrophils related mediators, by Elisa.
Results: Melanoma patient NDNs displayed an activated phenotype (increased percentages of $CD16^{+}Cd62L^{-}$ cells) and increased PD-L1 levels compared to HCs. Melanoma patients presented increased percentages of LDNs, which displayed higher levels of PDL-1 and an activated phenotype compared to HCs. NDN and LDN PD-L1 expression levels and activation status did not change during therapy. Melanoma patients displayed higher plasma concentrations of NETs compared to HCs. Kaplan Meier curves.
Conclusions: Neutrophils play important roles in immune-mediated clinical conditions such as infection, autoimmunity, and cancer. The activation status and PD-L1 expression in melanoma patients NDNs and LDNs were modified compared to HCs. In addition, both anti-PD-1 immunotherapy and anti-BRAF/MEK inhibitors did not modify these peculiar aspects. Further instigations are in progress to evaluate any prognostic/predictive significance for NDNs and LDNs in stage III melanoma patients undergoing anti-PD-1 immunotherapy or anti-BRAF/MEK target therapy.

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1895 – P2.08.41

Plasma cell hyperplasia in immune- cold triple negative breast cancer is prognostic of inferior outcome

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Triple-negative breast cancers (TNBCs) are characterised by an aggressive behaviour and limited treatment options compared to other subtypes of breast cancer. TNBCs with higher stromal tumor-infiltrating lymphocytes (sTILs) often exhibit a more favorable prognosis. This includes longer distant metastasis free survival (DMFS), particularly when accompanied by the presence of germinal center (GC) formations in the axillary lymph nodes (LNs). While immunotherapy holds promise for these TNBCs, the majority present with low sTIL infiltration and subsequently lack effective treatment options. This underscores the urgent need for innovative therapies tailored to target immune cold TNBCs and improve patient outcomes.
We analysed transcriptional data of four TNBC cohorts, totalling 618 cases, alongside sTIL scoring and immunofluorescence imaging of primary tumors, cancer-free, and involved LNs. Transcriptional data was deconvoluted using publicly available scRNAseq data of B, T lymphocyte and myeloid populations. We found that TNBC patients with the lowest DMFS were characterised by histologically low sTIL levels, elevated expression of plasmablasts/plasma cell genes and fewer GC formations in patient paired LNs. Plasmablast/plasma cell hyperplasia was confirmed in the primary tumour and involved LNs of these patients using multiplex immunostaining.
We characterised a series of orthotopic mouse models of TNBC and found that the most representative model of the human TNBC immune cold scenario also displayed plasmablasts/plasma cell hyperplasia within the tumour draining LNs at the expense of GC formations. Plasmablasts/plasma cell hyperplasia was unaffected in mice lacking SAP1/Sh2d1A, which is critical for GC B cell formation, possibly indicating an extrafollicular origin. Notably, targeted depletion of plasmablasts/plasma cells altered the phenotype of CD8 T cells leading to their increased functionality and effective impairment of tumour growth.
This study indicates that the induction of extrafollicular plasmablast/plasma cell hyperplasia within immune cold TNBC tumours disrupts tumor directed CD8 T cell immune responses. This discovery introduces a novel and unforeseen stratification of immune cold TNBC tumors, suggesting that therapeutic interventions targeting the depletion of plasmablast/plasma cells could potentially improve patient outcomes.

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1906 – P2.08.42

Hepatocellular carcinoma drives tissue-resident NK cell regulation of CD8+ T cells through the PD-L1 axis

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Purpose: Hepatocellular carcinoma (HCC) is the third leading cause of cancer-related deaths globally. Despite the shift to immunotherapy, sustained responses are limited, underscoring the need for novel treatment strategies. Tissue-resident lymphocytes have a critical role in tumour immunosurveillance but their interplay within the profoundly immunosuppressive tumour microenvironment (TME) in HCC is not well-characterised. We have previously identified a subset of liver-resident NK cells that can negatively regulate T cell function in the settings of chronic hepatitis B infection and in therapeutic vaccination through TRAIL, NKG2D and PD-L1. We hypothesize that intra-tumoral NK cells can similarly constrain anti-tumour T cells in HCC and that defining the pathways can identify new therapeutic targets.
Methods and results: We took advantage of our access to freshly isolated lymphocytes from human HCC surgical samples for *ex vivo* immune cell phenotyping by multiparametric flow cytometry. Within the tumour, CD8+ T cells expressed high levels of PD-1. Tissue-resident NK cells, representing 40% of the total infiltrating NK cell pool, expressed PD-L1 at higher levels than tumour-infiltrating conventional NK cells or NK cells from background liver or blood. *In situ* imaging of the TME visualised NK cells and CD8+ T cells localising in close proximity. To understand the drivers of PD-L1 upregulation on NK cells in HCC, we used *in vitro* co-cultures to test multiple features of the TME. Direct contact with HCC cell lines upregulated expression of PD-L1 on NK cells, a feature not driven by the HCC secretome. To test the functional role of this pathway *in vivo* we have established subcutaneous and orthotopic HCC mouse models and confirmed that these recapitulate the upregulation of PD-1 and PD-L1 on tissue-resident CD8+ T cells and NK cells respectively.
Conclusion: In summary, we demonstrate the upregulation of the PD-1/PD-L1 pathway on CD8+ T and NK cells in human HCC, showing that this may be driven by tumour cells within the TME. Our mouse models recapitulate our human findings and will allow us to investigate the therapeutic potential to boost anti-tumour T cell immunity by targeting tissue NK cell regulation.

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1966 – P2.08.43

Association of circulating inflammatory factors with postoperative complications after gastric cancer surgery

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Gastric cancer is the world's leading cancer in terms of mortality. Despite steady medical progress and a reduction in the incidence of gastric cancer over the past decades, this malignancy remains a major public health concern due to its high prevalence and the high mortality-to-morbidity ratio associated with its detection at advanced stages. Post-operative complications related to surgical removal of the tumour are known to be a common problem in the management of gastric cancer. During infection, the role of circulating CD14 and lipopolysaccharide-binding protein (LBP) molecules in the immune response is important. Together, CD14 and LBP play a key role in the initiation and amplification of the inflammatory response by facilitating the recognition and response to bacterial pathogens. The activation of these immune mechanisms is essential for the elimination of bacterial invaders and the restoration of homeostasis.

Evaluation of the circulating factors CD14 and LBP associated with post-operative complications, their pre- and post-operative levels and their correlation with post-operative complications, could help to determine the potential predictive value of these parameters in predicting post-operative complications.

Our aim was to evaluate the levels of circulating inflammatory factors CD14 and LBP in the serum of gastric cancer patients in order to investigate the potential predictive value of these parameters in predicting postoperative complications. To this end, we determined serum levels of circulating CD14 and LBP molecules by ELISA and compared them between patients with different types of postoperative complications. We also performed a fecal calprotectin test to check for inflammation in the intestines of the patients.

Serum circulating CD14 levels were significantly different between gastric cancer patients receiving chemotherapy and surgery (with a decrease after surgery), indicating that the immune response is enhanced against bacterial infections. The calprotectin test was significantly different between the baseline and surgical group (decreased after surgery), indicating a reduction in intestinal inflammation. Our results suggest that the identification of the factors CD14 and calprotectin in the prediction of early postoperative complications may help in the development of diagnostic tests for early detection and monitoring of the disease.

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Tumours of different invasiveness secrete small extracellular vesicles of distinct lipid composition influencing CD1a-restricted T cell responses.

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Purpose: Exosome-enriched small extracellular vesicles (sEVs) are one of the three types of extracellular vesicles (EVs). The size of sEVs falls into a range of 40-150nm in diameter, which allows for long distance cellular communication, with potential significance in tumour progression and evasion. We have previously determined that the membrane of sEVs may serve as a source of CD1a ligands and affect T cell responses. CD1a antigens may have a permissive (stimulatory) or non-permissive (inhibitory) effect on CD1a-restricted T cells depending on headgroup size and lipid fit into the CD1a binding groove and that a pathological state can affect their content in sEVs.
The project aims to investigate the lipid composition of sEVs secreted from benign and malignant tumours with potential differential effect in the downstream T cell responses.
Methods: sEVs were isolated from RT4 and T24 bladder cancer cell lines cultured in EV-depleted complete RPMI-1640. Conditioned media were collected after 24h and sEVs were isolated by serial centrifugation. Lipidomic analysis was performed by mass spectrometry to assess lipid composition of isolated sEVs. The effect of sEV supplied lipids on CD1a-restricted T cell responses was performed with IFNγ ELISpot assay with CD1a-transfected K562 cells serving as antigen presenting cells. Prior to ELISpot assay CD1a-K562 cells were pulsed with sEVs, which were digested by phospholipase A2 (PLA2) to liberate lipids from the sEV membranes.
Results: Lipidomic mass spectrometry detected that both types of lipids could be found in the sphingomyelin (SM; nonpermissive), and phosphatidylcholine (PC; permissive) classes, with differential content of nonpermissive lipids in tumorigenic lines in contrast to non-tumorigenic. We determined that the addition of lipid antigens supplied by sEVs to the K562-CD1a/T cell coculture affected CD1a-dependent IFNγ responses in comparison to the control unpulsed cells as determined by differential IFNγ secretion.
Conclusions: The results showed that sEV lipids secreted by tumours of different invasiveness differ in lipid content which contributes to differences in activation of CD1a-restricted T cells. Secreting differentially-composed sEVs by cancer cells could potentially constitute one of tumour immune evasion strategies.

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2093 – P2.08.45

NK-LGL comprise dominant pools of adaptive NK cells that help distinguish from NK cell expansions.

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Large granular lymphocytic (LGL) leukemia is a chronic malignant disorder characterized by the expansion of cytotoxic T- or natural killer (NK)-lymphocytes, accounting for 2-5% of chronic lymphoproliferative disorders. NK-LGL is subdivided as indolent NK chronic lymphoproliferative disorder (CLPD-NK), and rare aggressive NK-LGL leukemia (ANKL). It is currently difficult to distinguish CLPD-NK from reactive expansions of NK cells arising during viral infection or autoimmunity. Phenotypic analysis of NK-LGL patients revealed dominant populations of adaptive NK cells in both ANKL and CLPD. Characterization revealed loss of the PLZF transcription factor and signaling adapters FceRg, Syk and Eat-2 that distinguish adaptive NK cells but little of the common adaptive marker NKG2C. Transcriptomic and epigenetic analyses of sort-purified NK-LGL NK cells revealed dysregulation of oncogenic factors including RUNX1 and BCL11B. Several differentially expressed genes were supported by changes in chromatin accessibility specific to NK-LGL adaptive NK cells. Amongst these genes were known tumor drivers of LGL such as STAT3 together with putative candidates RUNX1 and RHOH. These were further evaluated in both knockdown and overexpression models in primary NK cells and NK-LGL cell lines suggesting a contribution to lymphoproliferative phenotypes. Our results refine a phenotypical definition that can aid diagnostics of CLPD-NK leukemia and further reveal dysregulated pathways that may underlie pathological lymphoproliferation.

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2211 – P2.08.47

Lamps and autophagy as predictive markers in colorectal cancer - A pilot study

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Long-term management of colorectal cancer (CRC) relies on effective chemotherapy and the prevention of metastatic disease, which ultimately is the main cause of mortality. However reliable prognostic biomarkers for better stratification and treatment of CRC patients, and thus prevention from relapse are currently missing. At present the fundamental role of autophagy in maintaining cell homeostasis is well defined with lysosomes playing a major role in it. Nevertheless, in oncogenesis, a dual role of autophagy is also indicated, either in promoting or inhibiting tumor growth. Tumor budding has been proposed as a poor prognostic factor but is not yet included in the routine diagnosis of CRC. The current study aimed to investigate and evaluate the relationship between the lysosomal proteins LAMP1 and LAMP2, tumor budding, and clinicopathological parameters, in CRC. The clinical significance of tissue expression, serum protein and gene expression levels from primary CRC and healthy patients were assessed. We found moderate to strong expression of LAMP1 and LAMP2 in the tumor stroma, parenchyma and tumor front in CRC. Moderate correlation and association between tumor budding, lymph nodes, lymphatics, and blood vessels were also detected. Specifically, intense LAMP1 and LAMP2 staining were observed in the front of tumor invasion compared with tumor parenchyma and noncancerous tissue. Upregulated LAMPS gene expression in WBC and elevated LAMP1 levels in sera from CRC patients were also noted. Here we show the concurrence of the spatial immunochemical expression of the lysosome-associated membrane proteins LAMP1 and LAMP2, and tumor budding in CRC patient samples, as well as their prognostic significance for the stratification, and treatment of CRC.

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2218 – P2.08.48

Significance of standard laboratory techniques in the monitoring of monoclonal gammopathies

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Plasma cell proliferative disorders often involve the synthesis and secretion of a monoclonal immunoglobulin. The clinical guidelines recommend serum protein electrophoresis, serum immunofixation and serum free light chain measurement as monoclonal gammopathy testing. This study aimed to assess the effectiveness of standard laboratory tests in monitoring monoclonal gammopathy.
We conducted a retrospective review of 404 immunofixation (IF) cases performed at the Clinical Immunology Laboratory of the Pasteur Institute of Tunis from August 2021 to July 2022 using the Sebia HYDRAGEL IF9 system. Quantitative Immunoglobulin Testing (QIg) and free light chain (FLC) assays were carried out using the Optilite automated system 5the Binding Site®).
Among the 404 samples, 50 showed a monoclonal band on IF, yet the M-protein was either barely detectable or not quantifiable on serum protein electrophoresis (SPEP). Based on the IF results, monoclonal gammopathies of the IgG type were most frequently observed (26 cases, 52%), with 22% classified as κ type and 30% as λ type. IgA type gammopathies ranked second (13 cases, 26%), while free light chain isotypes were identified in 8 cases. A FLC assay was conducted on 11 patients, revealing a pathological serum FLC kappa/lambda ratio (sFLCR) in 63% of cases. Urinary protein immunoelectrophoresis was performed on 28 patients, with 81% showing pathological results. Only 5 patients underwent QIg testing, and among them, 2 patients with an IgA monoclonal gammopathy on IF had IgA levels exceeding 3.5g/L.
Our findings suggest a concordance between the IF results and other assays, including QIg, FLC assay, and urine analysis. These diagnostic methods may provide valuable insights into disease progression, highlighting their significance in monitoring monoclonal gammopathies.

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2276 – P2.08.49

Strong YKL-40 expression in the invasive tumor front of colorectal cancer - A pilot study

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The poor prognosis of patients initially diagnosed at an advanced stage of colorectal cancer (CRC) and the heterogeneity within the same tumor stage define the need for additional predictive biomarkers. Tumor buds are proposed as a poor prognostic factor for CRC, however, they are still not implemented into routine pathology reporting. In turn, the chitinase-3-like protein 1 (CHI3L1) also known as YKL-40, is regarded as a candidate circulating biomarker and therapeutic target in CRC. The aim of our study was to investigate tissue YKL-40 localization and tumor budding in CRC. Thirty-one CRC patients and normal colonic tissues were examined. The correlation between YKL-40 levels, tumor budding and clinocopathological parameters was evaluated by polychoric correlation analysis. The immunohistochemical assessment revealed high YKL-40 expression in CRC in contrast to normal mucosa. Specifically, intense YKL-40 staining was detected in the front of tumor invasion compared with tumor parenchyma and noncancerous tissue. We present novel data for increased YKL-40 expression in tumor buds within the front of tumor invasion. We assume that the combination of this morphological parameter with the tissue level of the pleotropic YKL-40 glycoprotein could serve as a future prognostic biomarker for CRC stratification and treatment.

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2286 – P2.08.50

Histone 3 acetylation is altered in NK cells from metastatic breast cancer patients

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Purpose: NK cells are cytotoxic lymphocytes which play a major role in eliminating cancer cells and are a key focus of emerging immunotherapies. However, previous work from our lab and others has shown that NK cells from metastatic breast cancer patients are functionally and metabolically impaired. Epigenetics and metabolism are linked through acetyl CoA, a key metabolite which also acts as a substrate for histone acetylation reactions, an epigenetic modification which generally enhances gene transcription. Therefore, we sought to identify if altered metabolism can impact global levels of acetylated histone 3 in NK cells from metastatic breast cancer patients. Furthermore, we investigated the role of the enzymes acetyl CoA synthetase 2 (ACSS2) and ATP-citrate lyase (ACLY) as they have been shown to support histone acetylation by generating nuclear acetyl CoA in other cell types. In addition, we measured the effects of TGFβ, an anti-inflammatory cytokine which plays a direct role in NK cell dysfunction in the tumour microenvironment also.
Methods: Peripheral blood mononuclear cells (PBMC) from metastatic breast cancer patients and healthy controls were activated by overnight treatment with IL-12 (30ng/ml) and IL-15 (100ng/ml) in the presence or absence of TGFβ (10ng/ml) or small molecule inhibitors of ACLY (BMS-303141) and ACSS2 (ACSS2i). Acetylated H3 levels were measured by intranuclear flow cytometry.
Results: Healthy donor NK cells were shown to substantially upregulate H3 acetylation in response to IL-12/15 stimulation. In contrast, there was no increase observed in NK cells from metastatic breast cancer patients. Interestingly, use of a small molecule ACLY inhibitor blocked IL-12/15 induced upregulation of H3 acetylation while the ACSS2i had no effect. Finally, TGFβ caused a moderate decrease in H3 acetylation.
Conclusions: NK cells from healthy donors substantially increase H3 acetylation in response to IL-12/15 stimulation with ACLY derived acetyl CoA potentially playing an important role. In contrast, NK cells from metastatic breast cancer patients have altered histone acetylation patterns. Failure to upregulate H3 acetylation in response to cytokine stimulation may contribute to NK cell dysfunction in metastatic breast cancer.

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2293 – P2.08.51

Activated human macrophages suppress growth and kill cancer cells

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Purpose: The presence of tumor-associated macrophages (TAM) is well-documented. This fact, together with macrophages' role in clearing out dead cells and damaged tissues, make macrophages a highly interesting target for cancer immunotherapy. However, there is an ongoing debate on the role of TAMs in cancer since it has been reported both positive and negative correlations between TAM densities and patients' clinical outcome. This study aims at clarifying the ability of activated human macrophages to kill cancer cells *in vitro*.
Methods: We have established a time-lapse microscopy-based assay to visualize and quantify the killing of cancer cells by human macrophages. Macrophages were differentiated *in vitro* from monocytes isolated from the blood of healthy donors. Macrophages were activated with various stimuli such as interferons and toll-like receptor agonists, and co-cultured with several human cancer cell lines expressing fluorescent protein. The killing of cancer cells by activated macrophages was monitored over time and quantified by image analysis algorithms. A fluorescent caspase 3/7 probe was used to visualize apoptosis induction in cancer cells.
Results: We found that human macrophages can be triggered to either kill cancer cells or arrest cancer cell growth. This anti-cancer activity of macrophages was only seen in activated macrophages, and was dependent on the mode of activation. We are currently working on deciphering the cellular and molecular mechanisms involved.
Conclusions: Human activated macrophages can efficiently kill cancer cells of various origin and inhibit cancer cell proliferation. These experimental data support the potential of macrophages for cancer immunotherapy in humans.

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P2.09 IMMUNE RESPONSE REGULATION: CELLULAR MECHANISMS

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237 – P2.09.01

The interplay between intracellular pathogen proliferation and host cell death induction and metabolism during L. major infection

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Leishmania major (L. major) is an intracellular pathogen that is located mainly in macrophages during an established infection. We have shown previously that *L. major* parasites occur as high and low proliferating subpopulations, which seem to be correlated with differential host cell death induction in vitro and in vivo.
Whether activation of host cell death pathways is causally linked with the different proliferation rates of the infecting *L. major* parasites has remained unclear. To elucidate this question, we have generated killed but metabolically active (KBMA) *L. major* parasites, which are not able to proliferate, but retain their metabolic functions and the differentiation capacity from promastigote into amastigote upon infection.
By infecting macrophages with proliferation-competent versus KBMA parasites and comparing the infected macrophages to each other, we have determined the influence of the parasite proliferation on the host cell. Using flow cytometry and live cell imaging, significant differences in host cell death and expression of the lipid transporter CD36 and the uptake of long chain fatty acids and low-density-lipoprotein could be observed dependently of the intracellular pathogen proliferation state. CD36 expression also correlated with pathogen proliferation in experiments with an in vivo reporter system. Furthermore, we observed a differential activation of Caspase-1 versus Caspase-3 upon infection with proliferation-competent pathogen in vitro and in vivo.
These results suggest a differential influence of the proliferation rate of *L. major* parasites on their host phagocytes, highlighting the proliferation as an important factor influencing the host-pathogen interaction during an infection with *L. major*.

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341 – P2.09.02

Mitochondria in intercellular communication between stem cells and B cells

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Purpose: The significance of mitochondrial transfer is increasingly acknowledged as a vital immunomodulatory process employed by mesenchymal stem cells (MSCs). Although the effects on T cells and macrophages have been studied, the effects on B cells remain an area yet to be explored. This research focuses on understanding how MSC-mediated mitochondrial transfer influences the fate of B lymphocytes.
Methods: MSCs, either labeled with MitoTracker dyes or derived from mito::mKate2 transgenic mice, were co-cultured with syngeneic or allogeneic splenocytes. Flow cytometry was used to measure the transfer of mitochondria into different immune cell populations, to evaluate levels of reactive oxygen species (ROS), apoptosis, glucose uptake and mitophagy. RNA sequencing was then performed on CD19+ cells acceptors and non-acceptors of mitochondria. Changes in gene expression following mitochondrial uptake were identified by differential gene expression analysis. Furthermore, in an acute inflammation model induced by LPS, MSCs from mito::mKate2 transgenic mice were administered, and subsequent mitochondrial transfer and phenotypic changes in B cells from various organs were assessed.
Results: MSCs were observed to transfer mitochondria to CD19+ cells, although to a lesser extent compared to other immune cell types. Mitochondrial transfer was found to be associated with levels of ROS and was further affected by the induction of mitophagy. Cells that received mitochondria exhibited increased expression of CD69 and glucose uptake, indicating enhanced glycolytic activity. We also observed a reciprocal exchange of mitochondria, with immune cells transferring dysfunctional mitochondria to MSCs and receiving functional ones in return. CD19+ cells that acquired mitochondria displayed enhanced viability, proliferation and upregulation of genes linked to cell division and the downregulation of those involved in antigen presentation. The relevance of these findings *in vivo* was underscored by the transfer of mitochondria to B cells across various organs in a model of acute inflammation induced by LPS.
Conclusion: These discoveries reveal the complex interactions between MSCs and immune cells through mitochondrial trafficking and provide valuable insights into potential therapeutic applications of MSC-mediated immune modulation.

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343 – P2.09.03

Molecular mechanisms and therapeutic implications of T and B cell subpopulations in Common Variable Immunodeficiency with Granulomatous-Lymphocytic Interstitial Lung Disease

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Purpose: Granulomatous lymphocytic interstitial lung disease (GLILD) is one of the most serious non-infectious complications that can occur in up to 20 percent of common variable immunodeficiency (CVID) patients. An increase in follicular helper T cells type 1 (Tfh1) has been observed in these patients, which has recently been associated with an increase in Age Associated B cells (ABCs). It appears that interferon gamma (IFN-γ) may play an important role, however, the underlying molecular mechanisms are still not fully understood. This study investigates how treatment with rituximab and mycophenolate affects both subpopulations in a CVID patient with GLILD.
Methods: The patient received weekly intravenous Rituximab for 4 cycles along with daily mycophenolate. Blood was collected 1 week before treatment and 2 months after treatment. T and B cells were analyzed using 10-color and 8-color flow cytometry panels, and functional analysis was conducted with PMA and ionomycin.
Results: Clinically, the patient suddenly improves his pulmonary capacity and dyspnea. However, he relapses again 6 months later requiring a new dose of rituximab. Regarding T cells, total CD4 count and Tfh1 populations appear to be quantitatively unaffected by the treatment. Functionally, the percentage of CD4 cells synthesizing IFN-γ decreased with treatment. IFN-γ expression in Tfh1 cells couldn't be studied due to lost membrane markers with the *in vitro* conditions. Regarding B cells, before treatment, the patient exhibited elevated percentages of ABCs. After treatment, a marked reduction in CD21 high-intensity expressing cells was observed, while ABCs surprisingly persisted and increased in relative proportion.
Conclusion: Although Tfh1 cells did not show significant quantitative changes, a decrease in IFN-γ signal in CD4 cells after tretament suggested functional alterations. This would explain the immediate improvement of the patient.
On the other hand, ABCs appear to be resistant to treatment, since a relative increase in this subpopulation is observed after therapy. We speculate that the persistence of Tfh1 and ABCs causes the patient's relapse and would explain the need for chronic treatment with rituximab. However, further studies are needed to investigate the pathophysiological mechanisms Tfh1 and ABCs in GLILD. In this way, more specific treatments can be obtained.

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465 – P2.09.04

Mast cells switch their secreted cytokine profile from pro-inflammatory to pro-tolerant upon prologued IL-33 exposure

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Purpose: Upon activation, mast cells can release a myriad of factors, such as histamine, heparin, proteases, lipid mediators, and cytokines with which they influence their environment. IL-33 is well known to mediate a type 2 immune response upon allergen exposure in cells that carry its receptor ST2, such as mast cells. In the skin, these cells also express the Mas-related G protein coupled receptor X2 (MRGPRX2). Wasp venom and alum, which are repeatedly injected during desensitization therapy, can trigger an IL-33 release in the skin, and mastoparan in wasp venom can activate MRGPRX2. During desensitization, the immune response towards an allergen switches from a type 2 pattern with IL-4, IL-5, and IL-13 towards a tolerance response, with TGF-β and IL-10 being the main driving cytokines. It is however not clear, how this switch is initiated.
Methods: Unexposed or IL-33 pre-exposed LAD2 cells and primary skin mast cells were stimulated with IL-33 and *Hymenoptera* venom or Compound 48/80. Additional activation with FcεRI-crosslinkage was tested in LAD2 cells. We measured cytokine release by multiplex assay and ELISA, degranulation as increase in CD63 surface expression by flow cytometry, and intracellular signaling by a human phospho-kinase array.
Results: The combination of ST2 and MRGPRX2 stimulation was necessary to cause secretion of pro-inflammatory cytokines and TGF-β. IL-33 exposure for 4 days led to a switch in the secreted cytokine profile in LAD2 cells as well as primary skin mast cells upon stimulation. In IL-33 pre-exposed and stimulated LAD2 cells, the with-no-lysine [K] 1 kinase was activated, and the cells mainly secreted TGF-β. This switch in secreted cytokine composition happened independently of FcεRI-crosslinkage.
Conclusion: This different cytokine profile of mast cells could potentially skew other immune cells towards a tolerance response to the administered allergen during desensitization therapy.

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501 – P2.09.05

Activation of the Noncanonical Caspase-4/-5 inflammasome in Human Endothelial Cell Results in Secretion of Proinflammatory Proteins and Macrophage Activation

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The alterations in microbiome composition and heightened levels of circulating microbial components, known as metabolic endotoxemia, have been linked to the development of various chronic low grade inflammatory diseases, most notably cardiovascular diseases (CVD). Gut dysbiosis plays pivotal role in modulating the severity of metabolic endotoxemia and the nature of the ensuing inflammatory response. Nevertheless, the precise mechanism by which circulating microbial components, such as lipopolysaccharides (LPS), contribute to CVD pathogenesis remains elusive.
Cells detect microbial components via innate immune receptors, and their activation triggers intracellular communication through secretion of mediators, damage-associated molecular patterns, and extracellular vesicles (EV). We studied the activation of the noncanonical inflammasome induced by intracellular LPS in human coronary endothelial cells (HCAEC) and human umbilical vein endothelial cells (HUVEC). LPS was introduced into cells either by transfecting liposome-encapsulated LPS or by stimulating cells with bacterial outer membrane vesicles.
Intracellular LPS led to elevated expression of cell adhesion molecules, robustly increased protein secretion within the EV fraction, and induced pyroptotic cell death. This was dependent on caspase-4/5 as well as mixed lineage kinase domain-like (MLKL) but was independent of the NLRP3 inflammasome or Toll like receptor 4. Intracellular LPS induced significant secretion of EV-encapsulated proteins, without affecting the size or the quantity of the secreted EVs. Mass spectrometric analysis of EV fractions revealed over 2200 proteins, with more than 200 proteins exclusively identified following LPS transfection. The EV proteins included enzymes, kinases, peptidases, phosphatases, regulators of transcription and translation, transporter proteins, and transmembrane receptors. EVs derived from LPS-transfected HCAECs activated human primary macrophages, inducing expression of proinflammatory cytokines and interferon response.
LPS activates the noncanonical inflammasome in endothelial cells, resulting in secretion of EVs capable of macrophage activation, thus representing a potential link between dysbiosis, metabolic endotoxemia and vascular inflammation.

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545 – P2.09.06

Signaling lymphocyte activation molecule 7 (SLAMF7) in the differentiation of human, pathogen-specific T cells

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Misdirected T cell responses can be the cause of autoimmune diseases such as systemic lupus erythematosus (SLE). Understanding T-cell differentiation and the role of costimulatory molecules in pathological processes could open new ways to develop therapeutic approaches. As we found that a major inhibitory molecule on T cells - CTLA-4 - strongly controls the signalling lymphocyte activation molecule F7 (SLAMF7), a role for SLAMF7 in T cell differentiation was hypothesised.
We investigated the role of SLAMF7 in human T helper (Th) cell differentiation. Enriched naive $CD4^+$ T cells were antigen-specifically stimulated with heat-inactivated pathogens presented on APCs. In addition to functional assays, the activated human T cells were characterised by flow cytometry using characteristic expression of differentiation-associated molecules. Using a human model of antigen-specific T cell differentiation, we found that SLAMF7 is differentially expressed depending on the inflammatory cytokine milieu. While SLAMF7 is not expressed in resting Th cells, it is upregulated in activated cells with a strong correlation to the activation-associated surface molecule CD71. In addition to correlation to activation, the frequency of SLAMF7-expressing Th cells increases after completion of the first cell cycle. Notably, SLAMF7 expression is also age-dependent. This suggests a central role for SLAMF7 in Th differentiation. Its role in immune pathologies will be discussed.

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551 – P2.09.07

Targeting of SLAMF7 on human CD8+ T cells to enhance responsiveness against tumor-associated antigens

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In recent years, novel immunotherapy approaches, in particular immune-checkpoint blockade, revolutionized multiple therapy regimens against different type of cancer. Nonetheless, there are patients who don´t respond or only take little advance of it, while immune related adverse events occur frequently and often leads to limitation of the therapy. To improve anti-tumoral $CD8^+$ T-cell responses we identified the self-ligating receptor SLAMF7 as a promising target structure. SLAMF7 is expressed on various immune cells, where it´s activation leads to different immunoregulatory cues, depending on the intracellular adaptor molecule. On $CD8^+$ T cells we characterized SLAMF7 as a critical regulator during priming.
In this study, we investigated the effect of SLAMF7 activation on human $CD8^+$ T lymphocytes. Further, we examined whether activation of the SLAMF7 receptor in combination with an ICB leads to increased effectiveness of immunotherapy against tumour antigens. For this purpose we designed a method where we stimulated human $CD8^+$ T-cells, isolated from peripheral blood, via appropriate antibodies coupled on microspheres. As tumour antigen, we expose the T-cells to the cancer-testis antigen NY-ESO-1, which is re-expressed on wide range of malignant cells. The ICB is induced in APC/$CD8^+$ T-cell co-culture and is connected up- or downstream with targeting of SLAMF7. As read out we analyzed different surface and intracellular markers via FACS and functional assays (ELISpot, cytokine multiplex assay), which allow conclusions to be drawn about the activation status and effector function of the $CD8^+$ T cells.
We found that SLAMF7 is increasingly expressed on the surface of human $CD8^+$ T cells during activation. Thereby it´s expression is upregulated through proinflammatory cytokines, in particular IL-12. In addition, we demonstrated that activation of SLAMF7 via agonistic antibodies led to an increased expansion of $CD8^+$ T cells when simultaneously encountering their antigen presented on recombinant MHC molecules. This indicates a favourable effect of SLAMF7 during T cell activation. Taken together this research could provide and characterize SLAMF7 as a new target molecule for immunotherapeutic approaches to enhance anti-tumoral $CD8^+$ T-cell responses.

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622 – P2.09.08

Immunity of lipid-lowering drugs – how statins and PCSK9 inhibition shape immune phenotype and function

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High levels of pro-atherogenic low-density lipoprotein cholesterol (LDL-C) and triglycerides are significant risk factors for cardiovascular disease (CVD), the leading cause of death worldwide. Thus, lipid-lowering drugs are commonly prescribed, effectively reducing atherogenic inflammation and CVD incidence and mortality. There are currently two types of LDL-C inhibitors in use: statins and monoclonal antibodies (mAbs) targeting proprotein convertase subtilisin/kexin type 9 (PCSK9). Statins inhibit 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in cellular cholesterol synthesis effective also in immune cells. In contrast, therapeutic PCSK9 mAbs inhibit the interaction of PCSK9 with the LDL receptor in hepatocytes, interfering with receptor degradation. Consequently, systemic LDL-C is cleared without stalling cell-intrinsic cholesterol synthesis.
However, cholesterol as pleiotropic molecule shapes cell membrane composition and signalling capacity throughout the body. Its metabolites are essential precursors for broadly operating molecules, including bile acids, vitamin D and steroid hormones. Thus, cholesterol and cholesterol-targeting therapies exert complex and potentially contradictory effects in a variety of (patho)physiological mechanisms, involving cell proliferation, synapse formation, antigen presentation, vaccine response, cognitive function, tumour surveillance, osteoporosis, and depression. To understand the impact of lipid-lowering therapies on immune homeostasis and function, we analyse the longitudinal effects of HMG-CoA reductase and PCSK9 inhibition alone and in combination by 40-color spectral flow cytometry. We phenotype patient leukocytes in high quality and unprecedented detail prior to and three, six and twelve months after therapeutic intervention. Further, we perform *ex vivo* stimulation and functional assays to link immune phenotypes with functional outcome, including calcium fluxing and cytokine production. Furthermore, we analyse and correlate various clinical biomarkers, including circulating hormones, hepatic fat composition, bone density and skeletal microarchitecture. By gaining new mechanistic insights into how lipid-lowering therapies shape immune homeostasis and the function of other bodily systems, we hope to inform the development of novel patient-tailored therapeutic strategies.

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652 – P2.09.09

Role of the production of cytokines induced by excess mechanical stress in human periodontal ligament cells

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Purpose: Improper mechanical stress may induce various cytokines as side effects on the periodontal ligament cells during orthodontic treatment. If the roots and alveolar bones are extensively resorbed by cytokines such as IL-6 and RANKL following excess mechanical stress, unplanned tooth mobility and inflammation can occur. Although multiple factors are believed to contribute to the development of side effects, the cause depending of cytokines is still unknown. Sonic Hedgehog (Shh), one of the Hedgehog signals significantly associated with cell growth and cancer development, promotes osteoclast formation in the jawbone. Shh may be associated with root and bone resorptions during orthodontic treatment. In this study, we investigated the relationships among Shh, RANKL and IL-6 in human periodontal ligament (hPDL) cells exposed to improper mechanical force.
Methods: Weights were placed on hPDL cells and human gingival fibroblasts (HGFs), control to hPDL cells, for the optimal orthodontic force group (1.0 g/cm^2) and the heavy orthodontic force group (4.0 g/cm^2). A group: no orthodontic force was used as a control to force. B group: placing only glass plate was used as a control to weights. Real-time PCR, SDS-PAGE, and western blotting were performed to examine the effects of orthodontic forces on the expression of Shh, RANKL and IL-6 at 2, 4, 6, 8, 12 and 24 hours after placing glass plate and weights.
Results: The protein expression of Shh was not clearly induced by forces of 1.0 and 4.0 g/cm² compared with the controls in HGFs and hPDL cells. In contrast, RANKL and IL-6 gene and protein expression was significantly induced by weights, 1.0 and 4.0 g/cm² in hPDL cells for 6~24 hours. However, neither protein was expressed in HGFs. RANKL and IL-6 expressions in response to forces and in the controls were clearly inhibited by Shh inhibitors RU-SKI 43, cyclopamine and Gant 61.
Conclusion: Shh did not directly link to RANKL and IL-6 for root and bone resorptions by orthodontic force but associate with cell activities to be finally guided with the production of cytokines in hPDL cells.

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665 – P2.09.10

Cytokine profiling in mouse spleen cell culture supernatants using multiplexed bead-based immunoassay: comparison of flow cytometry and xMAP technology

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Purpose: This study aimed to characterize cytokine profiles, particularly Th1 and Th2 responses, in spleen cell culture supernatants using multiplex immunodetection methods, and to compare results obtained through flow cytometry and xMAP technology.
Methods: Splenocyte supernatants were collected after 24 hours culture of splenocytes isolated from female BALB/c mice immunized with SARS CoV-2 spike-derived antigens produced in a prokaryotic system and stimulated ex vivo with a SARS-CoV-2 S1 peptide pool. The cytokines analyzed included IL-5, IL-13, IL-2, IL-6, IL-10, IFN-γ, TNF-α, and IL-4, which are secreted by Th1 and Th2 cells. Cytokine production was assessed using two commercially available immunodetection assays, which utilized flow cytometry and xMAP technology for analysis.
Results: Using both multiplexed bead-based immunoassay analyses: flow cytometry and xMAP technology, significant increases were observed in the secretion levels of both Th1 and Th2 cytokines following in vitro stimulation with the peptide pool in mice immunized with the antigen compared to the control group. More specifically, there was a significant increase in the secretion of Th1 cytokines (IFNγ, IL-2, TNFα) and Th2 cytokines (IL-4, IL-5, IL-13), alongside a reduced secretion of cytokines associated with regulatory functions (IL-10/IL-6). It is worth mentioning that these responses exhibited consistency across both assays, despite the baseline secretion levels appearing higher in the flow cytometry assessments. The strong correlation, evidenced by a Pearson coefficient ranging from 0.8 to 0.99 for all tested cytokines, underscores the coherence and reliability of the data, highlighting the consistency between both tests.
Conclusion: Our findings demonstrate distinct cytokine profiles in spleen cell culture supernatants of immunized mice, with significant increases in both Th1 and Th2 cytokines following antigen stimulation. Most importantly, there is a high degree of correlation between both multiplexed bead-based immunoassay analyses: flow cytometry and xMAP technology indicating the robustness of multiplex immunodetection methods in cytokine profiling.

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670 – P2.09.11

The impact of growth factors and differentiation conditions on murine bone marrow-derived dendritic cells: Phenotypic and functional evaluation

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Purpose: Selecting a relevant and reproducible method for generating murine bone marrow-dendritic cells (BM-DCs) poses multiple challenges due to the plasticity of dendritic cells (DCs) and the multitude of experimental protocols. Consequently, assessing the characteristics of *in vitro* generated BM-DCs is crucial in order to tailor protocols based on particular research objectives while maintaining the functional and phenotypic traits of DCs.
Methods: We conducted a study to evaluate the phenotypic and functional characteristics of BM-DCs generated using GM-CSF with or without IL-4 and the impact of differentiation conditions on T cell responses. We analysed BM-DCs activation following LPS (lipopolysaccharide) stimulation by examining cytokine secretion and specific cell surface markers. In addition, we evaluated the ability of antigen-pulsed BM-DCs to prime naive T cells in an *in vitro* model consisting of a BM-DCs/T cells co-culture system. Also, to enhance antigen-specific responses, activated T cells were restimulated by new antigen-pulsed BM-DCs. Prime and boost stimulation were analysed using a multiplex assay in order to assess cytokines secreted by different subclasses of T helper cells.
Results: BM-DCs generated with GM-CSF alone exhibited high levels of costimulatory molecules indicative of a mature phenotype, but preserved the ability to respond to LPS maturation by cytokine production. Moreover, cytokine secretion patterns observed in the co-culture system indicate the potential of antigen-pulsed BM-DCs to prime naive T cells. In contrast, the addition of IL-4 in the developmental stages of BM-DCs led to cells with an immature phenotype, responsive to LPS-induced maturation but also promoted nonspecific T cell signalling, potentially impeding the induction of antigen-specific T cell responses.
Conclusion: These results show how different combinations of growth factors influence both the phenotypic and functional characteristics of BM-DCs. Furthermore, the study emphasises the importance of reviewing even widely used protocols for DCs differentiation and considering the impact of these protocols on experimental designs such as the evaluation of T cell responses.

This work was supported by the Ministry of Research and Innovation, Romania, PCCDI - UEFISCDI, number PN-III-P1-1.2-PCCDI-2017-0529 / 62PCCDI / 2018, from PNCDI III and the Nucleu Program, contract 25N / 2023, project PN 23 44 01 01.

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680 – P2.09.12

Human MAIT cell profiles biased towards IL-17 or IL-10 are transient effector states directed by the cytokine milieu

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Mucosal-associated invariant T (MAIT) cells are unconventional MR1-restricted T cells mediating rapid innate-like antimicrobial immunity. However, how the functional heterogeneity of human MAIT cell responses is controlled is largely unclear. Here, combining functional and transcriptomic analyses we define distinct MAIT cell effector programs directed by the cytokine milieu during antigen recognition. Activation by TCR signaling together with IL-12 or IL-23 drives c-MAF-dependent IL-10 production, with intermediate levels of IFNγ/TNF, and elevated expression of TIM-3, LAG-3, and PD-1. The MAIT-derived IL-10 mediates both autocrine and paracrine immune regulation. In active Crohn's disease, the MAIT cell IL-10 regulatory profile appears to be suppressed. In contrast to the action of IL-12, co-activation of MAIT cells with IL-18 strongly co-activates IL-17, GM-CSF, IFNγ and TNF, while counteracting IL-10 expression. Finally, TCR activation without cytokine co-activation drives primarily cytolytic arming. These activation states are transient and do not represent stable subsets. Altogether, these findings demonstrate how cytokine cues direct transient MAIT cell effector response programs.

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684 – P2.09.13

Macrophages treated with interferons induce different response in lymphocytes via exstracellular vescicles

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Limited information exists regarding the impact of interferons (IFNs) on the information carried by extracellular vesicles. This study aimed at investigating whether IFNα2b, IFNβ, IFN-γ, and IFN-λ1/2 modulate the content of EVs released by primary monocyte-derived macrophages (MDM).
Small-EVs (sEVs) were purified by size exclusion chromatography from supernatants of MDM treated with IFN. To characterize the concentration and dimensions of vesicles, Nanoparticle Tracking Analysis was used. SEVs surface markers were examined by flow cytometry.
IFN treatments induced a significant down-regulation of the exosomal markers CD9, CD63, and CD81 on sEVs, and a significant modulation of some adhesion molecules, major histocompatibility complexes and pro-coagulant proteins, suggesting IFNs influence biogenesis and shape the immunological asset of sEVs. Autologous PBMCs were treated with sEVS released by IFN-stimulated MDM, showing significant modulation of lymphocyte activation and IL-17 release.
Altogether, our results show that EVs composition and activity is affected by IFN treatment of MDM.

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695 – P2.09.14

Regulation of Natural Killer Cell Metabolism and Anti-Tumour Responses by Macrophages

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Oxidised cholesterol derivatives called oxysterols have diverse roles in many cellular processes. Their role in controlling NK cell metabolism and effector functions through inhibiting SREBP transcription factor activation is well-described. However, other SREBP-independent effects of oxysterols on NK cell responses are not well defined. For instance, oxysterols are known to alter plasma membrane structure through a number of mechanisms, which may impact many critical processes required for NK cell functions such as endocytosis, receptor signalling and degranulation.
Exposure of previously cytokine-activated NK cells to the oxysterols 25-hydroxycholesterol (25HC) and 27-hydroxycholesterol (27HC) for just 4 hours causes an impairment in cytotoxicity against K562 and RMA/S target cells despite no observed change in their expression of granzyme B, perforin or interferon-γ. The oxysterol-treated NK cells also had an accumulation of transferrin receptor at the cell surface, potentially due to oxysterol-mediated inhibition of endocytosis of the transferrin-iron complex. Furthermore, plasma membrane probes revealed alterations to membrane structure in oxysterol-treated NK cells, which may potentially impact endocytosis and degranulation.
Oxysterol-rich environments are known to arise due to pro-inflammatory macrophages in various infections. Ligation of TLR-2/6, -3 and -4 induce >10-fold increases in the expression of Ch25h (the enzyme the synthesises 25HC) in BMDMs stimulated with TLR agonists for just 6 hours. Culture of NK cells with stimulated BMDMs results in significant decreases in NK cell size, interferon-γ expression and expression of cytolytic molecules. These NK cell also showed significant impairments in their cytotoxicity. This suggests a new metabolic inhibitory pathway between macrophages and NK cells that could have implications for anti-viral and anti-tumour immunity.

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708 – P2.09.15

Dysregulation of the TLR3/IFIH1 pathway at juvenile dermatomyositis onset may implicate viral infection as a disease trigger

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Purpose: We previously reported elevated type I IFN responses, and an association with SARS-CoV-2 acute infection, in onset juvenile dermatomyositis (JDM) a rare autoimmune disease. Here we tested the hypothesis that dysregulation of specific viral nucleic acid sensor pathways may be implicated in the JDM onset.

Methods: We prospectively recruited 19 JDM patients at diagnosis and age- and sex-matched paediatric controls. Whole blood inflammatory profiles were assessed by single-cell phosphoproteomics using mass cytometry. Cellular responses of nucleic acid sensors were evaluated using standardized ex vivo whole blood stimulation assays (TruCulture) with Toll-like receptor (TLRs) agonists: R848 for TLR7/8, Poly(I:C) for TLR3/IFIH1 and ODN for TLR9. Cytokine secretion was quantified using Luminex and digital ELISA (Simoa) while transcriptomic signatures were determined by bulk RNA barcoding and sequencing (BRB-seq).

Results: We observed high heterogeneity in the phosphoproteome profile within JDM patients at diagnosis, with a sub-group of patients showing a strong over-activation as compared to controls. None of JDM patients at diagnosis had a similar phosphoproteomic profiles. Median levels of type I interferon and specific ISG proteins were 10 times higher in JDM patients at basal state compared to controls. Following TLR stimulation JDM patients showed a dysregulated response specifically to Poly(I:C) stimulation at both proteomic and transcriptomic levels. In contrast, responses to R848 and ODN stimulation were no different. Transcriptomic analysis identified an enrichment of the interferon pathway in JDM patients at basal state. IFIH1, but not TLR3, was up-regulated in JDM at diagnosis compared to controls.

Conclusion: JDM at diagnosis is characterized by a type I interferon (IFN-I) signature which was observed at both proteomic and transcriptomic levels. We identified a defective response to Poly(I:C) stimulation in JDM implicating the TLR3/IFIH1 pathway which may lead to the over-production of IFN-I. Further single cell studies will help to better understand the cellular specificities that drive the IFIH1 dysregulation and lead to JDM pathogenesis.

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779 – P2.09.16

Development of pooled CRISPR screening method for primary human macrophages

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Purpose: Macrophages are important in innate immune system, responding to pathogens or tissue damage. During pathogen infection, the pro-inflammatory cytokine, interferon-γ (IFNγ) is known to work with tumor necrosis factor α (TNF-α) or Toll-like receptor signaling to trigger macrophage cell death [1,2]. For human cells, CRISPR screens have been developed for immortalized myeloid cell line (U937 and THP-1) and iPSC-derived macrophages [3-5], but not yet for primary macrophages, impeding in depth biological study of macrophage functions in complex tissue microenvironment in human diseases. This study aims to develop a CRISPR screening method for primary human macrophages though overcoming main technical barriers, including low lentiviral infection efficiency, compromised cell survival after lentiviral infection, and inefficient Cas9 delivery into macrophages.
Methods: Lentivirus was generated using HEK 293T cells which were transfected with LentiGuide-Puro, psPAX2 and pMD2.G. Primary human monocytes were isolated from PBMCs of healthy donors. Monocytes were infected with lentivirus, Vpx virus-like particles (Vpx-VLPs) and polybrene. Cas9 protein was delivered into infected macrophages though electroporation. IFNγ, lipopolysaccharide (LPS) and TNF-α were used to induce macrophage cell death. Transduction efficiency of single-guide RNA (sgRNA), target gene knockout efficiency and live macrophage number were detected though flow cytometry.
Results: Firstly, efficient sgRNA transduction (30%) mediated by lentivirus has been achieved in macrophages though Vpx-VLPs. Secondly, macrophages could maintain survival and pro-inflammatory function post infection with low amount of lentivirus. Thirdly, efficient target gene knockout (20%) was achieved in sgRNA lentivirus infected macrophage though optimized program-mediated Cas9 electroporation. IFNγ combined with LPS or TNF-α stimulation induced around 50% and 20% macrophage cell death, respectively.
Conclusion: Here, we have overcome certain technical difficulties for the performance of CRISPR screening in primary human macrophages. Next, we will perform a transcription factor (TF) pooled CRISPR screening to identify overlapped or specific TFs involved in the synergism of IFNγ with TNF-α or LPS-induced macrophage cell death pathways.

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837 – P2.09.17

Dynamics of human T and B cells followingantigenic stimulation

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We are interested in dissecting immune T and B cell repertoires by integrating different datasets obtained through single-cell transcriptomics, proteomics, T cell receptor (TCR)/B cell receptor (BCR) sequencing and isolation of antigen specific T cell clones and monoclonal antibodies. This approach has led to the functional definition of different memory subsets characterized by distinct transcriptional profiles, clonal composition, and antigenic specificity, in addition to the characterization of populations of highly expanded memory B cells as well as CD4 and CD8 T cell clones with unique transcriptional profiles.
We are now focusing on the immune system's rapid response following Flu and SARS-CoV-2 infection or vaccination. To do so, we collected peripheral blood mononuclear cells (PBMCs) from different donors six days after antigen exposure and isolated both plasmablasts and recently activated T cells for ex-vivo analysis. In parallel, we in-vitro stimulated the total PBMCs and compared this immune response to a generic and strong stimulus with the antigen-specific one.
We show that, after infection or vaccination, recently activated Ki67+ cells represent family bursts of small antigen-specific clones that undergo vigorous expansion in vivo. In contrast, during steady state, recently activated T and B cells represent a random sample of the total repertoire and derive from most populations, ranging from naïve cells to terminally differentiated expanded cells. These findings are consistent with two modes of activation shared by T and B cells: i) the rapid proliferative response of specific clonal families following antigenic stimulation and ii) the homeostatic expansion of single cells driven by cytokines in an antigen-independent fashion.
Collectively our analysis performed on serial samples provides a snapshot of the dynamics of the immune response at the single-cell level.

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846 – P2.09.18

Investigating the effect of Fibroblast growth factor-23 on neutrophil migration

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Introduction: Fibroblast growth factor 23 (FGF23) is an endocrine hormone associated with vitamin D and phosphate homeostasis. We have previously shown an association with elevated FGF23 concentrations and increased mortality in critical illness. The effect of vitamin D on innate immune function is well described. However, little is known about how FGF23 interacts with innate immune cells. An array of FGF receptors (FGFRs) and binding partners (heparin, klotho), make unpicking the specific effects difficult. Neutrophils do not express klotho and are key cells in critical illness.
Methods: Neutrophils were isolated from venous blood (healthy donors) by density gradient separation. FGFR expression was assessed by flow cytometry. The effect of FGF23 pre-treatment (100ng/ml, 1 hour), on neutrophil migration towards IL-8 was assessed by Transwell migration assay and F-actin polymerisation using Phalloidin staining and confocal microscopy.
Results: Neutrophils predominantly express FGFR1 and FGFR2. FGFR3 had lower and variable levels of expression. When permeabilised only FGFR1 expression increased (28.02%, p=0.0165 n=8). FGF23 pre-treatment resulted in decreased migration through a Transwell insert to an IL-8 gradient, when compared with vehicle (veh=$5.6x10^4$, FGF23= $3.6x10^4$, p=0.0068, n=12). FGF23 pre-treatment resulted in decreased levels of F-actin accumulation, compared to vehicle, however this difference was not statistically significant (MFI: FGF23= 21.76, veh= 34.28, p=0.1457, n=5).
Discussion: FGFR1 and 2 are present on the cell surface of healthy peripheral blood neutrophils. FGFR1 expression increased after permeabilization, suggesting intracellular FGFR is present in the cytosol in homeostasis. FGF23 reduces neutrophil migration, demonstrating previously uncharacterised biological activity of FGF23 in these cells independently of klotho. We hypothesise that FGF23 reduces F-actin polymerisation and therefore prevent neutrophil migration. We have previously shown that elevated levels of FGF23 are associated with increased mortality, here we begin to show mechanism of how FGF23 may alter the neutrophil response.

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Contributors: NA conducted all laboratory studies, performed all analysis, and wrote the abstract. OST taught NA laboratory experiments. DT, AS, and DP are NA's supervisors and designed the project.

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894 – P2.09.19

Analysis of platelet monocyte complexes in patients with ST-elevation myocardial infarction

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Purpose: Direct physical interaction of platelets with leukocytes can lead to the formation of platelet leukocyte aggregates (PLAs) and is often associated with a bidirectional activation that enhances the proinflammatory state. Elevated numbers of PLAs can be found under inflammatory conditions, but the pathophysiological relevance of is largely unclear. Here, we investigated formation and functional alterations of PLAs in the blood of human patients after ST-elevation myocardial infarction (STEMI).
Results: Flow cytometry of blood samples revealed that the relative frequency of CD41+ as well as CD62P+ monocytes (MPAs) was strongly increased in the blood of STEMI patients on day 1 post MI compared to patients with stable coronary heart disease (CHD) or healthy volunteers. However, CD41+ neutrophils or lymphocytes were only slightly increased. Of note, MPA levels were elevated in patients up to six months post STEMI despite anticoagulant treatment. Analysis of MPA formation in monocyte subtypes revealed that MPA formation was mainly increased in classical and intermediate monocytes. Immunofluorescence-, transmission- and raster-electron-microscopy revealed that CD41+ monocytes are associated with intact platelets rather than CD41+ microvesicles or exosomes. Functional analyses showed an increased uptake of perfluorocarbon nanoparticles (PFCs) in CD41+ monocytes from healthy volunteers, CHD and STEMI patients. Migration towards gradients of the chemokine monocyte chemoattractant protein (MCP)-1 was increased in CD41+ monocytes from healthy volunteers, but not from STEMI patients. Intracellular TNFα expression as well as uptake of the glucose analogue 2-NBDG was higher in MPAs from STEMI patients compared to healthy controls. Finally, correlation analyses revealed that patients with a high number of intermediate MPAs have larger infarct sizes, increased endsystolic and enddiastolic volumes and higher CRP levels in the blood.
Conclusion: Here we show that particularly heterotopic aggregates of platelets with classical and intermediate monocytes are strongly increased in STEMI patients and remain elevated for up to six months. Monocytes associate with intact platelets which can lead to functional alterations such as increased phagocytosis of nanoparticles. However, future studies must unravel the precise functional consequences and the pathophysiological relevance of MPAs for the acute inflammatory and the subsequent healing reaction post STEMI.

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896 – P2.09.20

Tonsil organoids reveal that plasmacytoid dendritic cells modulate the humoral immune response to ChAdOx1 nCoV-19

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Purpose: To understand how vaccines induce productive immune responses, it is critical to study the processes that occur at the site of immune priming – secondary lymphoid tissues. However, access to such tissue is limited.
Methods: To overcome this, we have refined a recently developed human tonsil organoid model to explore the processes that regulate the induction of humoral responses to the ChAdOx1 nCoV-19 vaccine.
Results: Upon exposure to ChAdOx1 nCoV-19, tonsil cultures exhibited robust innate immune activation and cytokine release within 24 hours. This innate activation subsequently led to both global and antigen-specific B cell activation, along with the secretion of spike-specific antibodies during the ensuing 14-day culture period. Notably, exposure to an unrelated ChAdOx1 GFP vector did not elicit any detectable spike-specific antibody production. Among the immune cell types, plasmacytoid dendritic cells (pDCs) exhibited the highest levels of transduction and activation. Crucially, depletion of pDCs resulted in diminished innate and humoral responses. Blocking IFN-α had a similar effect to pDC depletion, while adding IFN-α to pDC-depleted cultures rescued humoral responses. Furthermore, IL-6 played a pivotal role in enhancing humoral responses, with its production being regulated in an IFN-α-dependent manner, even in the absence of pDCs, indicating intricate cross-talk signalling interplay.
Conclusion: In summary, this model underscores the essential role of pDCs in initiating humoral responses to ChAdOx1 nCoV-19, primarily through IFN-α. IFN-α appears to exert both direct and indirect effects by inducing IL-6 secretion to amplify the humoral response. Moreover, this model holds promise for investigating other vaccine platforms.

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897 – P2.09.21

Evaluation of HLA-G levels in human umbilical cord blood-derived mesenchymal stem cells after co-culture with PBMCs

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Mesenchymal stem cells (MSCs) are multipotent stem cells capable of differentiating into various cell types such as osteoblasts, adipocytes, chondroblasts, and neurons. They can be sourced from various fetal and adult tissues. MSCs exhibit immunomodulatory properties through the expression of soluble factors including human leukocyte antigen (HLA)-G, prostaglandin-E2, and transforming growth factor beta-1. These factors enable MSCs to suppress the proliferation of NK, B, and T cells, and inhibit the differentiation of dendritic cells and B cells.
In our study, we aimed to investigate the impact of co-culturing MSCs derived from human umbilical cord blood with stimulated and unstimulated peripheral blood mononuclear cells (PBMCs) on HLA-G levels. MSCs were co-cultured at ratios of 1:5 and 1:10 with PBMCs for 24 and 72h to induce an inflammatory environment. HLA-G levels in the co-culture supernatants were measured using an ELISA assay.
Results showed that when co-cultured at a 1:5 ratio with both stimulated and unstimulated PBMCs, HLA-G levels in CB-MSCs were significantly higher in 24 h cultures compared to 72 h cultures. In WJ-MSCs, there was a significant increase compared to 24 h in 72 h in co-cultured at a 1:5 ratio with both stimulated and unstimulated PBMCs.
In 72 h CB-MSCs with PBMC co-cultures at a 1:10 ratio were significantly higher compared to those at a 1:5 ratio in both stimulated and unstimulated groups. In the unstimulated group, there was a significant increase at a 1:10 cell ratio, while in the stimulated group, there was a significant decrease. In the co-culture of WJ-MSCs and PBMCs at a 1:5 ratio, a significant increase was observed at 72 h in both the unstimulated and stimulated groups, compared to the 24h.
In conclusion, our study highlights the dynamic nature of MSC immunomodulation in response to inflammatory stimuli. The duration of co-culture and the ratio of MSCs to PBMCs were found to play crucial roles in modulating HLA-G expression. These findings emphasize the importance of optimizing co-culture conditions for therapeutic applications in immune-related disorders.

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1042 – P2.09.22

Peripheral blood lymphocyte cell subsets in subjects with silicosis due to engineered stone

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Silicosis produced by Artificial Quartz Agglomerates or engineered stone (ES-silicosis) evolves more aggressively than the classical form of miners. This entity is emerging worldwide and a significant group of cases has been detected in the province of Cádiz (Spain) in recent years. Although the role of the cellular immune response in its pathogenesis is recognized, few studies have been reported on the lymphocyte subsets in patients with silicosis. In particular, none has been published on patients with ES-silicosis.
Purpose: to characterize lymphocyte subsets in the peripheral blood of patients diagnosed with silicosis due to silica agglomerates.
Methods: 91 patients diagnosed with silicosis due to silica agglomerates were included, 53 with simple silicosis (SS), 38 with complicated silicosis (CS) and 23 healthy unexposed as a control group (CG). All were men. Main age 40.1 ±7.7 (SS), 41 ± 6.2 (CS) and 36.4 ±8.3 (CG). Cell subsets from blood samples were analyzed by flow cytometry.
Results: The main subsets of leukocytes in peripheral blood, such as neutrophils and monocytes, progressively increased in patients with SS and CS compared with the CG. However, in the case of lymphocytes the opposite was observed, so that the percentage of lymphocytes in the GC was 31.3±8.2, 25.6±7.6 (SS) and 22.6±5. 8 (CS) with statistically significant differences. Analyzing the main subsets of lymphocytes, the following differences were observed in patients with silicosis compared to CG: i) a significant decrease in the percentage of memory B cells (30±13.6 [CG], 19.1±9, 4 [SS], 21.1±12.2 [CS]) and an increase in plasma cells (0.07±0.06 [CG], 0.11±0.08 [SS], 0.1± 0.04[CS]; ii) a significant reduction in the proportion of naïve Th cells (32.5±13.5 [CG], 23.5±10.1 [SS], 25.8±9.6 [CS]); iii) a significant increase in memory Th cells (43.6±13.2 [CG], 54.4±14.6 [SS], 53.3±12.5 [CS]).
Conclusions: Some subsets of lymphocytes are altered and could be targeted as possible new avenues of intervention to treat the disease.

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1064 – P2.09.23

Delineating the immunological responses in people admitted to the ICU with direct and indirect lung injuries using single-cell RNA sequencing

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Purpose: Acute lung injury (ALI) is characterized by acute inflammation in the lungs, with mortality of up to 46% in severe cases. There are two types of ALI, (i) direct lung injury, commonly caused by community acquired pneumonia (CAP); (ii) indirect lung injury caused by conditions including burns, pancreatitis, and non-thoracic trauma. While the immunopathology of direct lung injuries such as COVID-19 CAP, is relatively well understood, the complexities of immune dysregulation in indirect lung injuries remains poorly elucidated. This study aims to define the common and divergent inflammatory pathways associated with direct and indirect ALI.
Methods: Our cohort (N=33) included participants with (i) Direct ALI (N=11) caused by COVID-19 CAP. Criteria for CAP included symptoms of lower respiratory tract infection and radiological evidence as well as mechanical ventilation of less than 72 hours (ii) Indirect ALI (N=9) secondary to non-thoracic trauma with no secondary infection; (iii) ICU controls (N=13) or participants with no infection or lung inflammation within 72 hours of mechanical ventilation. We conducted serum cytokine profiling using the Luminex platform and single cell RNA sequencing on live CD45+ cells from peripheral blood on the BD Rhapsody Express system.
Results: Cytokine profiling demonstrated upregulation of IL-1R and IL-6 in the trauma compared to ICU controls. In contrast, IL-7, CCL-5 and IL-4 were found to be elevated in COVID-19 CAP compared to ICU controls. Transcriptomic analysis of peripheral blood revealed a large population of activated $CD4^+$ T cell expressing TNF-α and IFIT3 in the trauma group, whereas MAIT cells were predominant in COVID-19 CAP. Pathway analysis of these immune cells highlighted the upregulation of adaptive pathways such as IFN-γ signalling, TNF-α and MAPK signalling in COVID-19 CAP compared to trauma group.
Conclusion: This study highlights the divergent aetiologies and the immune landscape in direct and indirect lung injuries. Further delineation of the underlying mechanisms driving these distinct inflammatory pathways may lead to novel and or repurposed therapeutics. The COVID-19 pandemic demonstrated the success of immunomodulatory therapies in a subtype of CAP, promoting the potential to determine if similar translational strategies can be applied to treat other forms of ALI.

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1201 – P2.09.24

Recent thymic emigrants in SARS-CoV-2 infection and post-COVID-19

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Purpose: In severe COVID-19 thymic function and output are reduced. Naive T cells are affected in patients with post-acute sequelae of COVID-19 (PASC). Recent thymic emigrants (RTEs) are the youngest naive T cell population in the circulation, and represent thymic output. We examined the CD4+ RTEs and CD4+/CD8+ naive T cells in the blood of COVID-19 patients with and without PASC to check the thymic function in SARS-CoV-2 infection.
Methods: We recruited COVID-19 patients (n = 42) and collected blood at 1-3 timepoints. PBMCs were isolated, stained for FACS and analyzed. We divided the samples into acute (<30 d from symptom onset), convalescent (30-120 d) and recovered (>120 d) phases. We surveyed the patients for persisting impaired olfaction as proxy for PASC (n = 5 PASC+, n = 16 PASC-).
Results: We found that the proportions of naive CD8+ ($p = 0.049$) and Ki-67+ naive CD8+ T cells ($p < 0.001$) were negatively correlated with time since COVID-19 symptom onset. RTEs or CD4+ naive T cells did not show significant correlation. These findings were further proven using paired analysis, where the proportion of naive CD8+ T cells was higher in the acute than the convalescent disease phase ($p = 0.004$) and the proportion of Ki-67+ naive CD8+ T cells was higher in the acute ($p = 0.008$) and convalescent ($p = 0.0211$) phases than the recovered phase. We found a higher proportion of Ki67+ CD4+ naive T cells in the convalescent disease phase in PASC+ than PASC- individuals ($p = 0.031$). None of the other cell populations showed significant differences.
Conclusion: We found that CD8+ naive T cells and their proliferation were proportionally higher in acute disease than in convalescence. This is consistent with acute viral infection and, along with the stable RTE populations, suggests normal thymic function. The age-related findings of proportionally lower CD8+ naive T cells and higher CD4+ RTE proliferation are likely related to lymphopenia during COVID-19. The finding of higher proportions of proliferation in CD4+ naive T cells in PASC patients is preliminary, with a small cohort. Further research with larger sample sizes is required.

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Effect of long-term cryopreservation on peripheral blood mononuclear cell viability and immune cell subset frequency

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The aim of this study is to investigate the viability and the percentages of different immune cell subsets in peripheral blood mononuclear cells (PBMCs) after long-term cryopreservation and subsequent thawing to determine the optimal conditions for long-term storage of PBMCs. PBMCs were isolated from buffy coats using double gradient centrifugation and cryopreserved in a solution containing 50% RPMI, 40% FBS and 10% DMSO; 80% FBS and 20% DMSO; or 90% FBS and 10% DMSO. Cells were stored at -80°C and preserved in liquid nitrogen for up to 10 months. Flow cytometry analysis was performed on PBMC samples thawed at 1 month, 4 months and 10 months after cryopreservation. Monoclonal fluorescent antibodies targeting various immune cell surface markers were used for surface marker staining and analyses. The highest cell viability was observed when the PBMCS were stored in 90% FBS and 10% DMSO. The percentages of lymphoid and myeloid cell subtypes in PBMCs remained stable over the 10-month storage period. Percentages of cells positive for surface markers such as CD3, CD4, CD8, CD14, CD25, CD45, CD56 and HLADR showed stable levels, while slight fluctuations were observed in the percentages of CD15, CD16, CD19 and FOXP3 positive cell populations during long-term storage. Overall, the results suggest that surface marker expressions in PBMCs are effectively maintained under the indicated storage conditions.

This study demonstrates that long-term storage of frozen PBMCs is a reliable method to preserve their immunophenotypic properties.
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1255 – P2.09.27

Candida- Driven Modulation of Macrophage Polarization: Influence of Candida albicans and non-albicans species.

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Purpose: Macrophages are key players in the innate immune system, capable of polarization into classically activated (M1) and alternatively activated (M2) macrophage phenotypes based on microenvironmental signals. *Candida* species, fungal pathogens causing candidemia, intricately interact with the host's innate immune components, including macrophages. In this study, PBMC-derived monocytes (PBMC-DMs) were co-incubated with *Candida albicans* (SC5314), *Candida parapsilosis* (CLIB 214), *Candida auris* (AR 0381), *Candida glabrata* (CBS 138) to investigate their impact on the polarization of PBMC-DMs.
Methods: After the co-incubation, PBMC-DMs were immunolabelled using macrophage-specific (CD68), M1-specific (CD86), and M2-specific (CD163) fluorophore-conjugated antibodies. Their polarization was characterized using flow cytometry via fluorescence-activated cell sorting (FACS). Additionally, IFN-γ-activated M1 macrophages were also studied for their response against these *Candida* species through phagocytosis and killing assays.
Results: The result showed that PBMC-DMs infected with *C. albicans* showed no significant differences in their polarization to M1 or M2 macrophages. On the other hand, *C. parapsilosis* infection drives polarization into M2 macrophages. M1 macrophages resulted in lower phagocytic and killing activity against *C. albicans* and *C. parapsilosis*. However, this phenomenon was only significant in the case of *C. parapsilosis.*
Conclusions: These findings suggest that interaction of PBMC-DMs with *C. parapsilosis* could potentially contribute to M2 polarization, which may lead to immune evasion and disease progression. The reduced phagocytic and killing activity of IFN-γ-activated M1 macrophages against *C. parapsilosis* suggests compromised IFN-γ signaling, which may further compromise the immune response and favor the survival and persistence of this pathogen. In addition to the existing analysis, ELISA will be conducted to quantify and detect various cytokines including TNF-α, IL-10, TGF-β, and IL-6 induced by different *Candida* species. Furthermore, functional analyses are planned for *C. auris* and *C. glabrata* to provide more comprehensive understanding of the dynamics of non-albicans species.

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1285 – P2.09.28

Immunomodulatory effect of phenylhydantoin derivatives on myeloid leukemia cells

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Purpose: Leukemia is one of the most important cancers affecting a large proportion of the population, especially children. Despite recent advances to improve the efficiency of therapy, the range of effective drugs available is comparatively limited and there is a significant need for the development of new drugs. One of the most prominent biological functions of phenylhydantoin derivatives is the antitumor effect on cells of various cancers, although they are frequently used for the clinical treatment of epilepsy and inflammation. The unique physicochemical properties, especially the increased molecular surface area for interactions, make phenylhydantoin derivatives promising agents for biomedical applications. In this study, the immunomodulatory effects of phenylhydantoin derivatives on a chronic myeloid leukemia cell line (K562) were investigated.
Methods: Cells were exposed to treatment with five different concentrations of phenylhydantoin derivatives (from 0.1 to 100 µM), while cells were treated at two concentrations 1 and 10 µM for 72 hours to determine migration potential and concentrations of IL-1β and IL-6, MMP-2 and MMP-9, as well as gene expression of COX-2 and iNOS. The antitumor effects were evaluated by the influence of treatment on cell viability, oxidative stress parameters and migration potential, while the imunomodulatory effects were evaluated by the production of IL-1β and IL-6 and the gene expression of iNOS and COX-2.
Results: The results obtained indicate an intense effect on cell viability, suggesting a significant antitumor potential. After treatment with derivatives, increased nitrite production and iNOS expression was observed, especially in concentrations of 1 and 10 µM. Decreased superoxide anion production was observed after derivatives application, also in concentrations of 1 and 10 µM. In addition, the migration potential and MMPs expression levels were significantly reduced, as were the concentrations of IL-1β, IL-6 and COX-2 gene expression in K562 cells. These data indicate a significant immunomodulatory activity of the tested phenylhydantoin derivatives in K562 cells.
Conclusion: The tested phenylhydantoin derivatives exhibit considerable antitumor potential and show immunomodulatory effects on various parameters, which makes them suitable candidates for further research with the aim of achieving more effective results in antitumor therapy with less harmful effects on healthy tissue.

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1302 – P2.09.29

The pathophysiological implications of interleukin-6 concentrations associated with COVID-19 in human trophoblasts

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Purpose: Interleukin-6 (IL-6) is major pro-inflammatory cytokine with an exquisite ability to induce the acute phase response and cytokine storm. Proliferation, survival and invasion of extravillous trophoblasts on maternal tissue are essential for successful establishment of gestation. Cytokine storm is overproduction and uncontrolled release of pro-inflammatory markers, whereas IL-6 has role in regulating trophoblast phenotype and metabolism. COVID-19 represents major health issue globally, affecting hundred of millions of people worldwide in the past several years and causing the damage in various tissues. Proinflamatory cytokines are considered to be one one of the underlying mechanisms of COVID-19 inflamatory complications.
Methods: In this study was investigated the potential pathophysiological effects of concentrations of interleukin-6 present in clinical cases of COVID-19 on physiology of JEG-3 human trophoblast cells. The experiment was performed in atmospheric hypoxia (3% of O_2) conditions, correlating with oxygen environment of trophoblast in the first trimester of gestation. The cells were exposed to the series of concentration of interleukin-6 derived from clinical cases of COVID-19 patients (50 pg/ml to 500 pg/ml). The proliferation ratio, apoptosis index, inhibition of caspases, invasion potential, and the parameters of oxidative metabolism were evaluated in the study.
Results: Superoxide anion radical ($O_2^{\cdot-}$) production were significantly increased after several applied IL-6 concentrations, implying induction of intense redox imbalance. At all examined concentrations, IL-6 have stimulated the activation of caspases, executing enzymes of apoptosis, as demonstrated by an increase in Apostat fluorescence signal. The invasion capacity of JEG-3 cell was elevated at all applied concentrations, suggesting the promigratory role of IL-6. The results indicate that IL-6 in concentrations present in COVID-19 complications has disruptive effects on the optimal physiological functioning of first trimester trophoblasts in the aspects of cell survival, apoptosis level, invasion capacity and oxidative stress.
Conclusion: The obtained data suggest the potentially pathological effects of IL-6 on pregnancy progression due to inflammatory complications caused by COVID-19. The elevated levels of IL-6 induce various disturbances of trophoblast cell homeostasis and could be one of risk factors in development of pregnancy disorders, raising the concerns of infection impact regarding the effects on placenta and the offspring.

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1418 – P2.09.30

Interferon-β exposure and ARTS deficiency promote the generation of hyper-efferocytic Ly-6C+ macrophages during the resolution of inflammation

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During the resolution of inflammation, Ly-6C$^+$F4/80$^-$ monocytes differentiate to Ly-6C$^-$F4/80$^+$ macrophages that exert efferocytic properties and consequently convert to IFN-β-producing macrophages. Here, we report that exposure to IFN-β, or TGF-β, or a deficiency in the pro-apoptotic protein ARTS, results in the conversion of mature macrophages to a rejuvenated Ly-6C$^+$F4/80$^+$CCR2$^+$ phenotype. This phenotype appeared exclusively in peritoneal resolution phase macrophages and not their unchallenged peritoneal, splenic or bone marrow counterparts. Moreover, IFN-β-triggered rejuvenated macrophages were hyper-efferocytic and expressed higher levels of the efferocytic receptor CD36. Inhibition of CD36 ligation resulted in complete abrogation of efferocytosis ex vivo in both mature and rejuvenated macrophages. Altogether, our findings indicate an unprecedented phenomenon in which IFN-β promotes macrophage rejuvenation and efferocytosis that are limited by ARTS-mediated apoptosis during the resolution of inflammation.

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1471 – P2.09.31

The number of classical and non-classical monocytes are altered in the peripheral blood from patients with silicosis due to engineered stone

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Introduction: Silicosis by engineered stone (ES-silicosis) is a new, more aggressive form of silicosis with a shorter latency period than classical silicosis, which has appeared in workers exposed to high concentrations of crystalline silica (CS) particles as a result of handling ES, leading to rapid loss of lung function. The exact role of the immune system in the disease is not fully understood. Previous findings, our patient cohort has shown neutrophilia, lymphopenia and monocytosis, highlighting the need for further investigation into these cell subsets as potential contributors to the disease development and progression.
Purpose: In this study, we aimed to characterize monocyte subpopulations in peripheral blood from patients diagnosed with ES-silicosis and compare them with healthy controls.
Patients and methods: We used flow cytometry to assess monocytic subpopulations in 9 healthy controls and 18 patients diagnosed with ES-silicosis: 9 with simple silicosis (SS) and 9 with progressive massive fibrosis (PMF). Peripheral blood mononuclear cells (PBMCs) were isolated via density gradient centrifugation. The PBMCs were washed and resuspended in a staining buffer containing a combination of antibodies including CD16 (FITC), CD19 (PERCPCy5.5), HLA-DR (APC), CD14 (APCH7), CD11c (PECy7), CD45 (V500). Debris and dead cells were gated out, and monocytic subpopulations ($CD11c^{+}HLADR^{+}$) were selected following this gating strategy: classical monocytes ($CD14^{+}CD16^{-}$), intermediate monocytes ($CD14^{+}CD16^{+}$), and non-classical monocytes ($CD14^{dim/-}CD16^{++}$). Statistical analysis was performed using the SPSS software program.
Results: Flow cytometry data analysis reveals a statistically significant increase in the median of non-classical monocytes in patients with both SS (5.81) and PMF (6.25) compared to controls (2.98), accompanied by a significant decrease in classical monocytes. The proportion of intermediate monocytes is similar across all studied groups, and there are no differences in monocytic subpopulations between patients with SS and PMF.
Conclusions: There is an alteration in the distribution of classical and non-classical monocytes in patients with ES-silicosis. This study will allow us to focus on these subpopulations for further investigation into activation and polarization in the monocyte-macrophage axis, aiming to elucidate altered inflammatory processes and the effect of potential therapeutic targets.

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1499 – P2.09.32

Cross-reactivity to microbiota shapes natural CD4+ T cell responses against neo-antigens associated with acute lymphoblastic leukemia

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Purpose: Chromosomal translocations are frequent drivers of ALL in both children and adults which create a small and defined repertoire of mutated neoepitopes, serving as specific targets for anti-tumor T cell responses. Microbiota has a strong impact on anti-tumor responses but the mechanistic details and the microbial species involved are poorly understood. Here we aimed to pinpoint the properties of CD4+ T cell-dependent immunity against ALL neoantigens in healthy donors and leukemia patients, as well as to investigate the potential of microbiota—directed immunity to modulate the immune responses against ALL-relevant neoantigens through cross-reactivity.
Methods: We use the Antigen-Reactive T cell Enrichment (ARTE) protocol for identification and phenotypic profiling of antigen-specific CD4+ T cells. To deeply characterize antigenic specificity, affinity, and cross-reactivity of target lymphocyte populations, we made use of state-of-the-art single-cell- RNA-seq, expansion, and re-stimulation of rare antigen-specifically activated T cell clones, as well as TCR-repertoire profiling of antigen-specific T cells.
Results: We found that most of the investigated ALL-relevant neoantigens induce a T-cell memory response in healthy donors. A fraction of healthy donors demonstrated a strong Th17-polarized immune response towards UBTF-ATXN7L3 fusion antigen. This response was driven by T cells reactive to one common intestinal microbial species. UBTF-ATXN7L3-reactive T cells from leukemia patients demonstrated the same cross-reactivity pattern.
Conclusion: Our research serves as a proof of principle for microbiota-specific immunity to affect anti-leukemia immune surveillance by means of T-cell cross-reactivity. This will be relevant for the development of new immunotherapeutic approaches same as fundamental understanding of anti-tumor immune response mechanisms.

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1507 – P2.09.33

Comparing three methods for the isolation of exosomes from plasma in subjects with overweight and 3T3-L1 cell culture

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Purpose: This study aimed to isolate whole exosomes from plasma and supernatant of differentiated 3T3-L1 cells using three isolation techniques.
Methods: Plasma samples were collected from 120 individuals categorized based on body adipose tissue proportion (excess defined as more than 25% in men and 35% in women). Differentiated 3T3-L1 cells were cultured in DMEM supplemented with 10% FBS (fetal bovine serum depleted of microvesicles through overnight ultracentrifugation) and 10 µg/mL insulin for 20 days at 37°C and 5% CO_2. Furthermore, another set of 3T3-L1 cells was subjected to hypoxia for 24 hours following the same culture conditions.
We employed three isolation techniques: differential centrifugation (DC), size exclusion chromatography (SEC), and a commercial kit. Exosomes were characterized using cryo-TEM, TEM, and western blot (WB) for the tetraspanins CD9 and CD81.
Results: In the three isolation techniques, cryo-TEM and TEM analysis showed similar quality, quantity, and morphology of exosomes; at the same time, no difference was observed between individuals categorized by proportion of body fat.
In a more detailed analysis, we detected increased concentrations of CD9 and CD81 in plasma exosomes isolated by SEC and the commercial kit compared to DC. However, we observed an increase in background noise in WB with SEC.
Conclusion: We emphasize the importance of selecting the appropriate methodology for exosome isolation, which depends on the specific research objectives. When prioritizing exosome quantity and content, we suggest employing SEC or a commercial kit for plasma samples when seeking higher yields of intact exosomes. However, if purity is the priority, DC is the best method. In this study, exosomes were not obtained from the supernatant of 3T3-L1 cells. Therefore, our findings highlight the use of FBS depleted of microvesicles in 3T3-L1 cell culture. We recommend increasing the supernatant volume and/or the ultracentrifugation process or, alternatively, exploring a commercial exosome isolation kit designed specifically for cell culture samples.

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1585 – P2.09.34

Activated platelets rapidly induce pro-inflammatory phenotype and function in monocytes

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Background: Increased numbers of platelet-monocyte complexes have been implicated in a range of thrombo-inflammatory diseases such as myocardial infarction, type 2 diabetes, and Covid-19, with higher numbers relating to worsened clinical outcomes. Several studies suggest that platelets may alter a monocyte's inflammatory function. However, characterisation of these phenotypic changes and their functional implications following thrombotic stimuli is yet to be fully elucidated.
Aim: To investigate platelet-monocyte complex (PMC) formation and the resulting changes in phenotype and function.
Methods: Whole blood, or isolated monocytes/peripheral blood mononuclear cells (PBMCs), were incubated with washed platelets (1:100) and collagen related peptide (CRP-A; 1µg/ml) for up to 1 hour at 37ºC to form PMCs. Cell surface immunophenotyping (22 colour, 18 parameter spectral flow cytometry panel), phagocytosis ability (using pHrodo bioparticles) and ROS generation (using CellRox dye) of the complexes were all assessed by flow cytometry and compared to unbound monocytes. Data were analysed using FlowJo v.10 and Graphpad Prism.
Results: Platelet GPVI-specific stimulation leads to a rapid increase in platelet-monocyte complex (PMC) formation, observed both in whole blood and isolated cells. These PMCs display significantly elevated surface expression of CD16, CCR2, and CXCR4 ($p<0.05$) compared to unbound monocytes, indicative of a pro-inflammatory phenotype. They also demonstrate significantly elevated ROS generation ($p<0.05$) and a heightened phagocytic capability ($p<0.05$) in comparison to unbound monocytes, possibly facilitated by the increased surface expression of CD36 ($p<0.05$), which is known to play a role in phagocytosis. Additionally, PMCs exhibit an upregulation of CD142 (tissue factor), suggesting their potential involvement in thrombosis.
Conclusion: Our findings collectively indicate that platelet GPVI-specific stimulation by CRP triggers the formation of PMCs which rapidly (< 1 hour) transition to a heightened pro-inflammatory and pro-coagulative state compared to unbound monocytes. Additional work is required to explore these changes in order to further characterise platelet-monocyte complexes and their contribution to thrombo-inflammation.

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European Journal of Immunology Clinical-Basic-Translational

1654 – P2.09.35

Helminth infection modulates the immune response to SARS-CoV-2 vaccination

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Purpose: *Schistosoma mansoni* is a parasitic flatworm that infects almost 250 million people worldwide. *Schistosoma* and other helminths modulate the host immune response, with significant effects on allergy, atopy and vaccine responses. The SARS-CoV-2 pandemic resulted in the advent of a number vaccine strategies, including the first licensed mRNA vaccines. The efficacy of these vaccinations in African populations, particularly amongst people with existing parasitic infections has been debated. This study aims to resolve the question of the immunogenicity and efficacy of SARS-CoV-2 mRNA vaccination in helminth-infected hosts, through the use of a mouse model.
Methods: C57BL/6 mice were infected with *S. mansoni* and vaccinated 8 weeks later with Comirnaty (5µg and 1µg), followed by a boost immunization 4 weeks later. 1 week after boost immunization, the cellular and humoral immune responses were assessed by flow cytometry of splenocytes and lung cells, ELISA of re-stimulated splenocytes and measurement of spike-specific IgG responses and SARS-CoV-2 virus-neutralizing activity in sera.
Results: Between naïve and *Schistosoma*-infected mice there were minimal differences in humoral responses with similar antibody levels and SARS-CoV-2 virus neutralization capacities. The main finding was Th2 (IgG_1)-shifted humoral response, driven by the helminth infection. Whereas CD4+ T cell responses were similar between the groups, Spike-specific poly-functional CD8+ T cells were much fewer in helminth-infected mice. This CD8+ T cell suppression was accompanied by significant differences in the lung, where helminth infection shifted dendritic cell populations towards a cDC2 phenotype and a prominent shift in lung macrophage populations was also observed. Analysis is currently underway to identify the drivers and connections between the observed phenotypes.
Conclusion: Helminth infection –has a well-documented effect on host immune responses including in vaccination. Here we have assessed for the first time the effect of *Schistosoma mansoni* infection on the response to both SARS-CoV-2 and mRNA vaccination, finding a distinctly blunted induction of CD8+ T cell responses and extensive differences in antigen presenting cell populations. These findings are important to take into account for the development of vaccinations in regions where helminth infections are endemic.

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1665 – P2.09.36

Exploring the human immune response through tonsil-derived organoids

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Purpose: The *in vitro* study of the human germinal centre (GC) response has been challenged by the reproduction of sequential cellular interactions in different microanatomic niches, whose complexity surpasses B and T cell co-cultures. In this context, tonsil-derived organoids emerge as a physiologically relevant platform for dissecting these immune interactions. Despite being usually discarded following children's tonsillectomies, tonsils contain well-defined GC structures as secondary lymphoid organs, rendering them a valuable and readily accessible biological material. This project aims to establish tonsil organoids for studying cellular populations, their interactions and maturation/differentiation pathways while opening new avenues for further modelling the GC response with physiological significance.
Methods: To generate the organoids, biopsied palatine tonsils are dissociated down to a single cell suspension. Isolated cells are cultured at high-density onto a transwell, which promotes reaggregation while providing nutrients through the media in the outer chamber. The stimulatory antigen (e.g., bacterial toxin or vaccine) is directly loaded in the inner chamber along with the cells at the culture outset. Replicated cultures per donor are evaluated via flow cytometry and ELISA at different time points to assess cell proliferation and maturation/differentiation, as well as antibody and cytokine production.
Results: Cellular reaggregation occurs around day 2 in culture, resulting in macroscopically visible aggregates. Cytometry analysis at day 7 confirms that tonsillar organoids can sustain lymphocyte survival, proliferation and differentiation, recapitulating the cellular composition of germinal centres. Prior to culture, B cells are in higher proportion compared to T cells, whose ratio shifts following a week in culture. Nevertheless, B cells exhibit a more mature phenotype with expression of GC markers (i.e., CD27 and CD38) after the culture period, while T follicular helper cells have become activated (PD-1^{hi}). Cryopreserved tonsillar cells were shown to perform similarly to fresh cells in culture regarding viability and expression of GC markers.
Conclusion: This organotypic system recapitulates *in vivo* GC features of the adaptive immune response, holding potential for studying antigen specific responses and discriminating physiological from pathological conditions. Ultimately, this system enables the modelling of immune responses through polarization or inhibition of specific pathways.

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1744 – P2.09.37

Deciphering the pathological involvement of circulating immune complexes during viral infections

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In normal immune response, circulating immune complexes (CIC) consist of antibody bound to antigen participating in antigen clearance and infection resolution. In some viral infections, CIC contribute to the pathophysiology of disease by direct inflammatory mechanisms or by facilitating viral replication. The regulatory impact of CIC is typically ascribed to Fc fragments and their receptors, which are broadly distributed on immune cells. Mononuclear phagocytic cells (MPC) such as monocytes, macrophages and dendritic cells are especially involved in immune response triggered by CIC. Multiple factors may influence impact of CIC in diseases: size and composition of CIC themselves, the type of immune cells with which they will interact and their pre-activation state. However, these mechanisms remain poorly understood. In our study, we have used innovative methods and standardized protocols for qualitative, quantitative, and functional characterization of CIC.
We have developed experimental *in vitro* models to measure activation capacities of CIC on peripheral blood mononuclear cell (PBMC) or MPC. *In vitro* prepared CIC composed of adenovirus-derived-vectors and immunoglobulins trigger cell activation of PBMC and MPC, measured by expression of cells surface activation markers and cytokine secretion. Moreover, CIC size was determined after purification by PEG6000 and using Dynamic Light Scattering (DLS). Human plasma or *in vitro* generated CIC were tested in co-culture with monocytes and with or without other cells using transwell system.
When stimulated by CIC, PBMC exhibited an activation profile, characterized by CD38, CD169 overexpression. Using DLS, we were able to identify distinct peaks linked to CIC size. Datas collected in our experimental *in vitro* model suggest that activation of monocytes cells by CIC is an indirect process, other cells or cytokines are needed. Also, macrophages need pre-stimulation for stimulation by CIC.
In this study, we have developed a new model to characterized CIC and study their capacity to trigger monocyte activation *in vitro*. We are setting up a standardized procedure to better understand and predict how the structure and environment of CIC affect their immunomodulatory effect through interaction with MPCs. In addition, we provide new incite of MPC activation mechanism induced by CIC.

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1905 – P2.09.38

Unraveling glycan recognition: a cellular approach to studying lectin receptor-ligand interactions

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Introduction: Glycosylation, the enzymatic process attaching carbohydrates (glycans) to proteins and lipids, is crucial for cellular functions and signaling. Cancer cells exhibit altered glycosylation patterns compared to their normal counterparts, that directly contribute to hallmarks of cancer such as immune evasion and metastasis. These glycan structure changes contain biological information that glycan-specific receptors, or lectins, found in a wide range of cells, including immune cells, can decode. The interaction between altered glycans and lectin receptors may predict patient outcomes, underscoring the need to identify specific ligands. Lectin receptors are primarily classified as Type I and II single-pass transmembrane proteins, differing in orientation: Type I has a cytoplasmic C-terminus and an extracellular/luminal N-terminus, while Type II has the reverse orientation.
In this project we aim to develop cellular tools to identify lectin receptor ligands and study their triggering potential.
Methods: Utilizing Jurkat cells, we created a flexible pipeline to generate reporter cell lines for various lectin receptors. We designed constructs allowing for easy replacement of the receptors intracellular domain with activating motifs (ITAMs). Luciferase, that is regulated by the transcription factor NFAT, was used as a read out to measure activation.
Results: We produced reporter cell lines for myeloid receptors DC-SIGN and Siglec-7, examples of Type I and Type II membrane receptors. These were characterized using specific antibodies and glycan ligands. Both lines recognized and were activated by previously reported glycan structures, and by using specific antibodies. They could also distinguish subtle differences in glycan structures and were used to identify essential amino acids for their function. Co-culture with tumor cell lines demonstrated their ability to detect receptor ligands.
Conclusion: Our reporter cell lines for lectin receptors are flexible tools for identifying ligands and assessing their ability to induce signaling, offering valuable insights into receptor-ligand interactions and potential therapeutic targets.

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1996 – P2.09.39

NK Cell Regulation of Humoral Immunity in Therapeutic Vaccination

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Purpose: Therapeutic vaccination attempts to boost antigen-specific immune responses but effectiveness is hampered in chronic viral infections and cancer by dysfunctional T and B cell responses. A growing body of literature suggests NK cells are critical regulators of B cell mediated immunity via effects on CD4 help in the setting of murine and human infections. In this study we are therefore examining whether manipulating NK cell regulation can enhance the humoral response to therapeutic vaccination in chronic HBV infection.
Methods: To achieve this, we utilised a mouse model of persistent low-level HBV infection (*Huang et al, Gastro, 2012*) and treated animals with clinically relevant therapeutic vaccination regimens with or without NK cell depletion. We also cocultured human differentiated follicular helper T cells (Tfh) (*Locci et al, Nat Immunol 2016*) and isolated NK cells derived from chronically infected HBV patient PBMCs prior to analysing Tfh functional capacity.
Results: Using PBMC from healthy controls and patients with chronic HBV, we found that culturing CD4 T cells with previously published lineage-inducing cytokines (Activin-A and IL12) promoted canonical Tfh marker and effector expression to overcome limitations of low circulating Tfh. Purified NK cells reduced the frequencies of these *in vitro* expanded Tfh when cocultured *in vitro*. We probed the *in vivo* impact of depleting NK cells prior to therapeutic vaccination and found this significantly enhanced the expansion of splenic Tfh as well as germinal centre (GC) B cells, allowing further studies to determine relevant pathways.
Conclusion: We have initial evidence that NK cells act to inhibit the humoral response to vaccination in a mouse model of chronic HBV infection and in a human PBMC coculture system with key mediators of the response, Tfh cells. Further work will investigate whether NK cells limit the capacity of Tfh to give adequate help to HBsAg-specific B cells and how this affects anti-HBV nAb production using PBMC and lymph node samples from donors with chronic HBV infection. By examining the receptor ligand interactions that underpin these inhibitory NK pathways we will identify specific targets that could be blocked during a therapeutic vaccine regimen to improve patient responses.

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2012 – P2.09.40

TLR7/8 agonism reshapes hepatic immunity and improves the response to bacterial infection in liver fibrosis

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Purpose: Cirrhosis, a major healthcare burden, is characterized by liver fibrosis, immune dysregulation and impaired antimicrobial responses that contribute to increased infection risk and mortality in patients. Recent evidence has highlighted the therapeutic potential of Toll-Like-Receptor (TLR) 7/8-dual agonism (R848) in inflammatory pathologies including cancer. We therefore assessed in this study its immunomodulatory effects in a murine model of carbon tetrachloride (CCl_4)-induced fibrosis.
Methods: C57BL/6J 8-week-old male/female mice (n=6-8/group) received intraperitoneal (i.p.) injections of vehicle (oil) or CCl_4 (0,4 µl/g) twice-weekly (6 weeks) to establish fibrosis; during the 6th week, mice were treated i.p. with saline (PBS) or TLR7/8-L (R848; 1mg/kg). Liver non-parenchymal cells were assessed by flow cytometry (myeloid/lymphoid cells). In separate experiments, Escherichia coli (5 x 10^7 /20g body weight) was administered intravenously 24h before mice (n=8-11/group) were culled. Liver and spleen tissue lysates were plated on agar for colony-forming unit (CFU) quantification.
Results: Flow cytometry analyses showed that the number (cells /tissue gram) of VSIG$^-$ monocyte-derived macrophages expanded in PBS-fibrotic mice (Ctrl:33.063; PBS:123.975, p<0.0001) while TLR7/8-L administration reduced that number by ~35% (PBS:123.975; TLR7/8-L:79.398, p=0,0121). Conversely, increased dendritic cell (DC) numbers were observed in livers of TLR7/8-L-treated fibrotic-mice, compared to PBS-fibrosis (cDC1; PBS:11.193 vs. TLR7/8-L:42.184, p=0,0003; cDC2; 35.001 vs. 68.017, p=0,0225). No significant changes were observed in VSIG4$^+$ Kupffer cell numbers. TLR7/8 agonism further enhanced the numbers of CD4$^+$CD25$^-$FoxP3$^+$ T_{regs} in fibrotic mice (PBS:14.582 vs. TLR7/8-L:30.977, p=0,0005). Increased bacterial load (CFU/tissue gram) was detected in the liver and spleen of PBS-fibrotic mice, compared to vehicle-treated controls (Liver; Ctrl:145.245 vs. CCl_4:385.203, p=0,0026; Spleen; Ctrl:23.867 vs. CCl_4:66.212, p=0,0002). Notably, TLR7/8-dual agonism reduced CFUs in both tissues (Liver: PBS:385.203 vs. TLR7/8-L:217.944, p=0,0547; Spleen: PBS:66.212 vs. TLR7/8-L:24.873, p=0,0007).
Conclusion: Our findings demonstrate that, in the mouse fibrotic liver, TLR7/8 dual agonism mobilizes a reshaping of the intrahepatic immune compartment resulting in improved bacterial clearance after *E. coli* infection. Future studies are needed to better understand the immune intercellular crosstalk and underlying mechanisms of this TLR7/8-mediated immunomodulatory approach in liver fibrosis, aiming to ultimately translate TLR-targeted therapies to improve antimicrobial responses in patients with liver disease.

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2052 – P2.09.41

Induction of immunoregulatory responses at the injection site of vaccine adjuvant alum.

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A key consideration in the development of vaccines is to generate formulations that are both effective and safe, this relies on a fine balance between immunostimulatory capacity and tolerability of vaccine components. The vaccine adjuvant Alum, one of the most widely used adjuvants worldwide, has a well-established track record in both of these regards. Alum excels as an adjuvant capable of promoting protective antibody responses while also maintaining a robust safety record. Alum is not an optimal adjuvant for promoting cell mediated immune responses (CMI), particularly CD8 and Th1 cells, making it unsuitable for vaccines against pathogens which require CMI for protection. Despite being utilised in many licenced vaccines over the past century, aiding in protection against many infectious diseases including Human Papilloma Virus (HPV), Hepatitis A, Hepatitis B, Polio and Diphtheria, the specific mechanisms underlying the immune stimulating properties, its robust safety record and the effects of biological sex on responses to alum have not been fully resolved.
Our hypothesis is that a combination of immunoregulatory processes rapidly initiated at the site of alum injection, including the accumulation of regulatory T cells, a switch from classically inflammatory monocytes to a more anti-inflammatory phenotype, the production of anti-inflammatory cytokines such as IL-10 and the inhibition of Th1 cell polarising cytokines such as IL-12 underlie alums robust safety while also limiting the initiation of CMI. We present data supporting this hypothesis and the role of biological sex in dictating the extent of these responses.

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2249 – P2.09.42

Investigating the salivary gland tissue microenvironment in Sjögren's disease using Imaging Mass Cytometry™

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Purpose: Sjögren's syndrome (SS) is a complex autoimmune disease with destruction of glandular tissue as one of the major disease hallmarks. Novel Imaging Mass Cytometry™ (IMC™) can bring the investigation of the glandular tissue microenvironment into new dimensions by targeting multiple immune cell markers within the tissue simultaneously.
Objective: To study the immune cell subsets that are present in the microenvironment of minor salivary gland biopsies in a spatial dimension using Imaging Mass Cytometry.
Methods: Minor salivary gland biopsies from primary Sjögren's syndrome (pSS) patients and non- Sjögren's syndrome (non-SS) sicca controls were used. The patient cohort fulfilled the AECG/ACR-EULAR criteria for Sjögren's syndrome. To enable analysis of multiple patients and controls simultaneously, we created a tissue micro array (TMA) with cores taken from each individual biopsy and placed together in the same paraffine block. A tonsil was included for positive control. Region of interest was selected based on the dominating pathological structure of the gland. A section from the TMA was stained with 33 metal tagged antibodies, 3 segmentation markers and Iridium nucleic acid intercalator. The tissue was ablated with a laser beam in the Hyperion™ machine and data generated was first denoised to reduce hot pixels and artefacts, and metal spillover was compensated using Rstudio software. Further IMC downstream analysis were performed in Rstudio.
Results: The downstream analysis presented diversity in the detected immune cell subsets in the tissue among the patient and control participants. The follicular dendritic cells were detected in pSS patients with a more severe disease pattern and localized in close proximity to B cells in the larger focal infiltrates. Plasma cells were scattered interstitially and in the periphery of the infiltrates in the pSS cohort but was also detected in the non-SS control group. Further analyses are ongoing.
Conclusion: The analyzed data showed a difference between patients with a more complex and severe pSS disease compared to the patients presenting milder disease patterns.

Sources of contributed support: The IMC analysis was performed at the Flow Cytometry Core Facility, Department of Clinical Science, University of Bergen, Norway.

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2283 – P2.09.43

Transcriptomic Analysis Reveals Distinct Effect of Cigarette Smoke on Lung and Systemic Macrophage Populations

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Chronic obstructive pulmonary disease (COPD) is an inflammatory airway disease characterized by emphysema and chronic bronchitis and a leading cause of mortality worldwide. COPD is commonly associated with several comorbid diseases which contribute to worse patient outcomes. Cigarette smoke (CS) is the most prominent risk factor for COPD development and progression and is detrimental to effector functions of lung-resident immune cells, including phagocytosis and cytokine production. However, how CS mediates the various pathologies distant from the lung in COPD, and the biological effect of CS on systemic immune cells, is unknown.
Bone marrow cells were isolated from C57BL/6 mice exposed to CS for 8 weeks as an experimental COPD model and differentiated to bone marrow-derived macrophages (BMDMs) alongside room air (RA) controls. Alveolar macrophages (AMs) were isolated from the same CS-exposed and RA mice and bulk RNA-seq preformed. Bulk RNA-seq was also performed on BMDMs from CS and RA mice with and without LPS treatment. Gene ontology (GO) and Ingenuity Pathway Analysis (IPA)were used to perform pathway analyses and predict upstream regulators associated with the observed differentially expressed protein-coding genes induced by CS in each cell population. CS induced differential expression of genes involved in pathogen response, phagosome formation, and immune cell trafficking in AMs from CS-exposed mice compared to RA mice. Distinctly, CS induced alternative transcriptomic remodelling in BMDMs, associating with an upregulation of genes in the sirtuin signalling and oxidative phosphorylation pathways and with a downregulation of genes involved in histone and lysine methylation. Little overlap is observed in both differentially expressed protein-coding genes in BMDMs compared to AMs and their associated pathways, highlighting the distinct effects of CS on immune cells in different compartments. Additionally, BMDMs isolated from CS-exposed mice display a distinct transcriptomic signature upon stimulation with LPS compared to those from RA mice, with LPS treatment upregulating genes associated with RNA processing and splicing in BMDMs from CS mice. Our findings suggest that the systemic inflammation and symptom burden associated with COPD may arise from CS-affected bone marrow derived monocytes, which may in turn drive pathogenesis of diseases frequently comorbid with COPD.

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P2.10 IMMUNE RESPONSE REGULATION: MOLECULAR MECHANISMS

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93 – P2.10.01

ARID5B-mediated LINC01128 epigenetically activated pyroptosis and apoptosis by promoting the formation of the BTF3/STAT3 complex in antiphospholipid syndrome

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Purpose: Alterations of the H3K4me3 mark in monocytes are implicated in the development of autoimmune diseases. Therefore, the purpose of our study was to elucidate the role of H3K4me3-mediated epigenetics in the pathogenesis of antiphospholipid syndrome (APS).
Methods: H3K4me3 Cleavage Under Targets and Tagmentation and Assay for Transposase-Accessible Chromatin were performed to determine the epigenetic profiles. Luciferase reporter assay, RNA immunoprecipitation, RNA pull-down, co-immunoprecipitation, and ChIP were performed for mechanistic studies. Transmission electron microscopy and propidium iodide staining confirmed cell pyroptosis. Primary monocytes from patients with primary APS (PAPS) and healthy donors were utilized to test the levels of key molecules. A mouse model mimicked APS was constructed with β2GPI injection. Blood velocity was detected using murine Doppler ultrasound.
Results: H3K4me3 signal and open chromatin at the *ARID5B* promoter were increased in an *in vitro* model of APS. The epigenetic factor ARID5B directly activated LINC01128 transcription at its promoter. LINC01128 promoted the formation of the BTF3/STAT3 complex to enhance STAT3 phosphorylation. Activated STAT3 interacted with the *NLRP3* promoter and subsequently stimulated pyroptosis and apoptosis. ARID5B or BTF3 depletion compensated for LINC01128-induced pyroptosis and apoptosis by inhibiting STAT3 phosphorylation. In mice with APS, β2GPI exposure elevated the levels of key proteins of pyroptosis and apoptosis pathways in bone marrow-derived monocytes, reduced the blood velocity of the ascending aorta, increased the thrombus size of the carotid artery, and the release of interleukin (IL)-18, and IL-1β, tissue factor (TF). Patients with PAPS had the high-expressed ARID5B and LINC01128, especially those with triple positivity for aPLs, and there was a positive correlation between ARID5B and LINC01128 expression. Moreover, triple-positive patients with PAPS had the significantly lower C3, C4, and PLT levels compared to non-triple-positive patients.
Conclusion: This study indicated that ARID5B/LINC01128 was synergistically upregulated in APS, and they aggravated disease pathogenesis by enhancing the formation of the BTF3/STAT3 complex and boosting p-STAT3-mediated pyroptosis and apoptosis, thereby providing candidate therapeutic targets for APS.

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173 – P2.10.02

Neutrophil extracellular trap-associated glycosidases shape the immune response

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Purpose: Formation of neutrophil extracellular traps (NETs) is an anti-microbial activity of neutrophils and this is a cell destructive process. The NET structures are formed from nuclear and cytoplasmic components that can capture and kill pathogens. Here, we investigated NETs for the expression of functionally active glycosidases.
Methods: The pertinence of glycosylation on functional activities of immunoglobulins are well established. Thus, we assessed the effect of NETs on the functional activity of immunoglobulin G (IgG). NETs generated from healthy donors and pooled human intravenous Immunoglobulins (IVIg) were utilized to assess cytokine levels produced by cells stimulated with IgG-coated beads. Thereafter, the total IgG and IgG subclasses glycosylation patterns were assessed using MALDI-TOF and LC-ESI MS, respectively.
Results: NETs contain the glycan-modifying enzymes neuraminidase, β−galactosidase and hexosaminidase which are either bound to NETs or released during NET formation. Incubation of IVIg with NETs modulates their bisection and sialylation composition as well as their cytokine induction.
Conclusion: These findings show that the immunomodulatory effect of NETs has the potential to upregulate inflammation. This can have beneficial but also undesirable consequences. Targeting the glycosidases on NETs could be used for immune intervention.

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187 – P2.10.03

Impairment of alternative NLRP3 inflammasome pathway in peripheral blood monocytes from inflammatory bowel disease patients

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Introduction: Inflammatory bowel disease (IBD), comprising Crohn's disease (CD) and ulcerative colitis (UC), is characterized by chronic and relapsing inflammation in the gastrointestinal tract, and some hypotheses propose that damage to the intestinal mucosa occurs as a result of dysregulated innate immune response. NLRP3 inflammasome is rapidly emerging as a crucial regulator of intestinal homeostasis. This receptor mediates the assembly of the inflammasome complex in the presence of microbial ligands, triggering activation of caspase-1 and secretion of IL-1β and IL-18, and has been implicated in the pathogenesis of IBD, but the detailed role of NLRP3 inflammasome in IBD is still debated. Actually, the concept of detrimental inflammasome signaling in IBD is being evaluated due to recent reports that IL-1β and IL-18 production can confer protection against colitis. In the present work, we found a defective NLRP3 inflammasome activation by peripheral blood monocytes from IBD patients that supports the asociation of detrimental inflammasome signaling with the development of IDB.
Methods: Human PBMCs obtained from 32 healthy donors and 16 newly diagnosed IBD patients (including CD and UC) were stained with anti-CD3, CD56, CD19, HLA-DR, CD14 and CD16 fluorescent monoclonal antibodies to isolate CD14++CD16- monocytes by fluorescent cell sorting. After isolation, $1x10^5$ monocytes were cultured in RPMI media 10% FBS during 18 hours in the presence of lipopolysaccharide to activate NLRP3 inflammasome by alternative pathway and in the presence or absence of MCC950 a known NLRP3 antagonist. Cell free supernatants were collected to quantify several cytokines by multiplex immunoassays (ThermoFisher Scientific) for Luminex platform.
Results: We found significantly decreased levels of inflammasome dependent cytokines IL-1β, IL-18 and IL-1α in the supernatants of IBD patients monocytes compared to healthy controls after LPS treatment to activate NLRP3 inflammasome. However, the release of other inflammatory cytokines not dependent of inflammasome activation (IL-6, IL-8, TNF-α) was similar comparing IBD patients and healthy controls.
Conclusions: In the present work we found an impaired NLRP3 alternative pathway activation in peripheral blood monocytes from IBD patients compared to healthy controls. This result suggest that defective NLRP3 inflammasome activation could be asociated with the development of inflammatory bowel disease.

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189 – P2.10.04

The histone variant H2A.J shapes immune responses

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Purpose: Classical histones at promoter and enhancer regions can be replaced by histone variants, which consequently change the accessability of gene loci and therefore the overall gene expression. Multiple reports already demonstrated that histone variants control important cellular pathways like DNA repair and replication, or differentiation, however the role in immune cell responses remain largely unexplored. Previously it was found that the histone variant H2A.J, encoded by *H2AFJ*, controls inflammatory gene expression in senescent fibroblasts, however its function in immune cells and its impact on skin pathologies has not been investigated yet. In this project, we investigated the role of H2A.J in immune cell responses, with special focus on psoriasis.
Methods: *H2AFJ* mRNA and H2A.J protein level were assessed in skin biopsies from human psoriasis patients, compared to normal skin and non-lesional controls. For investigating the role of H2A.J in psoriasis *in vivo*, effects of imiquimod-induced, psoriasis-like skin inflammation was analysed by flow cytometry, gene expression analysis and ELISA from wildtype and *H2afj* knockout mice. This data was complemented by investigation of its function in T-cells and keratinocytes. For this purpose, primary cells were isolated from global *H2afj* knockout mice, activated and analyzed for changes in global gene expression.
Results: H2A.J expression is elevated in skin lesions of psoriasis patients. Moreover global deletion of H2A.J partially inhibits imiquimod-induced psoriasis-like skin inflammation, by inhibiting neutrophil and monocyte recruitment. This was likely due to the fact that H2A.J controls inflammatory gene responses in keratinocytes and $CD4^+$ T-cells.
Conclusion: With this work we discovered a new close connection between the expression of a histone variant and the immune response to the autoimmune disease psoriasis. Potentially H2A.J can be used as a biomarker for the chance to develop psoriasis. Next steps will be to unravel how H2A.J regulates the gene expression in immune cells and keratinocytes and if there is also a connection between H2A.J and other inflammatoy diseases.

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191 – P2.10.05

The immunomodulatory functions of long noncoding RNA DANCR in melanoma

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Cancer immunotherapy has emerged as a promising strategy for treating various malignancies, including metastatic melanoma. However, its efficacy remains variable among patients, necessitating a deeper understanding of the underlying mechanisms of tumor immune evasion. Long noncoding RNAs (lncRNAs) have recently garnered attention for their roles in gene regulation and disease pathogenesis. Among these, lncRNA DANCR (Differentiation Antagonizing Non-Protein Coding RNA) has emerged as a potential regulator of tumor progression and therapy resistance in multiple cancer types. In this study, we further investigated the roles of DANCR in melanoma immune evasion and tumor progression. Using TCGA skin cutaneous melanoma (SKCM) RNAseq data, we examined survival associations of lncRNA expression and identified DANCR as a prominent candidate (expression was categorized at the median value, n=230/grp). DANCR was found to be a poor prognosticator in SKCM, with its expression significantly associated with unfavorable outcomes. Furthermore, the analysis of previously published scRNAseq data revealed that DANCR was predominantly expressed in melanoma cells within the tumor microenvironment (TME), inversely correlating with the IFNγ response gene signature in immune-rich SKCM. In PTEN-high SKCM, low DANCR expression was associated with a positive prognosis (n > 112/group). In the literature, DANCR inhibited keratinocytes with differential expression of genes involved in PI3K signal transduction, EMT, and immunomodulation. Reduced DANCR levels decreased PD-L1 expression in nasopharyngeal cancer cells, indicating its potential as an immune checkpoint blockade therapy target. Notably, melanoma tumors responding to anti-PD-1 therapy exhibited decreased DANCR levels. Consistent with the literature, our receiver operating characteristic (ROC) analysis showed that DANCR predicted poor response to immune checkpoint blockade (ICB) in melanoma, highlighting its clinical benefit as a biomarker for treatment stratification. To evaluate these findings mechanistically and manipulate DANCR expression in melanoma cell lines we are leveraging CRISPR-dCas9 activation and inhibition system. The research, supported by TUBA-GEBIP, focuses on understudied regulatory pathways in tumor immunology and can reveal how melanoma progresses and achieves immunoevasion. Elucidating the role of DANCR in tumor immunoevasion could lead to the development of novel prognostic tools and therapy targets in melanoma and potentially other cancers.

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195 – P2.10.06

Phosphatases of regenerating liver balance F-actin rearrangements during T cell activation through WD repeat containing protein 1

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Purpose: Phosphatases of regenerating liver (PRLs) have been proposed to regulate actin dynamics in lymphoid cells. However, the mechanism mediating this regulatory role during T cell activation, IS assembly and effector function remained unknown.
Methods: Interaction among proteins was confirmed by both SILAC proteomics and immunoprecipitation analysis. Immunological Synapse (IS) formation was evaluated by high resolution confocal microscopy and early TCR signalling was studied by western blot. Secretion capacity was assessed by IL-2 detection by ELISA and degranulation assays by FACS. Edition of PRL-1 or PRL-2 expression was performed by CRISPR/Cas9 in Jurkat (JK) and primary T cells.
Results: We showed the interaction of the PRLs with the actin regulator WD repeat containing protein 1 (WDR1). This association was dependent on filamentous (F)-actin integrity and the proper recruitment of the PRLs to cell membranes through the CAAX motif. Endogenous PRLs and WDR1 were distributed to the IS, and knockout (KO) JK cells and edited primary T cells for PRL-1 or PRL-2 showed that the PRLs were required for proper distribution of WDR1 to F-actin at the IS. Further, perturbed expression of the PRLs or WDR1 resulted in defective IS assembly with aberrant F-actin rearrangements, altered positioning of LFA-1 and deregulated IL-2 production. Interestingly, balanced expression of PRLs was required for accumulation of CD3ε at the IS and early activating signalling.
Conclusion: We contribute experimental evidences indicating that PRLs regulate early T cell signalling and are required for proper F-actin dynamics by regulating the WDR1 access to F-actin networks. As a consequence, PRLs regulate LFA-1 positioning at the IS and proper cytokine secretion.

Sources
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222 – P2.10.07

End-Binding Protein-1 regulates the metabolic fate of CD4 T lymphocytes through the organisation of the mitochondrial network

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Purpose: This work focuses on the role of End-Binding Protein-1 (EB1), a protein that regulates tubulin polymerisation and dynamics and has previously been identified as a regulator of CD3 vesicle trafficking to the immune synapse. However, the regulatory role of EB1 in T cell metabolism after T-cell receptor activation is unknown.
Methods: EB1-silenced Jurkat T-cells, either transiently or stable clones and $CD4^+$ T lymphocytes were used to analyse the distribution of mitochondria and the cytoskeleton in the immune synapse by confocal microscopy and the mTOR signalling pathway, which regulates cellular processes such as cell survival and metabolism, was studied by Western blotting. Metabolic assays were also performed to investigate the mitochondrial respiration and glycolytic capacity, and the role of EB1 in cell death induced by activation through the Fas/FasL system was investigated by flow cytometry.
Results: EB1-silenced cells exhibit impaired polarisation of mitochondria and the actin and tubulin cytoskeleton towards the synapse site, as well as a defective metabolic responsiveness in activated T cells, suggesting a relevant link between the cytoskeleton and metabolism in response to TCR stimulation.
Conclusion: By linking the tubulin cytoskeleton and mitochondria during $CD4^+$ T cell activation, this work highlights the importance of EB1 in this context to regulate cell asymmetry, apoptosis and metabolic functions such as glycolysis and mitochondrial respiration.

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322 – P2.10.08

Exploring the role of calcium ion channels during mycobacterial infection

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Tuberculosis (TB) is considered a significant health concern, ranking among the top ten leading causes of adult mortality globally, highlighting the urgent need for continued attention and action to address this life-threatening disease. *Mycobacterium tuberculosis* **(***Mtb***)** is a highly competent pathogenic bacteria that proliferates within host cells as a prerequisite for progression to active TB by escaping the immune system of its victim through several approaches. Recently modulation of calcium ion (Ca^{2+}) channels has surfaced as an emerging immune invading mechanism for *Mtb*. Ca^{2+} channels belong to the vanilloid subfamily among the TRP superfamily. These ion channels are predominately expressed in immune cells, where they are co-localized within the endosomal-lysosomal continuum. Out of various ion channels, Ca^{2+} specific ones are involved in the regulation of cellular and lysosomal Ca^{2+} homeostasis affecting lysosome trafficking and autophagic flux. Despite the role of ion channel-mediated Ca^{2+} in lysosomal biogenesis and trafficking with putative relevance for host immune responses. Nevertheless, how Ca^{2+} channels are regulated in immune cells during mycobacterial infection remains poorly understood. We found that Ca^{2+} channel expression is modulated upon mycobacterial (*M. bovis* BCG) infection in murine macrophages and loss of function experiments resulted in dysfunctional lysosomes, lysosomal storage phenotype, and interference with phagosome maturation. Employing Ca^{2+} channel modulators to explore their role in mycobacterial infection of the endo-lysosomal continuum revealed upregulation of EEA1 and Rab5 but downregulation of Rab7. Ca^{2+} channel agonist followed by mycobacterial infection increased the number of acidic vesicles by promoting endo-lysosome fusion and consequently, reduced intracellular mycobacterial burden. In contrast, Ca^{2+} channel antagonist reduced acidic vesicle formation. Thus, our findings demonstrate that the Ca^{2+} channel plays a critical role in host cell responses against intracellular mycobacteria but can be modulated by mycobacteria to alter cellular Ca^{2+} homeostasis and putatively, phagolysosome formation to promote mycobacterial infection.

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336 – P2.10.09

Interplay of cytoskeleton and membrane morphology during formation of immunological synapse

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T-cells are key players of adaptive immunity, however is not fully understood how membrane distribution of critical molecules and membrane architecture itself influence their responses to antigens. Since contact between T-cells and APCs was found to involve microvilli, we propose that changes in surface morphology control the early events of T-cell activation. Specifically, we aim to understand how these events are driven by cytoskeleton.
Using advanced microscopy, we visualized formation of immunological synapse (IS) *in vitro* and observed robust accumulation of plasma membrane at the contact site between T-cells and APC. Cryo-TEM microscopy further revealed previously uncharacterized ultrastructural details of IS, which is actually formed by a surprisingly complex meshwork of membrane protrusions intertwined within the cavity of the interacting cells. This observation supports our assumption that increased membrane surface area within forming IS, including signalling proteins, appropriate geometry of microvilli and cytoskeleton, provide the optimal microenvironment for initial recognition of cognate antigen during T-cell:APC interaction.
Since we previously studied the critical role of spatio-temporal organization and dynamics of Lck and other signalling molecules in proximal TCR signalling, we have begun to focus on identifying their potential linkers to cytoskeletal network. We discovered α-actinin-1 and 4 as cytoskeletal components of Lck-RACK1 complexes, which are transiently formed during the initiation of T-cell activation and depend on Lck kinase activity. 3D-high resolution live-cell imaging revealed that in α-actinin-1/4 dKO T-cells, Lck was spatially arrested at IS periphery early after the start of synapse formation in contrast to Lck even distribution in WT controls. As expected, such disorganization resulted in reduced responsiveness of T-cells, indicating that α-actinins contribute to the stability of the forming IS.
Taken together, our data adds new pieces of the puzzle showing the importance and complexity of the interplay of cytoskeleton, membrane morphology and signalling molecules during IS formation.

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348 – P2.10.10

Analysis of TAM receptor expression in patients with Sjögren's disease

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Purpose: Receptor tyrosine kinases Tyro3, Axl, Mer (TAM) and their ligand Gas6 are involved in the phagocytosis of apoptotic cells, and defects in their signaling have been linked to various autoimmune diseases. Their association with Sjögren's disease (SjD) has also been investigated but their exact involvement remains elusive. We here analyzed expression levels of Tyro3, Axl, Mer and their ligand Gas6 in patients with SjD to explore if potential defects in clearance of apoptotic cells might be involved in SjD.
Methods: ELISA was used to examine plasma concentrations of the soluble forms of Tyro3, Axl, Mer and free Gas6 from 20 SjD patients and 20 age-matched healthy controls. mRNA levels were analyzed in peripheral blood mononuclear cells (PBMCs) from the same cohort by RT-PCR. We further analyzed TAM receptor expression of PBMCs from 16 SjD patients and 8 healthy controls using multi-color flow cytometry. In addition, immunohistochemistry was used to evaluate the expression of TAM receptors and their ligand Gas6 in labial salivary gland tissue from 8 SjD patients and 4 non-SjD sicca controls.
Results: While there were no significant differences in plasma, a significant decrease in the mRNA levels of Tyro3 and Mer were observed in patients. This significant decrease could not be confirmed at the protein level using flow cytometry, even though patients tended to have lower expressions of Tyro3 and Mer in most cell populations analyzed. In addition, we observed a significant reduction in the frequencies of different dendritic cell subsets in patients. In labial salivary glands, a slightly higher expression of Axl was observed in SjD patients than controls. Moreover, macrophages and dendritic cells were detected in higher numbers in patients compared to controls.
Conclusion: We found alterations in TAM receptor expression in SjD patients compared to healthy controls and non-SjD sicca controls, but further research is needed to understand their role in the pathogenesis of the disease.
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524 – P2.10.11

Influence of Neutrophils and Neutrophil Extracelluar Traps on Immunoglobulin G N-glycan modificatons over the time course of experimental sepsis

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Purpose: Sepsis is a life-threatening, multifactorial disease with a dysregulated host response. Neutrophils and Neutrophil Extracellular Traps (NETs) have been shown to contribute to the pathogenesis of sepsis, although the exact pathomechanism is not yet fully understood. Changes in the glycosylation of the Fc glycan of immunoglobulin G (IgG) have also been reported for sepsis. In this study we investigated the possible contribution of neutrophils/NETs to the alteration of IgG N-glycans in a mouse model of sepsis.
Methods: To examine the changes in N-glycosylation of IgG in a murine sepsis model in C57/BL6 and BALB/c mice over time, we employed mass spectrometry and specific lectin ELISAs. In addition, we analyzed the IgG subclass distribution, cytokine expression and activity of neutrophil elastase (NE) as a marker for NETs. Finally, we depleted neutrophils from the circulation of the mice using an antibody and assessed the IgG N-glycosylation using lectin ELISAs.
Results: Over the time course of the experimental sepsis, we detected an increased activity of NE as a surrogate for NETs and neutrophil-associated cytokines such as the keratinocyte chemoattractant (KC). With regard to N-glycosylation of IgG, we observed an increase in fucosylated and α1,3-galactosylated N-glycans and a decrease in sialylation, but not for all subclasses analyzed. After depletion of the neutrophils from the circulation, the exposure of fucose and α2,6-linked sialic acid was changed over time.
Conclusion: Induction of sepsis leads to increased activity of NE, expression of neutrophil-associated cytokines and changes in the N-glycosylation pattern of IgG, all of which are associated with inflammation. Depletion of neutrophils alters the exposure of the aforementioned N-glycans, indicating a role for neutrophils/NETs in these glycosylation pattern changes.

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562 – P2.10.12

Using biorthogonal (CLICK) chemistry to measure nutrient uptake flux in single immune cells.

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Nutrient uptake and utilisation are critical for the regulation of appropriate immune responses. Traditional technologies such as Seahorse-analysers and metabolomics rely on large numbers of pooled cells to measure metabolism. While these methods have revealed novel findings about immune cell metabolism, there is now a pressing need to accurately measure the metabolism of immune cells at the single cell level.
We have developed a new technology to accurately measure nutrient uptake, the first rate-limiting step for cellular metabolic pathways, using a biorthogonal chemistry (CLICK-chemistry) based approach. This involves using a minimally modified nutrient, containing a CLICK handle, that is taken up by the cell by the normal route. A fluorophore is then attached to the CLICK-nutrient after transport has occurred. This is achieved using a CLICK-reaction. This allows for fluorescence-based quantification of uptake into individual cells using flow cytometry.
This CLICK approach has been used to develop an accurate assay for SLC1A5 amino acid uptake; SLC1A5 is the major glutamine transporter in immune cells. Using this assay, we have demonstrated the metabolic heterogeneity of complex immune populations including splenocytes and developing thymocytes.
Current assays for measuring the uptake of fatty acids (FA) into cells rely upon BODIPY tagged fatty acid molecules. However, BODIPY is a highly hydrophobic molecule and has been show to have altered subcellular localisation characteristics. We have used CLICK-based technology to measure FA uptake using minimally modified oleic acid analogues, alkyne-oleic acid and Sterculic acid (StA). Uptake of both CLICK-FA was measured into diverse immune cell subtypes with single cell resolution. The highest uptake was observed in immune cells with known preferences for FA as fuels and those with highest expression of FA transport proteins such as CD36, such as macrophages and cDC1. Data using CD36-KO mice show that CD36 contributes to CLICK-FA uptake but other transport mechanisms are also involved.
Using multiple CLICK-based probes and configurations it is possible to perform 3 CLICK reactions in each individual immune cell. Using this we have simultaneously measured SLC1A5 uptake, FA uptake and rates of protein synthesis (using a CLICK-puromycin) providing 3-dimensional metabolic flux analysis in each individual cell.

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653 – P2.10.13

Spatiotemporal cellular dynamics related to germinal center reaction in regional lymph node of COVID-19 lungs based on in-situ hybridization at single-cell resolution

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In severe cases of acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the integrity of lymph node structures may be compromised, making the recovery process in convalescent subjects a vital area of study. Our study aims to unravel the nuances of structural recovery, dynamics of germinal center (GC), and their role in sustaining long-term immune memory in non-human primates recovered from SARS-CoV-2. Here, we employed the Xenium In-situ (10x Genomics) to analyze the composition of immune cells and the structure of mediastinal lymph nodes in the lung of macaques with COVID-19, tracking changes from the primary infection through to recovery and subsequent infection. Significant observations within lymphoid follicles include an increase in GC B cells during convalescence, and helper T and plasma cells during reinfection. In the paracortex area, reinfection leads to a decrease in NK cells but an increase in helper T and conventional dendritic cells. In the medulla, a reduction in macrophages is observed, accompanied by increased helper T, GC B, and plasma cells during reinfection. This study not only enhances our understanding of GC formation during COVID-19 recovery but also provides insights into immune cell repopulations within draining lymph nodes following reinfection. Such insights could be instrumental in refining vaccine approaches and developing targeted therapeutic strategies.

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699 – P2.10.14

Phosphatase of regenerating liver-1 regulates the activity of Lck during early T cell receptor signalling.

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Purpose: Activity of the Src kinase Lck has been proposed to be regulated by trafficking to signalling competent sites via the endosomal recycling compartment. We speculated that phosphatases of regenerating liver (PRLs), proteins trafficking through endosomal compartment and proposed to regulate Src kinases in cancer, might regulate Lck activity during initial T cell activation.
Methods: To study Lck regulation by PRLs, the PRL-1/2 expression were knocked out (KO) by using CRIRPR/Cas9 editing in the Jurkat cell line. Traffic of the active state of Lck (aLck) was evaluated, by measuring the phosphorylation of the activating tyrosine residue 394 (pY394-Lck). Lck activity was measured by initial ZAP70 phosphorylation upon TCR stimulation.
Results: A strong compensated expression of PRL-1 was found in PRL-2 KO, while PRL-2 KO showed a weak PRL-1 overexpression. PRL-1 KO reduced CD71 expression in plasma membrane and aLck traffic to CD71 compartment and cortical F-actin, a potential T cell activation site. Although PRL-2 KO reduced CD71 expression in plasma membrane, the aLck traffic was not affected. Evaluation of aLck levels in KOs and in cells overexpressing fluorescent-fusion PRL-1 and PRL-2 proteins indicated that catalytic activity and localisation of PRL-1 to plasma membrane promoted the aLck. Due to PRL-1 promoting role for Lck activity, PRL-1 KO hampered the delivery of aLck to nascent cognate interactions and early Lck activity. PRL-2 KO heightened Lck activity during later stimulation, a phenotype, probably originated from PRL-1 overexpression or from regulatory role of PRL-2 in Lck activity at later signalling. Moreover, immunoprecipitation revealed the interaction of PRL-1 and Lck in resting and stimulated cells, supporting an aLck regulation by PRL-1.
Conclusion: We propose PRL-1 is a regulator of CD71 expression at plasma membrane and is required for proper traffic of aLck to TCR signalling competent sites and for the initial TCR emanating signals. Although PRL-2 also regulate CD71 expression at the plasma membrane, our data points to PRL-2 as negative indirect regulator of aLck and ZAP70 signalling at later events during TCR stimulation. At any event, we propose that balance expression of PRLs, might contribute to appropriate TCR emanating signalling by regulating Lck activity.

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877 – P2.10.15

Vesicle transport inhibitors induce weak Nur77 expression in murine iNKT and B cells, potentially leading to false positive signals

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The Golgi transport inhibitors monensin and brefeldin A are widely used to facilitate the cytokine measurement in stimulated cells by intracellular cytokine staining. They block the intracellular vesicle transport at the trans-Golgi (monensin) or cis-Golgi (brefeldin A) network and accumulate cytokines intracellular which normally would have been secreted. Lymphocyte activation down-stream of the T cell receptor (TCR) or B cell receptor (BCR) can alternatively be measured by the rapid expression of the transcription factor Nur77 (NR4A1). However, Nur77 is also involved in endoplasmic reticulum (ER) stress-induced apoptosis. Given that vesicle transport inhibitors can induce ER stress, we hypothesized that the use of monensin or brefeldin A could also induce the expression of Nur77. Therefore, we incubated mouse splenocytes with monensin or brefeldin A with or without PMA/ionomycin stimulation and analysed Nur77 expression in conventional T cells, invariant Natural Killer T (*i*NKT) cells, and B cells by flow cytometry. PMA/ionomycin stimulation induced a strong expression (approx. three-log increase) of Nur77 in the majority of analysed T and B cells (>70% Nur77+ cells). However, the incubation of splenocytes with only monensin or brefeldin A for four hours *in vitro* also induced a weak expression (approx. 1/2-log increase) of Nur77 in approx. 4-8% of the *i*NKT cells and B cells. The increase of the Nur77-signal in conventional T cells did not reach statistical significance. Similar data were obtained with mouse mononuclear cells from mesenteric lymph nodes (mLNs). The direct link between ER stress and Nur77 expression is currently explored. In summary, our data indicate that the widely used vesicle transport inhibitors monensin and brefeldin A can induce a weak expression of Nu77 in *i*NKT and B cells in the absence of antigenic activation. As such a weak Nur77-expression could be wrongly interpreted as a sign of cell stimulation, our data highlight a source of potential false positive signal.

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1070 – P2.10.16

Maternal microbial metabolites in the fetal intestine and their effects on immunomodulation and epithelial function

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Purpose: Maternal microbial metabolites, transported across the placenta, may play a crucial role in modulating the development of the intestinal immune system already during the fetal development. Using non-targeted LC-MS/MS (QTOF) metabolomics, we have shown that 2200 metabolites were significantly less abundant in fetuses and placentae of germ-free (GF) mice compared to specific pathogen free (SPF) mice. Approximately 150 metabolites were undetectable in GF fetal tissues. Many of these metabolites showed strong correlations with the expression of genes related to host-microbe interactions, innate immunity, and intestinal epithelial barrier in fetal intestine of SPF mice.
Methods: We have created a new LC-MS/MS dataset comparing the fetal metabolomes across mammalian species, including human newborn meconium and amniotic fluid, and fetal intestines from mice, pig, and cattle. The new, more precise Orbitrap data enables the identification of many of the previously unannotated metabolites, using MS/MS libraries in MS-DIAL 5 and structure prediction by substructure annotation in the Sirius software. Integration with our previous data from mice allows us to identify potential microbial metabolites with immunomodulatory roles in the large mammalian dataset. To asses if these metabolites activate aryl hydrocarbon receptor (AhR), an essential sensor of microbial metabolites, we use human hepablastoma cell line HEPG2. Effect of these metabolites on immune responses and the intestinal epithelium are studied using macrophage-like cells derived from THP-1 and U937 and the Caco-2 cell line. As an efficient in vivo model of intestinal immune system development, we will use GF zebrafish embryos.
Results and conclusions: We observed many known immunomodulatory microbial metabolites in the newborn and fetal intestinal samples, including indoles, serotonin, glutamyl dipeptides and bile acids. In addition, we detected multiple metabolites which have not been previously explored in this context, but are likely modulated by the maternal microbiota, based on significantly lower levels in GF mice. Our observations suggest that the development of the intestinal immune system in humans and other large mammals is modulated by a rich array of maternal microbial metabolites already before birth.

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1129 – P2.10.17

Development of an Immunoassay for Quantification of Soluble Human CD40L (CD154) in Plasma Samples

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Purpose: Autoantibody production by autoreactive B cells are central aspects in autoimmune diseases such as Systemic Lupus Erythematosus (SLE). Signaling through the CD40-CD40L pathway provides a fundamental co-stimulatory signal for B cell activation. Dysregulation of the CD40L:CD40 axis is associated with inflammatory and autoimmune diseases and increased levels of soluble CD40L (sCD40L) in the blood is implicated in many diseases involving cardiovascular diseases, autoimmune diseases as well as some types of cancer. Thus, sCD40L could serve as a valuable marker of disease; however, to do so, it is of utmost importance to be able to precisely measure and quantify sCD40L levels in a human blood sample.
Methods: We have developed a sandwich-type time-resolved immunofluorometric assay (TRIFMA) for quantification of sCD40L in plasma and serum samples. This was done by generating 29 different monoclonal mouse anti-human CD40L antibodies. From these, we selected the optimal combination of a capture and detection antibody to produce an sCD40L assay. To test the robustness of the assay, we tested the influence of the sample type (comparing different blood collection tubes for serum and plasma sampling), the influence of freeze-thaw cycles, the influence of circadian rhythm on the samples, as well as the influence of sample centrifugation.
Results: We successfully developed an immunoassay for quantification of sCD40L. We found a very similar level of sCD40L in paired EDTA plasma and serum samples (linear correlation, R_2=0.995). In blood samples from 100 healthy blood donors, 61 had a level of sCD40L below the detection level of the assay, while 39 samples had ranging levels of sCD40L from 1.14 to 33.14 ng/mL.
Conclusion: We present a time-resolved immunofluorometric assay (TRIFMA) based on paired monoclonal antibodies, and show high specificity, sensitivity, and homogeneity. The assay presented uses Eu^{3+}-labelled streptavidin in the detecting step, which provides for consistent assay readouts and a broader dynamic range than seen in standard enzyme-linked immunosorbent assays. This assay paves the way for specific and consistent quantification of sCD40L in human plasma and serum samples, allowing for the use of sCD40L as a trustworthy marker of disease.

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1161 – P2.10.18

Similarly reshaped cytokine profile in malignant and autoimmune hematologic disorders

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Purpose: Deregulated immune system plays a crucial role in pathophysiology of hematologic diseases particularly autoimmune disorders like immune thrombocytopenia (ITP) and malignancies such as lymphomas and leukemias. Multiple studies have separately indicated a proinflammatory state in ITP and in hematologic malignancies (HM) in which inflammatory cytokines influence the proliferation of both normal and pathogenic cells. In our study we examined pro- and anti-inflammatory cytokines and their ratios in patients with HM and ITP.
Methods: We quantified the concentrations of plasma cytokines TNF-a, IL-17, IL-6, IL-4 and IL-10 in HM and ITP patients and age-matched healthy individuals to compare cytokine production and cytokine ratios. Statistical analysis was performed using Prism Graph Pad and SPSS software. Mann-Whitney U-test was used to compare different study groups: ITP and HM patients and controls.
Results: Our analysis revealed elevated concentrations of TNF-a, IL-6 and IL-4 in both ITP and HM patients compared to the age-matched healthy controls. Conversely, IL-17 level was significantly reduced regardless of the pathology. There was no alteration in IL-10 levels within any patients groups. We observed significantly decreased ratio of TNF-a/IL-6 in HM patients, while this tendency was insignificant in ITP group. However, TNF-a/IL-4, IL-17/IL-4, IL-17/IL-10, TNF-a/IL-6, IL-17/IL-6 and IL-10/IL-6 ratios were significantly reduced regardless the pathology.
Conclusion: Cytokines as major inflammatory mediators instigate the similarly reshaped immune microenvironment in different pathologies. Better understand of cytokine profile similarity or heterogeneity in HM and ITP will serve to identify new effective therapeutic targets for hematologic diseases.

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1383 – P2.10.19

Implications of PD-1/PD-L1 pathway as a new immunological paradigm for juvenile idiopathic arthritis

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Background: Juvenile Idiopathic Arthritis (JIA) is the most common pediatric arthritis disease affecting children below the age of 16 characterized by persistent pain and swelling of joints. It is a group of heterogeneous T-cell mediated autoimmune disease with symptoms of premature aging of the immune system that can lead to significant joint deformities which can cause deterioration of bones, tendons and ligaments and hampering the growth of children through adulthood.
Objectives: The aim of the study was to determine the expression of PD-1, PD-L1, and CTLA-4 in subsets of JIA patients, elucidating their underlying immunomodulatory mechanisms.
Methods: The expression of PD-1, PD-L1, CTLA-4 and FOXP3 was determined by flow cytometry in blood and synovial fluid of 43 JIA patients. ELISA was also performed on all JIA patients to measure the level of soluble form of PD-1, PD-L1, and CTLA-4 in blood samples. The patients' demographic data and treatment will be recorded. JIA will be classified according to the ILAR criteria. JIA activity will be assessed using the JADAS-10 tool.
Results: There was male preponderance (28/43; 65.11%) in our study. Among 43 patients, 12 patients were diagnosed with oligoarticular JIA, 12 with polyarticular JIA, 5 with systemic JIA, 13 patients had enthesitis-related arthritis and one psoriatic arthritis. Expression of PD-1 on CD3+ CD4+ T-cell and CD3+ CD8+ T-cell was found more in synovial fluid than peripheral blood of the patients using flow cytometry. Positivity of PD-1 was different in all the JIA groups. Expression of PD-L1 was decreased in SF and PB of JIA patients as compared to PD-1 expression.
Conclusion: Our finding showed the expression of PD-1/PD-L1 pathway that might provide the elucidation of disease pathogenesis, and the development of potential targeted therapeutic strategies in JIA which may be more specific as compared to conventional disease modifying anti-rheumatic drugs (c-DMARDS).

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1403 – P2.10.20

Crosstalk between innate immune system and bone metabolism is influenced by cell-free DNA quantity and mediated by cyclic GMP-AMP synthase

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Background & Objectives: The ability of blood monocytes (PBM) to differentiate into osteoclasts is a crucial link between innate immunity and bone metabolism, particularly significant in autoimmune-related chronic inflammation, where heightened osteoclast generation/activity leads to pathological bone resorption. Circulating cell-free DNA (ccfDNA) has immunomodulatory properties and its abundance increases during chronic inflammation. While inflammatory cytokines are known to influence osteoclast differentiation, the potential role of ccfDNA in this process remains unknown. This study aimed to investigate the role of serum ccfDNA in osteoclasts differentiation from PBM and the effect of quantitative and qualitative properties (nuclear versus mitochondrial origin) of ccfDNA on osteoclastogenesis.
Methods: To measure nuclear (nc-) and mitochondrial (mt-) ccfDNA content in sera of 47 human subjects, ccfDNA was isolated and quantified using absolute qPCR of single-copy and *MT-ND2* genes. Concentration was calculated from Cp values using control standard curves prepared from whole blood. Pools of sera with varying ccfDNA content were used to culture PBM isolated from healthy individuals (N=25). Two *in vitro* experimental models were employed: 1) serum pools digested by deoxyribonuclease I alongside their undigested counterparts were applied on PBM during osteoclastogenesis, and 2) PBM were cultured in undigested sera after pre-inhibition with G140, a selective inhibitor of cyclic GMP-AMP synthase (cGAS). At the end of culture, osteoclasts were identified by histological staining and counted.
Results:
In contrast to mt-ccfDNA, the number of differentiated osteoclasts positively correlated with nc-ccfDNA concentration in undigested sera ($r=0.51$; $p<0.01$). Osteoclastogenesis decreased 1.8-fold after culturing PBM with sera treated with deoxyribonuclease I ($p<0.0001$), and 1.7-fold in culture where PBM were pre-inhibited with G140 ($p<0.05$). The trend towards a greater effect of ccfDNA digestion by deoxyribonuclease I compared to cGAS inhibition by G140 reflects higher concentration of nc-ccfDNA present in pooled sera specifically used for digestion experiments ($p<0.05$).
Conclusion: These results suggest novel connection between innate immunity and bone metabolism and indicate that serum nc-ccfDNA co-stimulates differentiation of monocytes into osteoclasts in presence of osteoclastogenic signals, at least partially via cGAS. Further study of this phenomenon could provide useful insights for treatment of autoimmune diseases.

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1440 – P2.10.21

Deciphering tissue-specific epigenetic signatures governing memory T lymphocyte residency

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Memory T lymphocytes, especially tissue-resident memory T lymphocytes (Trm), are central to immunological memory, yet their residency mechanisms across diverse tissues remain elusive. Here, we present a comprehensive analysis of tissue-specific epigenetic imprints on memory T lymphocytes. Utilizing 58 "reduced representation bisulfite sequencing" (RRBS) datasets encompassing 22 distinct tissue-derived and blood-borne human memory CD4+ and CD8+ T-cell populations, we investigate the methylome profiles. Our findings unveil a nuanced landscape of tissue-specific and shared differentially methylated regions (DMRs) within Trm populations across various tissues. Notably, Trm cells in different tissues exhibit distinct hypo- and hyper-methylated DMRs, highlighting the orchestrated nature of their residency. These DMRs encompass key genes encoding chemokine receptors, integrins, effector functions, and transcription factors. Importantly, we observe enrichment of tissue-specific transcription factor binding sites in hypomethylated DMRs, suggesting their regulatory role. The substantial correlation between DMRs and gene expression may significantly contribute to tissue-specific recruitment, maintenance in steady state, and effector gene expression upon reactivation, including cytokine expression memory. Our study underscores the significance of epigenetic imprinting in shaping immune memory and provides novel insights into Trm dynamics in secondary immune responses. By utilizing tissue-specific DMR signatures, we have demonstrated, for the first time, the mobilization of Trm and their consequential role in systemic secondary immune reactions. This work advances our understanding of memory T lymphocyte residency and opens avenues for deciphering the intricate interplay between epigenetic regulation and immunological memory.

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Phospho-flow based immunometabolic biomarkers for pharmacodynamic evaluations: translation from bench to bedside

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Pharmacodynamic evaluation of investigational drugs targeting immunometabolic pathways is technically challenging, which hampers early clinical evaluation of such compounds. Though various functional assays are described in the public domain, the clinical application of these assays is limited since most work is based on immortalized cell lines, with a completely different immunometabolic profile. We aimed to identify, develop, and validate functional human cell-based pharmacodynamic biomarkers, focused on key junctions in immunometabolic pathways, for future implementation in early phase clinical pharmacology studies.
Human peripheral blood mononuclear cells (PBMCs) were isolated from healthy human donors. PBMCs were challenged *ex vivo* by nutrient starvation or immunometabolic modulating compounds such as dorsomorphin and rapamycin. Pharmacodynamic effects on immune cells were evaluated, such as cell viability and apoptosis, but also more in-depth evaluation such as phosphorylation of mTOR (T2446), RPS6 (S235/236), Acetyl-coA (S79) and AMPK (Thr172) by phospho-flow. Staining's for monocytes and lymphocytes were included allowing differentiation between PBMC cell subsets. Cultures of 24 hours were prepared in media with serum for unstimulated and compound conditions, while glucose- and FBS-deprived media were used for starvation conditions.
Starvation induced cell death in lymphocyte and monocyte populations resulting in 10 to 20% viability drop compared to the unstimulated conditions. A mild increase in apoptosis was observed for all conditions compared to unstimulated. Rapamycin at 200nM strongly inhibited phospho-RPS6 and phospho-mTOR on monocytes, while no inhibitory effect was observed on lymphocytes. Dorsomorphin only strongly inhibited phospho-mTOR in monocytes upon starvation, while no effect was observed on lymphocytes or unstimulated monocytes.
Starvation resulted in a strong increase in phospho-AMPK and a mild increase in phospho-Acetyl-coA. Interestingly, starvation strongly induced phospho-RPS6 while it suppressed phospho-mTOR. Lymphocytes appeared to be unresponsive to all test conditions, even to the well documented control compounds. This may be explained by the naive state of lymphocytes derived from healthy donors.
Monocytes showed a robust response to all test conditions, indicating a clear difference in metabolic activity between monocytes and lymphocytes.

Based on the technical and physiological insights obtained in these experiments, cell-based immunometabolic assays can be selected for pharmacodynamic evaluation of investigational compounds targeting these pathways.

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1713 – P2.10.25

CTLA-4, PD-1, LAG3, TIM3 and TIGIT display distinct vesicular distributions in murine T cells

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Purpose: T cell function can be inhibited by transmembrane inhibitory receptors. Different inhibitory receptors have different signalling pathways. However, their intracellular distribution remains unclear. These receptors play a crucial role as primary therapeutic targets in cancer immunotherapy. This study investigates how five co-inhibitory receptors, CTLA-4, PD-1, LAG3, TIM3 and TIGIT, differ in their subcellular localisation.
Methods: Confocal and TIRF microscopy were used to study the subcellular and interfacial distribution of inhibitory receptors in murine T cells. APEX2, which catalyses the biotinylation of proximal proteins, was used to elucidate the inhibitor-receptor-containing vesicles by proteomic analysis. Immunoprecipitation was used to detect the trafficking regulators that interact with the inhibitory receptors. Immunofluorescence was used to study the co-localisation of inhibitory receptors and cellular compartment markers.
Results: We observed that a significant amount of CTLA-4, PD-1, LAG3, TIM3 and TIGIT were localised in the intracellular vesicles. TIRF imaging confirmed the distinct spatio-temporal patterns of the above inhibitory receptors. The distinct clustering of the lumenal proteomes containing CTLA-4, LAG3, and TIM3 is visualised by t-SNE analysis. Furthermore, immunoprecipitation revealed that different inhibitory receptors bind to common sorting complexes such as AP-1, AP-2 and ESCPE-1. CTLA-4 distribution was most pronounced, preferentially associated with lysosomal vesicles and the ESCPE-1 machinery.
Conclusion: The five inhibitory receptors were shown to have different subcellular distributions when compared using a variety of metrics, including intracellular clustering, dynamics of plasma membrane insertion, spatial and temporal distribution on the cell surface, and molecular protein neighbourhood. In the absence of evidence supporting the existence of distinct vesicle subtypes to account for the differing distributions of inhibitory receptors, we propose that these distributions are influenced by different trafficking patterns through a shared collection of vesicular compartments. This comprehensive examination of the subcellular distribution of five inhibitory receptors in juxtaposition with one another provides the basis for studying their molecular trafficking and their potential therapeutic applications.

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The effects of the hypoosmolar environment developing in hyponatremia on immune system cells

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Purpose: Hyponatremia or hypotonicity is characterized by a plasma sodium concentration of less than 135 mEq/L due to the relative or absolute increase in water concentration in the extracellular environment. While the effects of a hypoosmolar environment, especially on astrocytes surrounding neurons and the resulting clinical manifestations, are known, the effects of a hypotonic environment on the immune system remain unclear. This study aims to elucidate the effects of a hypoosmolar environment resulting from hyponatremia on immune system cells.
Method: The study included 20 symptomatic hyponatremia patients suspected of being corrected with water restriction. Serum and urine osmolarities of all patients were analyzed. Peripheral blood samples were obtained from all patients before and after treatment. Lymphocyte, monocyte, and granulocyte subsets were evaluated using a flow cytometer. Additionally, serum concentrations of cytokines including IL-4, IL-5, IL-6, IL-9, IL-17A, IL-21, IL-22, TGF-β, Taurine, and TNF-α were assessed using the ELISA method.
Results: According to the flow cytometry results, the levels of pE2 (pre-effector memory 2) and E (effector) cells, which can produce high levels of inflammatory molecules such as perforin and granzyme B among the CD8+ T cell subsets, increased, while the levels of pE1, EM1 (effector memory 1), and EM4 cells, which have a lower ability to produce these molecules, decreased. There is a pro-inflammatory profile in terms of memory CD8+ T cells in the hypoosmolar environment. An increase in Th1 and Tc1 cells was observed in hyponatremia. A significant increase in Th22/Tc22 cells and a trend of increase in Th17/Tc17 cells were observed. In ELISA analyses, an increase in Th2, Tc2, Th17, and Tc17-associated cytokines was observed during the disease. After treatment, IL-5, IL-6, IL-10, IL-17A, and TGF-β levels decreased, while Taurine levels increased.
Conclusion: According to all analyses, there is a trend of increase in Th17,22 and Tc17,22 cells, which may have pathogenic effects enhancing inflammation. The increase observed in Th22 and Tc22 cells may be a response to tissue damage caused by the stress condition in which the cells are located. ELISA results indicate that, in addition to the pro-inflammatory profile, it also affects type 2 responses.

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Functional dissection of RNA modification Pseudouridine in T cells

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Gene expression is regulated at multiple levels and their orchestrated action is crucial for cell differentiation, homeostasis and function. To this end, RNA undergoes extensive chemical modification, the sum of which is referred to as the epitranscriptome. Of all RNA modifications, pseudouridylation - the conversion of uridine into Pseudouridine is most prevalent. Despite their conservation and causative function in various human diseases, mammalian epitranscriptomics research is only emerging. Pseudouridine is introduced by 13 non-redundant Pseudouridine synthases (PUS). The abundance of Pseudouridine along with the dynamic expression of PUS suggests this modification as a regulatory layer in processes where transcriptomic/proteomic rewiring is necessary such as T cell development and functional differentation.

To dissect the role of pseudouridylation, we use *in vivo* pooled CRISPR screening. To this end, hematopoietic stem cells from $CD4^{Cre}$ $Cas9^{fl\text{-}STOP}$ mice are transduced to express sgRNAs targeting the PUS and then transplanted into lethally irradiated recipient mice. Subsequently, T cell subsets from the engrafted mice are subjected to NGS to assess gRNA representation. Underrepresented gRNAs indicate that knockouts of these PUS have negative consequences for the respective T cell subset and hence identify essential PUS for T cell-relevant pathways.

We identified PUSL1 as potentially ‘essential’ in different T cell subsets - from double-positive T cells in the thymus to regulatory T cells in the small intestine. Our preliminary data suggests PUSL1-mediated pseudouridylation to be crucial in T cell development and activation/function, making it a strong candidate for further characterization.

Using arrayed CRISPR/Cas9 screening *in vitro* and *in vivo* combined with scRNA-seq, we seek to further understand PUSL1-mediated pathways crucial for T cell development and function. Thus, paving the way for further epitranscriptomics research in immunology.

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Investigation of the effects of bevacizumab treatment on the immune system in age-related macular degeneration

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Purpose: Age-related macular degeneration (AMD) is characterized by a progressive decrease in central visual acuity in patients aged 50 years and older, manifested by pigmentary and atrophic changes in the macula. Bevacizumab, used in the treatment of wet AMD, blocks angiogenesis by binding to all subgroups of VEGF and is usually used intravitreally. In this study, we aimed to investigate the effects of Bevacizumab, which is used in the treatment of wet AMD, on immune system cells.
Method: Patients with wet AMD (n: 18) who were admitted to and/or followed up at the Department of Ophthalmology, Uludag University, Faculty of Medicine, Bursa, and who were to receive Bevacizumab treatment and cataract surgery patients (n: 10) as the control group were included in the study. Peripheral blood samples were collected from patients with wet AMD before Bevacizumab treatment and after 3 injections. Peripheral blood samples were also collected from patients with cataract.
Results: $CD4^+CD27^+CD28^+$ EM_1 T-cell frequency was significantly lower in AMD patients compared to cataract patients (p:0.006), while $CD4^+CD27^-CD28^-$ EM_3 T-cell frequency was significantly higher (p:0.018). B10 cells in AMD patients were found to be higher than in cataract patients (p:0.026). On the other hand, it was observed that $CD4^+CD279^+$ exhausted T cells increased (p:0.012) and $CD62L^{low}CD16^{high}$ neutrophil levels decreased (p:0.029) in AMD patients after Bevacizumab treatment.
Conclusion: In AMD patients, EM_3 T-cells, which can produce inflammatory mediators such as IFN-γ at high levels, increased, while EM_1 T-cells, which exhibit low effector activity, decreased. In addition, the high level of regulatory B10 cells in AMD patients indicates that the immune system is trying to stabilize homeostasis. The administration of 3 doses of Bevacizumab in the treatment of AMD seems to mediate the regulation of inflammatory responses by directing proinflammatory T-cells, which were high before treatment, to exhaustion. Similarly, the levels of activated $CD62L^{low}CD16^{high}$ neutrophils, which can produce cytokines such as IL-1β, decreased after treatment. Taken together, the data suggest that an inflammatory state is present in patients with AMD and that Bevacizumab treatment improves these inflammatory processes.

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Role of Irisin in Autosomal Dominant Polycystic Kidney Disease

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Purpose: Autosomal dominant polycystic kidney disease (ADPKD) is the most common inherited cause of end-stage renal failure in adults. It is seen with a frequency of 1/400 to 1/1000 in the population. PKD1 and PKD2 gene mutations are observed in 85% and 15% of affected families, respectively. Decreased intracellular 5′ AMP activated protein kinase (AMPK) activity is one of the most important features of the disease and drugs that increase AMPK activity are being tried in treatment.
Irisin is a protein identified in muscle tissue that causes energy expenditure by converting white adipose tissue into brown adipose tissue. Irisin is a myokine formed from fibronectin type III domain-containing protein 5 (FNDC5) by degradation as a result of AMPK activation and is negatively correlated with oxidative stress and inflammation.
In this study, irisin levels in early stage ADPKD were compared with healthy control group.
Methods: A total of 90 early stage ADPKD patients and 70 healthy controls were included in the study. All patients were recruited from the Turkish Society of Nephrology Cystic Kidney Disease Study Group Data Sheet and had a positive family history. Patients with GFR-EPI less than 60 ml/min and patients with active infection were excluded. Sera from ADPKD patients and healthy controls were portioned and stored at -20°C until the study date. Irisin protein level was determined by ELISA kit (BT LAB, China).
Results: In patients with ADPKD, irisin level (ng/ml) was found to be significantly lower than in healthy controls ($p<0.001$) (Figure 1). In addition, a significant inverse correlation was observed between irisin levels and blood urea nitrogen (BUN, mg/dL) and creatinine (mg/dL), while a significant positive correlation was found with glomerular filtration rate (GFR, ml/min) in ADPKD.
Conclusion: In our study, we showed that irisin levels were significantly lower in ADPKD. This decrease may be related to low AMPK activation, which is a phenotypic feature of the disease. In future studies, the relationship between oxidative stress and inflammation and irisin should be investigated.

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Dissecting the immune microenvironment in a High Grade Serous Ovarian Cancer murine model following Platinum treatment and epigenetic modulation

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Purpose: High Grade Serous Ovarian Cancer (HGSOC) is a challenging disease characterized by TP53 mutations and defects in DNA repair (dDR). Debulking surgery and platinum-based chemotherapy is the standard care. The introduction of PARP inhibitors (PARPi) as first-line maintenance treatment for patients with dDR changed patients' prognosis, but eventually resistance occur. dDRs alter immune response, suggesting that tumor immune microenvironment (TiME) can be exploited in HGSOC therapy. G9a/GLP histone methyltransferase, overexpressed in HGSOC, negatively regulates gene expression, promotes DNA damage repair and perturbs the TiME. This study aims to dissect HGSOC TiME contribution in response to PT/PARPi, and the involvement G9a inhibitors (G9ai) in harnessing the TiME through combinatorial therapy.
Methods: TP53-PTEN-KO ID8 murine ovarian cancer cells were injected *i.p.* in syngeneic C57BL/6 mice. 4 days after ID8 injection, mice were treated with saline, PT, G9ai and their combo three times/week and sacrificed at 11 or 21 days of treatment. Treated tumor-free mice were used as control. Flow cytometry analysis of ascites, spleen, lymph nodes and bone marrow was performed to assess modulation of myeloid and lymphoid cell populations.
Results: No significant differences were noted in tumor-free mice receiving the different treatments. In tumor-bearing mice, the major changes were observed in ascites, whose volume decreased in the combo-treated mice. Most alterations in TiME are noticeable at early treatment and related to the expansion of regulatory T cells (Tregs), mast cells (MCs), and type 1 dendritic cells. Here, Tregs coproduce IL-10 and IL-17. We also found an increase of $Ki67^{+}OX40^{+}CD8^{+}$T cells and Granzyme B producing $CD49b^{+}$NK, as if these cells were early activated after the combo. No significant changes were observed in the other tissues considered. Conversely, immune cells are drastically reduced in combo-treated mice at late time-point, suggesting that early modifications can shape tumor growth.
Conclusion: In our *in-vivo* model, the PT-G9ai combo therapy shapes the TiME towards activation. The possible skewing of Tregs into a Th17-like phenotype and the concomitant accumulation of MCs, that can counteract Treg suppressive activity fostering Treg-Th17 differentiation, suggest of deeply investigating the MC-Treg-Th17 network in our setting.

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1922 – P2.10.31

Role of autophagy in antitumor activity of human NK cells

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Purpose: Natural killer (NK) cells are cytotoxic lymphoid cells that play a crucial role against different tumors. Indeed, NK cells are considered a great promise in cellular immunotherapy. On the other hand, cancer cells can inhibit NK cell function by creating a hostile microenvironment. Consequentially, tumor microenvironment (TME) results in tumor resistance and decreased efficacy of NK cell-based immunotherapy. Autophagy is a self-degradation process crucial for maintaining cellular homeostasis. Although it has been shown that autophagy regulates the immune system, its role in human NK cell biology remains to be clarified. Autophagy is activated by stress and physiological conditions such as nutrient deprivation, hypoxia, cell differentiation, or activation. In TME, the NK cells are subjected to stress conditions. Thus, regulating autophagy may affect the NK anti-tumor activity and improve cellular stress response in the tumor microenvironment.
Methods: To evaluate the autophagic flux in different NK functional states, the expression of genes involved in the critical steps of autophagy was analyzed in NK cells isolated from peripheral blood (pb-NK) and expanded NK cells (ex-NK) upon cytokine stimulation. To investigate autophagy on NK cell homeostasis and cytotoxic activity, the autophagic flux was manipulated by drugs and genetic manipulation of the autophagy-related gene (ATG).
Results: NK-activating cytokines can modulate the autophagic status. Indeed, deprivation of pro-survival and pro-cytotoxic cytokines induces autophagy in both pb-NK and ex-NK cells. However, pharmacological and genetic modulation of autophagy can affect the NK cytotoxic activity.
Conclusion: Autophagy influences the NK anti-tumor performance, modulating their cell cytotoxicity. The regulation of autophagy represents a crucial tool to counteract the inhibitory effect of low pro-survival factors in the TME. The modulation of autophagy in NK cells represents a novel strategy to boost NK cell-based cancer immunotherapy.

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1933 – P2.10.32

Investigating the role of E3 ubiquitin ligase Pellino1 in B cell antigen uptake

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Long-term protective antibody responses depend on B cell activation through a specific interaction between the B cell receptor and its cognate antigen. Upon binding, the BCR-antigen complex is also internalised resulting in antigen processing and presentation via MHC class II to initiate T cell responses. The process of BCR-antigen internalisation – while essential for normal immune function – is also crucial to prevent hyperactivation. Thus, internalisation influences BCR signalling and vice versa, but the regulators of this critical interconnection remain unknown.
A recent genome wide CRISPR screen identified E3 ubiquitin ligase Pellino1 (Peli1) as a novel regulator of BCR trafficking. This project aims to identify the targets of Peli1 in B cells, determine its role in BCR uptake and signalling events. We demonstrate increased BCR uptake and antigen presentation in primary and Ramos B cells upon Peli1 knockout – the first example of negative regulation of the BCR by an E3 ubiquitin ligase. Despite increased antigen presentation, murine germinal centre responses upon immunization were significantly reduced.
Using SILAC mass spectrometry, we have identified specific cellular pathways coordinated by Peli1 in human Ramos B cells, including endocytic and structural proteins, metabolic regulators and signalling components. Subsequently, via the Seahorse assay we have validated novel roles of Pellino1 as a metabolic regulator in B cells with Peli1 knockout cell lines exhibiting increased basal metabolism, maximal mitochondrial respiration, and upregulation of known metabolic regulators such as GSK3α/β via western blot. Finally, we have seen increased intracellular Ca^{2+} signalling upon Pellino1 knockout.
Taken together our findings highlight an important role of Pellino1 in B cell metabolism and signalling, with a potential to bridge the gap between BCR signalling and endocytosis. Its role as a negative regulator of B cell activation opens further research questions and therapeutic potential in malignancy and autoimmunity.

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1950 – P2.10.33

AKNA is a novel RNA-binding protein that localizes to the centrosome and regulates the germinal center reaction

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The presence of RNA at the centrosome has been known for a long time, and more recently also RNA-binding proteins have been found associated with this organelle, but a functional role remained unclear. These RNA-binding proteins might be involved in transporting RNAs to the centrosome, where local translation can occur. The AKNA gene has been implicated in several diseases including microcephaly and Sjögren's syndrome. Originally, the AKNA protein was proposed to localize to the nucleus, and since it harbors an AT-hook, it was thought to interact with AT-rich DNA or potentially also RNA. A recent study revealed that AKNA is rather a cytosolic, centrosomal protein that regulates microtubule organization during neurogenesis.
In this study, we establish that AKNA is a novel RNA-binding protein. We show that the AT-hook localizes the protein into the RNA/protein-adducts containing interphase after orthogonal organic phase separation (OOPS) of UV-crosslinked cells. Furthermore, individual-nucleotide crosslinking and immunoprecipitation (iCLIP) in immune cells suggests binding of AKNA to mRNAs encoding for proteins of the cytoskeletal machinery and actin-nucleation complex. The strong upregulation of AKNA early during lymphocyte activation and the localization to the centrosome in B and T cells during immune synapse formation suggest an importance for adaptive immunity. Indeed, in mixed lymphocyte reactions, AKNA is required in antigen-presenting cells (APCs) for effective T cell activation and proliferation. Upon immunization of AKNA knockout mice, we find an impaired germinal center reaction, which depends on tightly controlled interactions between lymphocytes with APCs to generate high-affinity antibody-producing plasma cells and memory B cells. Currently, we study how AKNA regulates B cell-T cell interactions and immune synapse formation via its RNA-binding activity. In ongoing experiments, we aim to decipher previously unrecognized immune regulatory mechanisms by centrosomal RNA-binding proteins.

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2004 – P2.10.34

Deficiency in nitric oxide production by myeloid cells during LPS-induced inflammation in naked mole-rats H. glaber

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Naked mole-rat (NMR) is a long-lived rodent highly resistant to cancer and age-associated disorders. The immune system of NMR possesses a unique cellular composition with the prevalence of myeloid cells. It was reported that NMR macrophages activated with lipopolysaccharide (LPS) and interferon-gamma exhibit reduced nitric oxide (NO) production *in vitro* as compared to mouse macrophages. Moreover, upon classical macrophage activation genes involved in arginine metabolism demonstrate different expression patterns between the two species. We hypothesized that under inflammatory conditions NMR myeloid cells located in the blood and tissues also have limited capacity for NO induction due to species-specific metabolic adaptations.
To address inflammation-induced species-specific profile of NO metabolism in vivo, we tested two doses of LPS (1.5 or 30 μg/g) in a model of acute systemic inflammation. Blood cellular composition as well as intracellular NO production 24 h following LPS administration were assessed by FACS. Also, the expression of nitrogen metabolism-associated genes and cytokines was analyzed in the spleen, liver and kidney.
NMR demonstrated sickness behavior such as reduced locomotion and food intake, especially pronounced for the high-dose group in correlation with an increase in inflammatory cytokine gene expression in the spleen and the liver. The percentage of $CD11b^+$ $CD14^+$ in the blood was elevated 24 h after LPS challenge in both species. However, intracellular NO levels changed significantly in $CD11b^+$ blood cells 24 h after LPS administration in mice, but not in NMR. Relative expression of *Nos2* increased in a dose-dependent manner in murine liver and spleen, while only high dose LPS upregulated *Nos2* in NMR liver. Interestingly, relative expression of *Gatm* in NMR liver remained unchanged in LPS-treated group, unlike in mice. Since AGAT is a consumer of arginine as a substrate for the first step of creatine synthesis, its expression during inflammation in NMR may imply the predominance of other pathways of arginine utilization than iNOS-catalyzed reactions.
Our findings suggest that the naked mole-rats can tolerate higher doses of LPS due to metabolic adaptations that are associated with altered iNOS activation in myeloid cells.

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2022 – P2.10.35

Combining single-cell short and long-reads sequencing for dissecting immune cell states

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PURPOSE: T-cell populations are heterogeneous cell types with unique cell states that shape adaptive immunity. T-cell differentiation stages can be studied in detail through the characterization of gene expression signatures, where single-cell technologies offer unprecedented resolution. Although short-read data has been widely used to study these signatures at the gene level, alternative splicing and polyadenylation have not been dissected yet thoroughly at single-cell resolution. To address this, we here combine both short and long-read single-cell technologies to characterize transcript-level expression across T-cell states.
Methods: We isolated the following T-cell subpopulations: αβ CD8+ naïve, central memory, effector memory, terminal effectors, and γδ T-cell populations from healthy donor peripheral blood mononuclear cells (PBMCs). We performed 5' scRNA-seq using short-read and long-read sequencing on those sorted populations and total PBMCs. We annotated assembled transcripts, systematically compared high-quality cells across technologies and performed differential gene/transcript expression and differential transcript usage (DTU) analysis.
Results: The number of genes and UMI counts detected per cell was highly correlated between short and long-reads data and we observed reproducible prediction of cell identities with the classifier CellTypist. Transcript categorization from long-reads using *gffcompare* revealed above 70% of reads with complete matches against known transcripts in the GENCODE database, indicating low presence of artifacts. Using this set of annotated transcripts, we were able to detect DTU events. Some of these are well characterized, such as the one affecting the *PTPRC* gene, encoding CD45, which is alternatively spliced between naïve and memory T-cell states.
Conclusion: With the addition of the transcript-level expression layer, we have started to build a comprehensive map of post-transcriptional events across fine-grained cellular states. As a proof of concept, we have compared the protein expression of CD45 against transcript expression in specific sorted T-cell subsets, adding depth to our understanding of this key locus. Furthermore, we studied patterns of transcript usage transcriptome-wide in the continuum of unsorted T cells from peripheral blood. In the long run, this type of analysis holds the promise to not only unravel dynamics of transcript-level expression but also uncover novel isoforms in the context of development or disease.

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2117 – P2.10.38

Dissecting immune cell states in healthy and CMV-infected newborns through single-cell sequencing technologies

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Purpose: The adaptive immune system begins to develop in prenatal life but only fully matures years after birth. Unique features, such as lower cytotoxicity or tolerogenic bias, have been reported to characterize neonatal adaptive immunity. Nonetheless, the mechanisms controlling these processes are poorly understood. Neonates encounter a myriad of microorganisms post-birth with diverse outcomes that shape their immune system. Congenital Cytomegalovirus (cCMV) infection, the most common congenital infection (0.2-2.0% of newborns globally), can lead to permanent disabilities, such as hearing loss and neurological deficits. Previous reports have shown that neonatal responses to CMV include activation of natural killer cells and T cells, however, there is a lack of comprehensive evaluation of the immune response in these subjects.
Methods: Here, we present an in-depth analysis of the neonatal immune cell landscape and its changes in cCMV using single-cell genomics (CITE-seq and scV(D)J-seq) to deeply characterize peripheral blood cell phenotypes and antigen receptor features (healthy n = 10; cCMV n = 2). We assessed the frequency and the phenotype of immune cell subpopulations in healthy and CMV-infected neonates compared to adult controls. To this end, we employed automated and manual cell type annotation, differential composition, and differential gene expression analysis.
Results: Our preliminary data indicate that naïve populations within the T and B cell compartments are more prevalent in neonates, as expected. Interestingly, we also find that naïve cells across age groups show differential activation of specific gene programs. In the cCMV neonates, apart from naïve lymphocytes, we also detect the presence of effector memory cytotoxic T cells, which are not evident in their healthy counterparts, opening the possibility to dissect antigen-driven responses in early life.
Conclusions: Overall, we have generated deep molecular profiles of cellular subpopulations in neonates, both healthy and cCMV-infected. We have identified gene signatures unique to neonatal cell states and activated cell states in congenital infection. Future work will on the one hand be focused on increasing our cohort size across age groups and on the other hand on dissecting the role of neonatal specific gene programs in steady state and upon viral infection.

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2222 – P2.10.40

Alterations in the natural autoantibody network during pregnancy in Hashimoto's thyroiditis

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Purpose: Hashimoto's thyroiditis (HT) stands as a prevalent autoimmune disorder predominantly observed in women of reproductive age. Although HT is classically considered a T cell-mediated disorder, the presence of pathological anti-thyroid autoantibodies indicates the dysfunction of B cells, which may result in the imbalance of the natural autoantibody (nAAb) network. The regulatory role of nAAbs in tolerance induction has been described, thus the network may also be important in the establishment of the immunological tolerance to the fetus. Consequently, we aimed to investigate nAAbs in pregnant women with HT (HTP) and healthy pregnant women (HP).
Methods: Serum levels of nAAbs in HTP and HP against mitochondrial citrate synthase (CS), heat shock proteins (Hsp60 and Hsp70) were measured using in-house developed ELISA, while anti-cytokine autoantibodies were measured by a Milliplex multiplex immunoassay. Serum samples of patients with Hashimoto's thyroiditis (HT) and healthy, non-pregnant women (HC) were also collected as controls.
Results: We found that nAAb levels of IgM against CS were higher, while levels against Hsps were lower in HP than in HC. Additionally, IgG nAAb levels against Hsps were elevated in HT compared to HC but decreased during the pregnancy of HT patients. An increase in anti-cytokine autoantibodies against TNFα, IFNγ and IL-8 was determined in the first trimester in HP compared to HC, which was not observed in HTP.
Conclusion: We hypothesize that in pregnancy, the physiological nAAb network exhibits a moderate plasticity to regulate immune response and tolerance, which is disturbed in HTP. Our results also support the regulatory role of autoantibodies in the availability and activity of Th1 cytokine IFNγ and inflammatory cytokine TNFα which are considered to be central players in early pregnancy.

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2232 – P2.10.41

Validation of inflammatory markers' association with rheumatic heart disease in an African cohort

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Introduction: Rheumatic heart disease (RHD) is a significant contributor to premature morbidity and mortality in sub-Saharan Africa and the global south. Literature supports prompt diagnosis of early stages of RHD with appropriate treatment strategies to prevent disease progression; however, the optimal approach to doing so is contested. Using mass spectrometry and machine learning (ML) algorithms, we recently identified inflammatory proteins likely to be involved in the pathogenesis of ARF/RHD. If validated, these proteins present an opportunity to improve early recognition of ARF/RHD, by incorporating them in the development of a bedside diagnostic tool. Here we document the validation of the previously identified inflammatory proteins in people with severe RHD utilizing immunological assays .
Methods: Serum levels of adiponectin (ADIPOQ), complement C7, gelsolin (GSN) and ficolin-3 (FCN-3) were measured using commercial sandwich ELISA in people with severe RHD (n=70) and healthy controls (n=59). ELISA was performed and quantified according to the manufacturer's instructions, in duplicate; the mean of the duplicates was used for statistical purposes. Statistical analysis was performed using GraphPad Prism 10.0.2 (GraphPad Software, USA). Nonparametric Mann-Whitney and Kruskal-Wallis tests were used to compare differences in protein concentration between the experimental groups. For all results, p-values less than 0.05 were considered significant.
Results: Three proteins (ADIPOQ, C7, GSN) were significantly increased in RHD cases in comparison to controls (p-value: <0.05 respectively). Two of the proteins (ADIPOQ and C7) confirmed the *in-silico* predicted findings of an association with RHD, while GSN contrasted with the negative correlation suggested by the mass spectrophotometry. The fourth protein, FCN-3, showed no significant difference between cases and controls despite a high ranking in our prior ML algorithm.
Conclusions: Preliminary data emanating from this study presents proteins may serve as potential markers for the development of a diagnostic tool that could identify individuals at risk for RHD. The data presented here also provides further evidence that *in-silico* derived data requires laboratory-based validation.

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2295 – P2.10.42

Functional analysis of elongation factor 1 alpha 1 in M1 macrophage differentiation

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Purpose: This study investigates the role of elongation factor 1 alpha 1 (EF1α1) in the differentiation of M1 macrophages. We identified EF1α1 as a protein associated with signal transducer and activator of transcription 1 (STAT1) by co-immunoprecipitation using an anti-STAT1 antibody. Although it is widely recognized that STAT1 is essential for the differentiation of M1 macrophages, the mechanisms underlying its interaction with EF1α1 are not yet fully elucidated. Furthermore, the significance of this interaction in the differentiation and functional capabilities of M1 macrophages remains unclear. Therefore, the purpose of this study is to clarify the biological significance and functional roles of the STAT1-EF1α1 interaction, thereby enhancing our understanding of the regulatory mechanisms governing M1 macrophage differentiation.
Methods: Murine RAW 264.7 macrophages were cultured with lipopolysaccharide (LPS) and interferon-gamma (IFN-γ) for 24 hours to induce M1 macrophage differentiation. Immunoprecipitation using an anti-STAT1 antibody was then performed to explore interactions of STAT1 with other proteins following stimulation. Simultaneously, RAW cells were transfected with EF1α1-targeting siRNA to reduce its expression. After a similar 24-hour exposure to LPS and IFN-γ, transcriptional levels of key genes associated with M1 macrophage differentiation, including EF1α1, were measured using quantitative polymerase chain reaction (qPCR). This experimental setup enabled evaluation of the effects of EF1α1 downregulation on the STAT1 pathway and related inflammatory markers in differentiated M1 macrophages.
Results: RAW 264.7 cells treated with LPS and IFN-γ for 24 hours demonstrated increased expression of M1 macrophage markers. Immunoprecipitation using anti-STAT1 antibodies showed co-precipitation with EF1α1, confirming their association. Additionally, EF1α1 silencing via siRNA led to a pronounced decrease in the mRNA levels of iNOS and NF-κB expression.
Conclusion: The findings of this study suggest the potential interaction between STAT1 and EF1α1 during the differentiation process of M1 macrophages. Additionally, silencing EF1α1 significantly affected the expression of key inflammatory markers such as iNOS and NF-κB, indicating a possible role of EF1α1 in the regulatory pathways of M1 macrophage activation. Further analyses are underway to elucidate EF1α1's role in M1 macrophage differentiation and function.

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P2.11 IMMUNE SENESCENCE

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454 – P2.11.01

Ageing impacts the innate immune response to vaccine adjuvants

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Immunosenescence encompasses the changes in the immune system with age. Although most commonly associated with a decline in adaptive immunity, immunosenescence also affects innate immunity, which plays a key role in priming robust adaptive responses. These immune alterations can result in a decreased ability to fight infections and reduced vaccine efficacy in later age. Vaccine adjuvants can improve the immune response induced by immunisation; consequently, designing and testing novel adjuvants and understanding their mechanism of action becomes imperative to develop more effective vaccines, particularly for older people.
Purpose: This study aims to assess the effect of ageing on the innate immune response (e.g., the NLRP3 and TLR4 pathways) to vaccine adjuvants using in vitro models of human macrophages from older and younger individuals.
Methods: Fresh peripheral blood mononuclear cells were isolated from healthy younger (20-30 y.o.) and older (>65 y.o.) donors. CD14+ monocytes were then differentiated into macrophages by exposure to macrophage colony-stimulating factor (M-CSF) for seven days. After day 7, these monocyte-derived macrophages (MDMs) were stimulated for 6 h in vitro with different vaccine adjuvants. Control cells were stimulated with LPS and Nigericin. Cell viability was measured using the Cytox96 nonradioactive cytotoxicity assay, and secretion of IL-1β, IL-18 and TNF-α monitored using ELISA.
Results: Human monocyte-derived macrophages (HMDM) from older donors released more IL-1β when exposed to QuilA saponin, Aluminium Hydroxide (Al_2OH_3) or adjuvant combinations MPLA+QuilA and MPL-A+Al_2OH_3 compared to younger individuals. A similar trend was seen in HMDM from older donors when stimulated with LPS+Nigericin. MPL-A alone promoted lower release of IL-1β in older donors compared to younger donors. Interestingly, the adjuvants and LPS+Nigericin stimulation induced lower levels of cell death in HMDM from older donors.
Conclusion: These findings suggest that adjuvants that can activate the NLRP3 inflammasome elicit a more pronounced inflammatory response in older adults. Also, we observed a potentially defective response to TLR4-mediated signalling in the context of ageing, indicating that the choice of adjuvant may need to be tailored to the recipient age bracket. Further assays are necessary to validate the increased NLRP3 activation in older adults and its potential impact on vaccine effectiveness.

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961 – P2.11.02

Protective effect of cortistatin upon thymic regression

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Thymic involution is a hallmark of immunosenescence, the innate and adaptive immune dysfunction that accompanies aging and causes ineffective responses to infections/vaccines, cancer and autoimmunity. Age-related thymus degeneration plays a key role in this process, due to the decline in T-cell production and the imbalance of T-cell proportions, leading to a decreased capacity to sustain immune competence. Additionally, thymus undergoes transient involution as a result of acute insults such as infections, stress or chemotherapy; these effects can be especially detrimental considering the context of an aged-involuted thymus in the elderly population.
Although various cytokines/chemokines, growth factors and neuropeptides/hormones have been proven to regulate thymic regression, the identification of new factors and mechanisms involved in this critical process is an urgent need. The aim of this work is to investigate the role of cortistatin, an immunomodulatory neuropeptide with potential effects in thymic function, in both acute and chronic thymus involution, by mainly using a model of aged cortistatin-deficient mice exposed to systemic infection.
We firstly confirmed the expression of cortistatin in the four main mouse thymocytes populations (CD4+CD8+, CD4+CD8, CD4-CD8+ and CD4-CD8-),and of the cortistatin-receptors SSTR2 and GHSR in thymocytes. Cortistatin deficiency was associated with accelerated age-related thymic atrophy (along 6-24 months), with involvement of changes in CD4/CD8, CD25/CD44 thymocyte populations. Remarkably, we found that 6-month-old cortistatin-deficient mice showed comparable thymic weight/cellularity than that showed by 12-month-old wild-type mice. RNA-sequencing analysis comparing cortistatin-deficient and wild-type thymocytes of 3-/6-/12-month-old mice revealed 450-900 differentially expressed genes, with an enrichment in ribosome biogenesis, ncRNA metabolic and cellular senescence processes.
Moreover, the earlier thymic regression related to cortistatin deficiency may lead to impaired responses to immune challenges. Using an experimental polymicrobial sepsis model, we found that 12-month-old cortistatin-deficient mice showed enhanced thymic involution, dysregulated inflammatory response, more severe clinical/histopathological signs and increased mortality compared to wild-type mice. Furthermore, systemic administration of cortistatin for 4-5 weeks in aged mice appeared to revert thymic involution (higher weight/cellularity, reduced thymocyte apoptosis), and improved immune response to sepsis caused by subsequent polymicrobial infection. Overall, cortistatin seems to play a beneficial role in immunosenescence by attenuating thymic regression.

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1289 – P2.11.03

How an acute stress impacts immunity in elderly patients?

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Purpose: Our society faces a major challenge with the management of the health and socio-economic burden caused by aging of the population (older than 75 years). As society ages, the incidence of physical limitations is dramatically increasing, which reduces the quality of life and increases healthcare expenditures. In western society, ~20% of the population over 60 years is confronted with moderate or severe physical limitations. This fragility results in a higher morbidity and mortality where the deleterious role of inflammation is often debated. In this context, we used hip fracture (HF) as an acute stress model that accelerates the progressive course of aging. Nowadays, this trauma, which affects around 1.6 M patients worldwide, is still associated with poor clinical outcomes in the elderly (20-30% one-year mortality; 50% inability to walk). This emphasizes the value of assessing biological factors that may predict clinical outcome after HF.
Our aim is to decipher mechanisms taking place during this medical situation, by comparing immunity from patients with different clinical outcomes (autonomy or death) in order to decrypt the respective pathways involved.
Methods: We analyze longitudinally immunological parameters evocating of the Immune Risk Phenotype in sequential pre- and post-surgical samples collected from HF patients over 75 years of age. Clinical outcomes (death and capacity to walk) were collected retrospectively. The different markers, such as white blood cells count, circulating T- B- & NK-cells (naïve/ memory/activation status), CMV responsiveness, and inflammatory molecules were screened by flow cytometry and Luminex to determine the immune status of such patients.
Results: The study revealed that HF is associated with a profound impairment of immunity. Comparing healthy elderly individuals and HF elderly patients, we found a transient T-cell leucopenia and an acute hyper-inflammation early post fracture. Among this signature, we pinpoint a central role of neopterin (an immune activation marker) which predicts the loss of autonomy and death.
Conclusion: Both innate and adaptive immunity are affected transitory during this medical event which leads to different immune trajectories. The identification of these pathways could result in the development of new therapeutic strategies for better care of the geriatric population.

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1291 – P2.11.04

Shorter supplementation of vitamin D is associated with an inflammatory and senescent immune profile in older people

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Purpose: Vitamin D is discussed to positively influence several health aspects and sufficient vitamin D levels may help to enhance healthy ageing. Ageing is associated with increased inflammation and immune senescence, comprising changes in the immune cell composition. The risk to develop vitamin D deficiency is particularly high in older individuals. Therefore, the aim was to evaluate the association between vitamin D and immune cell composition in a well characterized cohort of older people.
Methods: Cross-sectional analysis of 24 immune cell populations of 840 participants (mean age = 75.8 years) of the Berlin Aging Study II (BASE-II, mean follow-up of about 7.4 years as part of the GendAge study) was performed using flow cytometry. Blood was sampled across all seasons. All donors were stratified into different groups of serum vitamin D sufficiency, and confounding factors like season of blood collection, morbidity, and vitamin D supplementation were considered by applying strict matching strategies. The participants were predominantly healthy and only n = 23 were vitamin D deficient.
Results: Seasonal changes in vitamin D levels and immune parameters, particularly CD4+ T cells and CD8+ memory T cells (CD8+ CD45RA-), were demonstrated. Independent of season or morbidity, the number of inflammatory monocytes (CD14+ CD16+) was higher with increasing vitamin D levels. Intermediate monocytes (CD14++ CD16+), CD8+ naïve T cells (CD8+ CD45RA+ CCR7+) and CD8+ memory T cells were positively associated with vitamin D levels and even stronger with morbidity. Morbidity was higher in supplemented participants. Short-term supplemented donors (supplementation started between baseline and follow-up) exhibited higher amounts of CD8+ T_{EMRA} (CD8+ CD45RA+ CCR7-), inflammatory and intermediate monocytes as well as decreased CD4/CD8 ratio.
Conclusion: A direct association between vitamin D sufficiency and immune ageing cannot be confirmed. Contradictory, in short-term supplemented donors, a tendency towards an inflammatory and senescent immune profile was detectable. It cannot be excluded that supplementation because of newly diagnosed diseases is linked to this observation as a secondary effect. This study sheds light on the associations between vitamin D, seasonality, morbidity, and immune cell variations during healthy ageing.

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1478 – P2.11.05

Cross-talk between overweight and immunosenescence in healthy adults

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Purpose: Several studies have demonstrated that obesity and high body mass index (BMI) accelerate inflammaging and are associated with a higher risk of cardiovascular diseases, diabetes type II, and neoplastic processes. The aim of the study was to evaluate whether overweight affected some markers of T-lymphocyte senescence.
Methods: Peripheral blood samples from 29 clinically healthy individuals (12 male and 17 female) aged 21 to 55 were analyzed. Participants were distributed in two groups according to CDC weight category: group A (healthy weight; n=18), BMI (mean, ±SD) 21±2.4 and group B (overweight; n=11), BMI 29±4 (p=0.0001). The percentage distribution of lymphocytes, T-, B-, and NK cells, naïve (CD45RO-CD27+), memory (CD45RO+CD27-) and effector (CD45RO-CD27-) CD4 and CD8 T lymphocytes, proapoptotic (CD57+CD27-) CD8 T, T cells with inhibitory potential (TIGIT+) and activated (HLA-DR+CD38+) T lymphocytes were analyzed by multicolor flow cytometry (FACSCanto II, FACSDiva 6.1.2).
Results: Group A and B participants were similar in age (mean, ±SD) 33±7 and 36±10 (p>0.05). The higher BMI was associated with a higher absolute count of leucocytes (mean, ±SD) 7200±1200 vs 5800±1000 (p=0.006), lymphocytes 2340±474 vs 2026±360 (p=0.05) and B-cells 306±133 vs 209±104 (p=0.02). Group B was characterized with decreased percentage of naïve CD8+ T cells, accumulation of effector memory CD4+ T-cells (23±14 vs 35±10, p=0.03; 38±12 vs 29±10, p=0.05) and elevated proportion of TIGIT+CD8+ T lymphocytes with decreased proliferative potential (42±15 vs 27±9, p=0.01). The BMI inversely correlated with percentage of naïve CD4+ T cells (Rho= -0.48, p=0.005) and was associated with elevated expression of TIGIT+ on helper T-cells (Rho= 0.36, p=0.05).
Conclusion: Our results suggest that a high BMI may accelerate the process of immune ageing, most probably through ongoing inflammation inducing the activation, differentiation, and depletion of CD4 and CD8 T -lymphocyte clones.

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1504 – P2.11.06

Beyond the obstruction: immunological perspectives on aortic stenosis and coronary artery disease

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Purpose: It has been shown that the process of aortic valve calcification that occurs in aortic stenosis (AS) shares similarities with the systemic vascular atherosclerotic process leading to coronary artery disease (CAD), indicating a common pathogenesis. Moreover, traditional cardiovascular risk factors like hypertension, hypercholesterolemia, smoking, or diabetes are associated with both AS and CAD. The progression of AS and CAD, like atherosclerosis, involves inflammatory processes, highlighting the complex immunological interplay between these conditions.
Methods: We performed an immunological characterization of both innate and adaptive immune populations in peripheral blood from sex/age matched AS patients (n=58), CAD patients (n=20) and healthy donors (HD, n=55).
Results: Our analysis revealed distinct immune profiles between AS and CAD cohorts. CAD patients exhibited an overall increase in the frequencies of terminally differentiated effector memory T cells (CD4, CD8 and TcRγδ) when compared to AS and HD individuals (p<0.001). Additionally, both frequencies and absolute numbers of CD14+CD16+ monocytes and CD56bright NK cells were increased in CAD patients (p<0.01). In contrast, AS patients exhibited an increased CD4:CD8 T cell ratio compared to CAD patients and HD (p<0.01). Furthermore, AS patients had higher frequency of $CD4+CD28^{null}$ and $CD8+CD28^{null}$ T and an enhanced expression of CX3CR1 and CD57 surface markers in all T cell subsets (p<0.01) in comparison with HD and CAD patients. Moreover, we found lower absolute numbers of dendritic cells (DCs) and their subsets ($p < 0.01$) in AS when compared to CAD patients and HD.
Conclusion: Although AS and CAD share the same pathogenic mechanisms, our research reveals significant differences regarding the immune populations present in the peripheral blood of these patients. Our analysis suggests that CAD patients exhibit an expansion of immune populations associated with inflammation. On the other hand, AS patients show higher level of immunosenescence than CAD patients. These differences suggest the necessity to further investigate the role played by the immune system in the atherosclerotic progress, which varies between these diseases.

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1597 – P2.11.07

Role of β2 integrins on dendritic cells in immunosenescence

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Purpose: This study aims to investigate the multifaceted impact of age-related changes (immunosenescence) in dendritic cells (DC) on the immune system. Specifically, we aim to elucidate the intricate role of β2 integrins, in modulating immune cell function and intercellular communication within the context of aging. By employing a mouse model with a DC-specific knockdown of β2 integrins, we seek to delineate the mechanisms underlying age-associated alterations in DC function (intrinsic effects) and their consequential effects on other leukocytes (extrinsic effects).
Methods: We generated a mouse strain with a DC-specific conditional knockdown of β2 integrins (CD18cKO) by breeding mice with a floxed CD18 gene locus (CD18fl/fl) with CD11c-Cre (DC-restricted expression of Cre recombinase) mice. Leukocytes derived from old (>52 weeks) and young (8-12 weeks) CD18 cKO and WT (CD18fl/fl) mice were compared. Immunophenotyping of primary DC and other immune cells was conducted by flow cytometry, cytokine detection and metabolic assays. Additionally, bone marrow-derived DC (BMDC) were assessed for genotype- and age-dependent effects on DC function, including antigen presentation and T cell activation. Moreover, single-cell RNA sequencing (scRNA-Seq) of spleen cells was performed to better understand the observed intrinsic and extrinsic effects and to find underlying mechanisms.
Results: In old CD18cKO mice, primary DC exhibited lower activation marker expression but increased cytokine production as compared to wild type and young mice. This pattern was also observed in the according BMDC cultures. Furthermore, old CD18cKO BMDC induced stronger IFN-γ production in T cells after three days of co-culture. Additionally, β2 integrin deficiency increased mitochondrial metabolism in BMDC, regardless of age. Moreover, stimulated splenic T cells and B cells from aged CD18cKO mice showed elevated activation marker expression and cytokine production indicative of DC-dependent regulation of either cell type under basal conditions. ScRNA-Seq revealed age- and genotype dependent alterations in immune cell frequencies and gave insight to potential mechanisms of the observed differences.
Conclusion: These findings highlight the significant role of β2 integrins in regulating immune cell function, particularly in the context of immunosenescence. Understanding the mechanisms underlying age-related immune dysregulation could offer insights into therapeutic interventions aimed at restoring immune homeostasis in elderly.

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1756 – P2.11.08

Homelessness and Low Socioeconomic Status Drive Broad Immune Dysregulation and Impair Vaccine Induced Immunity to SARS CoV2

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Purpose and Methods: Sharp social gradients in disease outcomes observed during the Covid-19 pandemic have renewed interest in the social-to-biological processes underlying health inequalities. The linked studies, Assessing Effects of SES-Associated Psychosocial Stress on Vaccine Induced Immunity to SARS CoV-2 (AESS-Vax, n=164) and Premature Ageing in long Term Homeless Adults- Immune (PATH-I, n=59) were designed to examine psychosocial adversity as a potential biological conduit for the socioeconomic determination of health and ageing. Detailed sociodemographic, health and medical data were recorded in both studies along with psychosocial inventories of stress and wellbeing. Blood samples were collected at time of recruitment and in AESS-Vax, at multiple timepoints following vaccination with BNT162b2 (Pfizer-BioNTech). Serum and plasma samples were banked for multiplex cytokine analysis, quantification of anti-SARS CoV-2 antibodies and for confirmation of CMV serostatus. Peripheral Blood Mononuclear Cells (PBMCs) were isolated for immunophenotyping and functional analysis by spectral cytometry. Whole blood samples taken 4-6 weeks and 12-16 weeks after a third dose of BNT162b2 were used to conduct Interferon-Gamma Release Assays (IGRA) to profile antigen-specific responses.

Results:

- Total Anti-SARS CoV2 Spike antibodies are significantly lower in low SES individuals with no history of infection 6-8 and 12-16weeks after vaccination.
- IFN-γ production following stimulation with Spike peptides and whole Spike protein was higher in High SES participants 4-6 weeks and 12-16 weeks following a third dose of vaccine.
- Eight circulating cytokines are significantly elevated in homeless versus housed participants (TNF-a,IL-1B, IFN-g,IL-12p70, IL-18, IL-4, IL-10,IL-2)
- Low SES participants had diminished naïve T cell populations, enlarged TEMRA populations and higher expression of surface markers indicative of T cell exhaustion and Immunosenescence.

Conclusions:

- SES and housing status are associated with diminished humoral and cell-mediated immunity to SARS CoV2 after vaccination with BNT162b2.
- Homeless participants display elevated levels of circulating proinflammatory cytokines and cytokines resembling patterns seen in accelerated, immune driven ageing. Associations are independent of age and sex.
- T Lymphocyte populations in low SES participants display a more mature phenotype, possibly affecting functionality and responses to novel antigens. Further analysis of T cell function and of myeloid immunophenotype will follow.

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P2.12 INNATE LYMPHOID CELLS

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377 – P2.12.01

Metabolic heterogeneity between tissue-resident NK cells

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Numerous studies have now concluded that cellular metabolism is crucial for effective Natural Killer (NK) cell responses using *ex vivo* or *in vitro* approaches. However, the degree to which this is true of NK cell responses *in vivo* is less well understood. Recent studies have demonstrated large discrepancies between the metabolic parameters of immune cells measured *in vitro* compared to those measured *in vivo*. This study aimed to understand the metabolic configurations of NK cells in their in vivo niche and how in vivo metabolic features change following immune stimulation.
High-resolution quantitative proteomic analysis was performed on splenic NK cells that were activated *in vivo* and isolated for analysis directly *ex vivo.* Analysis of these proteomes revealed that a robust metabolic response was induced following *in vivo* activation. This included the increased expression of nutrient transporters, such as the amino acid transporters Slc1a5 and Slc7a5, increased expression of machinery for glycolytic and glutamine-metabolism pathways, aswell as increased expression of electron transport chain components and other mitochondrial proteins.
Next, I used single cell flow-based metabolic analyses, developed in the Finlay lab and by others, to confirm what we see at the proteomic level. I have also applied these single cell technologies to explore potential metabolic heterogeneity of tissue-resident NK cells based on the tissue of residence. Tissue-resident NK cells were studied from the spleen, uterus, bone marrow, adipose tissue, salivary gland, liver and lung.
The data shows that tissue resident NK cells differ substantially in their metabolic configuration dependant on their tissue location, both basally and upon challenge with virus and/or bacteria. The data show that the activities of two key amino acid transporters, Slc1a5 and Slc7a5, are quite distinct in NK cells residing in different tissues. I then studied the impact of deleting either of these amino acid transporters, specifically in NK cells, on the homeostasis/function of NK cells is different tissues.
This study is revealing the in vivo metabolic features of tissue resident NK cells across a range of tissues, and how these metabolic configurations change to respond to immunological challenge.

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489 – P2.12.02

Divergent roles for STAT4 in shaping differentiation of cytotoxic ILC1 and NK cells during gut inflammation

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Purpose: Natural killer (NK) cells and type 1 innate lymphoid cells (ILC1) play pivotal roles in host defense and tissue homeostasis, relying on the transcription factor STAT4 for effector functions. Our study aims to elucidate the role of STAT4 in regulating the effector differentiation of NK cells and ILC1 within the intestinal microenvironment during inflammation.
Methods: In this study we generated a novel mouse model enabling the deletion of *Stat4* in *Ncr1*-expressing cells. We then utilized the widely adopted mouse model of intestinal inflammation, namely dextran sulfate sodium (DSS)-induced colitis, to study the effects of STAT4 deletion in large intestinal lamina propria (lilp) *Ncr1*-expressing cells in colitis settings. Additionally, we employed single-cell transcriptomic analysis to deconvolute how *Stat4* deletion shapes the transcriptomic programs of lilp NK/ILC1.
Results: Deletion of *Stat4* in *Ncr1*-expressing cells had detrimental effects on colitis severity, with increased weight loss and colitis score in $Ncr1^{iCre}$ $Stat4^{fl/fl}$ mice, compared to wt $Stat4^{fl/fl}$ littermates. At cellular and molecular level, we showed that STAT4 is necessary for the terminal differentiation of NK cells, as well as for driving the effector responses of both NK and ILC1. Intriguingly, this deficiency paradoxically amplifies the generation of cytotoxic ILC1 during intestinal inflammation. Transcriptomic analyses reveal a distinct gene expression profile in Stat4-deficient ILC1, characterized by the upregulation of gene modules typically regulated by STAT5. This dysregulated transcriptional landscape translated into aberrant effector differentiation, particularly evident upon IL-2 stimulation. Furthermore, STAT4 expression in NCR+ innate lymphocytes limited the severity of colitis by curtailing the pathogenic production of IL-13 from CD4+ T cells within the large intestine.
Conclusion: Our study elucidates the intricate regulatory network orchestrated by STAT4 in governing the effector functions of NK cells and ILC1 in the intestinal milieu. These findings not only highlight shared and distinct mechanisms of STAT4-mediated transcriptional control but also underscore potential therapeutic avenues for modulating innate lymphocyte responses in the context of inflammatory bowel disease.

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548 – P2.12.03

Oxysterols modulate iNKT cell function

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Invariant NKT (iNKT) cells are a distinct subtype of lymphocytes characterized by their reactivity to lipids presented by CD1d. They are relatively unique amongst T cells because of their ability to rapidly produce a variety of cytokines, while also having cytotoxic capabilities. The tumour microenvironment (TME) is known to be suppressive and affects immune cells in different ways. Lipids and other metabolites are increased in the TME and can induce immune cell dysfunction.

Oxysterols are a family of oxidized derivatives of cholesterol whose levels are elevated in some tumors and are known to impact immune cell function. In our work we investigated how oxysterols affect iNKT cell function. Splenic iNKT cells were activated ex-vivo in the absence/presence of two different oxysterols: 25-hydroxycholesterol (25-HC) and 27-hydroxycholesterol (27-HC). We found that both oxysterols inhibited iNKT cell cytokine production. We Investigated the molecular mechanisms underlying the immunosuppressive effects of these oxysterols, including effects on metabolism and signalling pathways. Oxysterol can modulate of two families of transcription factors, SREBP and the LXR. However, neither PF429242 (pharmacological inhibitor of SREBP activation) or GW3965 (a synthetic LXR agonist) affected iNKT cell function in our short-term ex-vivo activation model.

We also analysed the activity of mTORC1 by staining the phosphorylated S6 ribosomal protein (rpS6) and protein synthesis via puromycin incorporation as metabolic readouts. While neither oxysterol affected the rate of protein synthesis, oxysterols impaired the phosphorylation of S6 ribosomal protein. These data suggest that oxysterols might be affecting iNKT cell metabolic signalling.

The lipid microenvironment in the plasma membrane is crucial for signal transduction. Because the presence of oxysterols can change the membrane fluidity, we investigated if alterations in the membrane order might be responsible for the effects on iNKT cell function. The analysis of Di-4-ANEPPDHQ staining showed increased membrane disorder on oxysterol-treated cells, suggesting that a disturbed membrane is underlying the reduced function of iNKT cells.

Understanding the specific role of 25HC and 27HC in inhibiting different immune cells' functions may pave the way for the development of novel pharmacological interventions aimed at modulating immune responses in various pathological conditions.

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890 – P2.12.04

Novel FFAR2 agonist prevents development of type 1 diabetes in C57BL/6 mice

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Type 1 diabetes (T1D) is a chronic autoimmune disease that usually develops in early childhood. The recent market approval of the anti-CD3 monoclonal antibody (teplizumab) is a major advance in protecting at-risk patients from the onset of T1D. In addition to the presence of autoantibodies, T1D-prone individuals exhibit alteration in the gut microbiota and the intestinal immune response. As intestinal epithelial and immune cells express receptors for free fatty acids (FFAR), and it was shown that stimulation of FFAR2 can down-regulate inflammation during Crohn's disease, we have tested the efficacy of the novel agonist for FFAR2 (Cpd1) in the prevention of T1D induced by five injections of low-dose of streptozotocin in C57BL/6 mice. Oral application of Cpd1 (applied from the 1st day of T1D induction for 20 days in total) resulted in a significant reduction of T1D incidence and lowered mean glycemia levels in the treated mice. Accordingly, these mice had lower insulitis in the pancreas and higher insulin production. *Ex vivo* analysis on the 8th day post T1D induction showed higher proportions of innate lymphoid cell type 3 (ILC3) that produced IL-2 within the small intestine (SI) lamina propria of Cpd1-treated mice. The increase in IL-2 correlated with higher Treg proportions detected on day 12 in the SI lamina propria. Similar findings were observed in NOD mice, a spontaneous T1D model, treated with Cpd1 on a daily basis (from 8 weeks until 12 weeks of age). Cpd1 increased the presence of ILC3 in the SI lamina propria of NOD mice, and more specifically, the proportion of Nkp46^{-} ILC3. In total, these results demonstrate that Cpd1 increased the population of ILC3 and Treg by activating FFAR2, thereby modulating the anti-inflammatory immune response and protecting the pancreas from the imposed damage.
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1114 – P2.12.05

Cobalt exposure enhances group 2 innate lymphoid cell apoptosis in vitro

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Purpose: ILC2 is an environmental sensor that can aggravate allergic diseases by expressing type-2 cytokines, but it also participates in tissue repair by secreting Amphiregulin. The homeostasis of ILC2 in the body regulates the inflammatory status of the mucosal site, which is where most environmental pollutants are encountered. Heavy metals are harmful environmental pollutants that can accumulate in the human body through bioaccumulation. Cobalt is primarily absorbed through the lungs and digestive tract and its exposure is associated with allergic contact dermatitis and occupational asthma. When inhaled, cobalt-containing dust can lead to a pathological response in the lung parenchyma. However, the direct effects of cobalt exposure on immune toxicity are still unclear. This study investigates the impacts of cobalt exposure on ILC2, including cell proliferation, apoptosis, and function *in vitro*.
Method: We expanded ILC2s *in vitro* and treated them with varying concentrations of $CoCl_2$ (less than 1 μM levels). We then stimulated the cells with rmIL-33 and analyzed the cell numbers of ILC2s using Cell Counting Kit-8. We also used flow cytometry to measure the Ki-67 proliferative activity and Annexin-V^+ apoptosis. Additionally, we measured the type-2 cytokine expression abilities of ILC2s using enzyme-linked immunosorbent assay. Finally, we evaluated the expression of tissue repair-related genes by Real-time PCR analysis.
Results: ILC2 cells treated with 0.1 or 1 μM $CoCl_2$ exhibited a significant decrease in cell count and proliferation capability. Upon stimulation with rmIL-33, there was a significant increase in the number of apoptotic ILC2 cells after 6-hour $CoCl_2$ treatment. However, no significant changes were observed in the expression levels of the *Areg* gene, which is associated with tissue repair, and *Mapk1* gene, which is related to cell proliferation, in ILC2 cells.
Conclusion: Cobalt treatment *in vitro* increases ILC2 apoptosis without affecting their function. This may lead to decreased tissue repair in inflamed mucosal sites. However, the mechanism and health implications of cobalt exposure requires further investigation.

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1515 – P2.12.06

High-Dimensional Single-Cell Analysis of Human Natural Killer Cell Heterogeneity

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Purpose: Our initiative aims to propose a standardized terminology and provide a basis for subsequent studies of the role of human Natural killer (NK) cells in health and disease.
Methods: Our comprehensive analysis, based on a joint consensus of 20 leading laboratories specializing in NK cell research, integrates proteomic (CITE-seq) and transcriptomic data (scRNAseq) from a total of 224,689 NK cells isolated from 718 donors. Our study starts in the blood of healthy donors and expands by analyzing the heterogeneity of NK cells in 3 healthy tissues (tonsil, lung, and intestine). Finally, we analyze the distribution of NK cell subsets in tumor tissues and blood from 22 cancer types.
Results:
- Three prominent NK cell subsets:
Our analysis delineates three distinct NK cell subsets: NK1, NK2 and NK3. Their gene profiles extend beyond the peripheral blood of healthy individuals and reveal the heterogeneity of NK cells in different tissues. Our results describe the unique molecular features, key transcription factors, major biological functions, significant metabolic traits, cytokine responses of each subgroup, and ontogenetic origins of NK cells leading to distinct transcriptional trajectories.
- Two distinct NK cell trajectories:
Our study documents for the first time unique transcriptional pathways for the NK1 and NK2 populations.
- Relationships between NK cells and other innate lymphoid cells:
The different ILC subsets identified in tonsils, lungs, and intraepithelial lymphocytes show different relationships with the three NK subsets. For example, the NK2 subset shows similarities with the precursors of innate lymphoid cell precursors.
- Composition of the NK compartment in pathological conditions:
The distribution of NK cell subsets in 22 tumor types varies by tumor type and shows no strict correlation with the distribution in blood.
- A user-friendly classification system:
We have developed a classification system that is available through an easy-to-use web portal. It is both intuitive and robust and facilitates the accurate categorization of NK cell populations in different samples.

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Conclusion: This classification is intended to serve as a reference point for future studies, enabling a more uniform approach to the understanding and use of NK cells in both research and clinical settings.

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1601 – P2.12.07

Analysis of the mechanisms regulating ILC2 immune checkpoints expression and function in the tumor microenvironment

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Background: ILC2s are a subset of innate lymphoid cells involved in the type 2 immunity. ILC2s are mainly resident at mucosal barriers and are specialized in the secretion of type 2 cytokines IL-4, IL-5, and IL-13 in response to IL-33, IL-25 and TSLP. Although the expression of immune checkpoints has been reported on ILC2, little is known about their regulation and function in these cells. We found that ILC2 in the tumor microenvironment (TME) display higher levels of immune checkpoints expression, compared to ILC2s freshly isolated from healthy donors' peripheral blood. In particular, we observed that ILC2 in the pleural effusions from lung cancer patients express PD1 and CTLA4. These data suggest that the TME regulates ILC2 response through these receptors. The immune checkpoint ligands are released as soluble molecules in the TME and expressed on the membrane not only of tumor cells but also of immune cells such as macrophages.
Purpose: The aims of my project are to study the molecular mechanisms involved in the expression and function of PD1 and CTLA4 in ILC2 and to investigate the contribution of tumor-resident macrophages in this regulation.
Methods: To achieve the objectives of the project, we performed in vitro experiments using primary activated ILC2 cultures as a model reproducing ILC2 phenotype in the TME. We performed co-culture experiments with monocytes-derived macrophages (MoM) in presence and absence of immune checkpoint inhibitors (ICI). We analysed ILC2 phenotype, cytokine production and gene expression by RNA sequencing.
Results: We identified the targets of PD1 and CTLA4-mediated inhibition in ILC2. We found that ILC2/MoM co-culture results in a reduction of PD1 and an increase of CTLA4 expression. We also demonstrated that MoM downregulate the expression of type 2 cytokines by ILC2.
Conclusions: Collectively our data show that MoM are able to limit ILC2 effector functions and suggest that the immune checkpoints PD1 and CTLA4 play a distinct role in controlling ILC2 response in the TME.

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P2.14 LYMPHOCYTE DIFFERENTIATION

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476 – P2.14.01

Role of the transcription factor Foxo1 in the differentiation of naïve CD4 T lymphocytes

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Following activation by antigen presenting cells in the periphery, naïve CD4 T cells (CD4 T_N cells) can differentiate into a variety of well documented T-helper (TH) cell subsets, such as TH1, TH2, TH17 or induced regulatory (iTreg) T cells, characterized by their cytokine production profiles and specific effector functions. The immunological context in which CD4 T_N cells are immersed at the time of their activation is known to guide TH cell lineage commitment and thereby to adjust the quality of T-cell responses to the nature of the stimuli. We recently observed that Foxo1-deficient CD4 T_N cells are prone to differentiate into TH1 and TH2 cells *in vitro*, even in the absence of exogenous polarizing cytokines in the culture medium (IL-12 for TH1 polarization and IL-4 for TH2 polarization). In line with these results, higher percentages of memory CD4 T cells from the secondary lymphoid organs of $Foxo1^{TKO}$ mice compared with control mice are able to produce IFN-γ (TH1) and IL-13 (TH2) *ex vivo*. The aim of the present project is to decipher the mechanisms underlying the enhanced differentiation of CD4 T_N cells into TH1 and TH2 effector cells in the absence of Foxo1 expression. Indeed, inadequate responses might lead to immune disorders such as asthma, allergy or autoimmune diseases. To answer this question, we performed RNA-seq and ATAC-seq of Foxo1-deficient CD4 T_N cells to determine their transcriptomic signature and compare it with the transcriptomic signatures of the different TH cell subsets in the literature by using Ingenuity Pathway Analysis and GSEA. The results obtained suggest that the $Foxo1^{TKO}$ CD4 T_N cells are already commited towards the Th1/Th2 cell lineages. It is currently accepted that IL-2 promotes the differentiation of naïve CD4 T_N cells into Th1/Th2 cells while inhibiting Th17-cell development. Interestingly, our latest results strongly suggest that Foxo1 deficiency makes CD4 T_N cells more sensitive to IL-2, by promoting STAT5 signaling pathway.

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789 – P2.14.02

Preliminary results of the fluctuation of peripheral follicular T cells and their subsets in lupus nephritis

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Purpose: Follicular T (TF) cells are implicated in the pathogenesis of Lupus Nephritis (LN) by mediating the selection of high-affinity B cells in germinal centers. They classically reside in secondary lymphoid organs; however, T cells with similar markers can be detected in the blood, the exact origin and function of which have not yet been specified. Herein, we describe the population of circulating TF cells in patients with LN and their association with disease hallmarks.

Methods: Peripheral blood was collected from 25 LN patients (LN) and 25 healthy controls (HC). Flow cytometry was performed to assess cTF (CD4+CD45RA$^-$CXCR5+) cells, and their subsets, defined as cTF1 (CD4+CD45RA$^-$CXCR5+CXCR3+CCR6-), cTF2 (CD4+CD45RA$^-$CXCR5+CXCR3-CCR6-), cTF17 (CD4+CD45RA$^-$CXCR5+CXCR3-CCR6+) and cTF-ICOS+ (CD4+CD45RA$^-$CXCR5+ICOS+).

Results: Here are presented preliminary results analyzed in 15 LN patients (age = 38±8yrs) compared to 9 HC (age = 31.5±7yrs). The percentage and total number of cTFH cells were similar between LN and HC [11(0.6-22.4) % and 41.6(3.4-412.7) cells/μL, vs. 8.8(1.9-12.1) % and 43.5(13.3-69.6) cells/μL, respectively]. cTFH-ICOS+, cTFH1 and cTFH2 cells were upregulated in LN compared to HC [0.9(0-5.2) vs. 0.256(0-1) cells/μL, 6.7(0.2-25.9) vs. 1.9(0.4-13.3) cells/μL, and 9.88(0-14.5) vs 1.82(0.46-32) cells/μL, respectively], while the cTFH17 compartment was obviously condensed [6.58 (0.79-213.8) vs 16.9(0.6-29.2) cells/μL, respectively]. Increased SLEDAI-2K score (>6) was associated with increased cTFH [75(14-413) vs. 34(3-99) cells/μL], cTFH1 [12.45(2-26) vs. 3(0.2-18) cells/μL], cTFH2 [10.38(5-14) vs. 2.27(0-11) cells/μL], cTFH17 [23.8(0.8-214) vs. 1.6(1-44) cells/μL], and cTFH-ICOS+ [1.13(0.5-5.19) vs. 0.552(0-1.18) cells/μL]. Increased levels of proteinuria were characterized by increased cTFH1 [12.4(2.08-25.88) vs. 6.7(0.2-18.17) cells/μL] but reduced cTFH17 [3.7(0.8-42.3) vs. 6.6(1.06-213.8) cells/μL] and cTFH-ICOS+ [0.22(0-5.19) vs. 0.9(0-3.7) cells/μL].

Conclusion: Most of the subtypes of cTFH were increased in active LN patients, while proteinuria levels were associated with increased cTFH1 but reduced cTFH17 and cTFH-ICOS+, suggesting the involvement of different pathogenic mechanisms and the possibility of an appealing therapeutic target for LN.

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937 – P2.14.03

Data-independent acquisition mass spectrometry reveals the protein landscape of human regulatory T-cells

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Regulatory T cells (Tregs) are critical in regulating the immune response. In vitro induced Treg cells (iTregs) have significant potential in clinical medicine. However, applying iTregs as therapeutics is complicated due to their poor stability and variable suppressive activity. To gain a better understanding of the molecular characteristics specific to iTregs, a data-independent proteomics analysis was performed. A group of Treg signature proteins was identified, including FOXP3, EOS, IL2RA, CTLA4, PDCD1, IKZF3, LAG3, RUNX1 and HIC1, all consistent with the known molecular characteristics of Tregs. These Treg signature proteins were validated using targeted proteomics and TaqMan assay. In addition, we discovered that Leupaxin (LPXN) is upregulated in response to Treg differentiation. The LPXN deficient cells showed impaired expression of Treg protein markers, FOXP3, EOS, and IKZF3 and reduced ability to suppress effector cells. Collectively, this study characterized the proteomic signature of human iTregs and identified a novel role of LPXN in the development and suppressive activity of Tregs. Our data provides a resource for further research into Treg cell biology.

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978 – P2.14.04

Aryl hydrocarbon receptor signalling contributes to the enhanced ability of human gut-topic T-cells to acquire a Trm phenotype.

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Purpose: Memory precursor T-cells recruited into tissues become tissue resident memory cells (T_{RM}) under the influence of local cues; some precursors show pre-commitment to a T_{RM} fate. The human intestine contains T_{RM} which play a key role in inflammatory bowel disease (IBD). Modulation of the immune system by environmental factors is pivotal to IBD onset and natural history. Here we test the hypothesis that the aryl hydrocarbon receptor (AHR), a key environmental sensor responsive to dietary and microbial ligands, regulates the generation of T_{RM} from gut-tropic circulating precursors.
Methods: Peripheral blood mononuclear cells were obtained from healthy human donors and conventional (αβTCR+) T-cells FACS purified into: naïve (CD45RA+), gut-tropic memory (CD45RA-β7integrin+) and non-gut memory (CD45RA-β7-) fractions. Expression of *AHR* and its target gene *CYP1A1*, as well as genes associated with T-cell residency, were quantified by qRT-PCR. Generation of T_{RM}-like cells (CD69+CD103+) was assessed following sequential culture in IL-15 and TGFβ, with or without the AHR antagonist CH223191.
Results: All sorted populations expressed AHR, but mean relative expression was significantly higher in memory (both β7+ and β7-) compared with naïve populations (0.0081 vs 0.0027, p = 0.0002; n=6). In contrast, expression of *CYP1A1*, indicative of active AHR signalling, was significantly greater in gut-tropic (β7+) than β7- memory cells (4.5x10-5 vs 1.7x10-5, p = 0.02; n=6). Expression of *CYP1A1* was similar in naïve and β7- memory cells. CD69+CD103+ T_{RM}-like cells with reduced expression of the tissue egress genes *S1PR1* and *KLF2*, were generated from memory T-cells *in vitro*. Significantly more β7+ than β7- memory cells acquired a T_{RM}-like phenotype (CD8+: 23% vs 11%, p=0.02 & CD8-(CD4+): 3.8% vs 1.1%, p =0.007; n= 5). The generation of T_{RM}-like cells was significantly reduced by AHR antagonism (β7+CD8+: 8.79% in CH223191 vs 23% in vehicle, p=0.02 & β7+CD8- (CD4+): 0.33 vs 3.92, p =0.007, n= 5) with corresponding preservation of tissue egress gene expression.
Conclusion: AHR signalling enhances the generation of human T_{RM}-like cells *in vitro*. Memory T-cells expressing β7 integrin, with the potential to traffic to the intestinal mucosa, have a more active AHR signalling pathway and enhanced T_{RM} potential.

Wellcome Trust, BRUK.

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1092 – P2.14.05

In-depth characterization of human induced regulatory T cells by mass cytometry

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Regulatory T cells (Tregs) are responsible of the maintenance of immunological homeostasis and self-tolerance. Unlike other T helper cells, Tregs suppress immune responses via contact dependent and humoral factor-mediated mechanisms. However, abnormalities in Treg numbers, frequencies, and suppressive function can trigger autoimmune diseases while Treg accumulation in tumors suppresses anti-tumor immunity. The aim of this study was to perform an in-depth characterization of early human induced Treg cell differentiation by high-dimensional single-cell mass cytometry. For this purpose, a panel of 25 markers was designed and validated in Tregs, differentiated *in vitro* from naïve human umbilical cord blood derived $CD4^+$ T cells. The expression of these markers was further studied in Tregs compared to activated control Th0 cells over time. Our results revealed that Tregs cluster distinct from Th0 control cells. Tregs upregulated the key transcription factor Foxp3 and several co-stimulatory and -inhibitory molecules (i.e. CCR4, CCR7, CXCR3, TIM3, CD73, PD1, CD137 and CD103) during early human Treg cell differentiation. In summary, our study provides valuable insights into the phenotypic characteristics of human induced Tregs during early differentiation, and their potential therapeutic applications.

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1402 – P2.14.06

Unraveling the contribution of B cells on the differentiation of specific CD4+ T cells using a novel MHC class II multimer platform

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CD4+ Tfh-B-cell interaction is necessary to induce affinity-matured plasma and memory B cells, resulting in a high-affinity antibody response. Vice versa B cells might also affect the phenotypic differentiation of CD4+ T cells in the lymph node. Recently, we found that CD4+ T cells in multiple sclerosis (MS) patients on B cell depleting (anti-CD20) therapy upregulated the activation markers HLA-DR and CD38 after second SARS-CoV2 vaccination compared to untreated MS controls [Verstegen *et al.* JNNP 2024]. We aim to unravel the role of B cells in the formation and phenotypic differentiation of specific CD4+ T cell responses by performing *ex vivo* deep-phenotyping on spike-specific CD4+ T cells 7 days after second SARS-CoV2 mRNA vaccination in MS patients with or without anti-CD20 therapy. Major histocompatibility complex (MHC) class II tetramers or dextramers might be used to detect antigen-specific CD4+ T cell responses directly *ex vivo*. However, the current reagents are often limited in the signal intensity and binding affinity to the antigen-specific T cell receptor. Therefore, we developed novel peptide-specific MHC class II multimers based on Ig Fc-fusion protein framework to optimize the detection of antigen-specific CD4+ T cells. We proved that our multimers stained antigen-specific CD4+ T cells with much higher signal intensity and specificity than commercial reagents. Besides, multimers of the MS dominant HLA type HLADRB1 15:01 conjugated with spike peptide 866-880 showed specific staining in HLADRB1 15:01 positive donors compared to negative ones. Furthermore, no staining was observed in COVID19 naïve individuals. Using these MHC class II multimers, spike-specific CD4+ T cells are sorted from MS patients, either untreated (n=7) or on anti-CD20 therapy with (n=7) or without an anti-RBD IgG response (n=7). During sorting, proteomic data on memory and T helper phenotype of the spike-specific CD4+ T cells is acquired. In combination with single cell RNA-sequencing data of these sorted cells, we expect to obtain insight in the effect of lack of B cell interaction on the CD4+ T cell differentiation during a recently developed immune response.

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1617 – P2.14.07

Thymic cell competition relies on efficient bone marrow contribution to prevent T cell acute lymphoblastic leukemia

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T lymphocytes differentiate in the thymus from hematopoietic progenitors of bone marrow origin, which progressively lose multipotency, commit to the T cell lineage and differentiate. This is a process characterized by high cell turnover and, at steady state, mouse thymocytes are all virtually replaced every 4 weeks. We have shown that thymus turnover is partly regulated by cell competition involving thymocytes at the double negative (DN) 2 and 3-early stages of differentiation. Specifically, young DN2 and DN3-early with a shorter time of thymus residency outcompete and replace old DN3-early with longer times of thymus residency. If thymus seeding by competent progenitors is temporarily impaired, thymocytes can self-renew and autonomously maintain thymopoiesis. However, such property bears a heavy burden on the organism because it causes T cell acute lymphoblastic leukemia (T-ALL). Here, we show that thymus autonomy and T-ALL can be triggered as result of inefficient bone marrow reconstitution of γ_C-deficient mice. Interestingly, the risk of T-ALL was inversely proportional to the level of engraftment of the bone marrow, i.e. the lower the efficiency of wild type bone marrow engraftment, the higher the incidence of T-ALL. Our data reveal that aberrant thymocytes, probably the precursors of leukemia, emerge very early, and we establish a safe threshold for the input of healthy HSPCs and bone marrow reconstitution capable of inhibiting thymus autonomy. Altogether, our data reveals the leukemogenic potential of thymocytes upon bone marrow correction of γ_C-deficiency, and the requirements to prevent such unwanted outcome.

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1870 – P2.14.08

DNA methylation profiles guide development and differentiation in T cells in the thymus and in the periphery, but epigenetic recoding of proliferation only occurs after full maturation

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Epigenetic mechanisms are known drivers of T lymphocyte development and function. This is attributed to epigenetic changes on gene regulatory elements like promoters and enhancers, which control the expression patterns of their target genes and with this, imprint cell type-specific transcriptional profiles. In addition, in previous studies, we also observed a progressive DNA methylation loss in the heterochromatic parts of the genome in human T cells from the peripheral blood, as a lasting epigenetic record of undergone proliferation episodes. Here, we addressed the epigenetic remodeling of the DNA methylome during the thymic development of T cells, which so far has not been clarified in detail.
We generated genome-wide DNA methylation profiles of well defined, human ex vivo isolated thymocyte populations at various stages of T cell development. We observed two waves of epigenetic remodeling taking place: the first from the early double-negative stages to phases of TCR rearrangement and the second one after finishing TCR rearrangement to the mature state. Although cells during TCR rearrangement undergo a series of phenotypic and transcriptomic changes, the DNA methylome during this phase is stable, until the next major rearrangement to the mature state. Furthermore, we did not observe significant loss of DNA methylation in the heterochromatin throughout thymic development, although cells undergo episodes of strong proliferation. This finding is in stark contrast to proliferating mature cells from the periphery, which display a clear loss of heterochromatic DNA methylation, which we could identify to be driven by DNA replication speed. Cells displaying increased proliferation-induced loss of heterochromatic DNA methylation were predisposed to memory differentiation, indicating that the proliferation history of cells influences their further developmental fate.
Taken together, we could show that remodeling of the DNA methylome occurs during thymic T cell development as well as during memory generation in the periphery, however, proliferation episodes seem to be only imprinted into the DNA methylome after full maturation. Then, however, cells with an accumulated proliferation history being recorded in their DNA methylome, are predisposed for further differentiation into memory phenotypes.

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2031 – P2.14.09

T cell-intrinsic PKD3 fine-tunes the development into CD8+ central memory T cells

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The orchestration of a T cell response is tightly balanced, with both proper T cell activation as well as memory development being critical to ensure protection from pathogens without leading to autoimmunity.
Recently, we have identified Protein Kinase D3 (PKD3) as a novel regulator of this process. Thus, T lymphocytes from conventional PKD3-deficient mice are hyper-reactive upon polyclonal stimulation *in vitro* or immunization *in vivo*. However, this might be explained by the mice's substantial skewing in their T cell compartment towards an effector/memory phenotype, already under steady-state conditions.
In our current study, we have complemented our initial observations with mouse models that either lack, or overexpress a constitutive active form of PKD3, specifically in the T cell compartment. Combining these model systems, we surprisingly observed T cell-intrinsic PKD3 to be dispensable for the cell's activation. Moreover, we were able to specify that the strongly skewed T cell compartment of PKD3$^{-/-}$ mice is largely caused T cell-extrinsically, whereas T cell-intrinsic PKD3 specifically fine-tunes the development into CD8^{+} central memory T cells.

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2151 – P2.14.10

Mixed phenotype acute leukemia: a case report

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Mixed phenotype acute leukemia is a rare form of leukemia that combines two different types: acute myelocytic leukemia (AML) and acute lymphoblastic leukemia (ALL). This leukemia has an aggressive profile, and presents a disjunctive in treatment, as there is no standardized treatment protocol.
We present a case of an 18-year-old female patient with a lymphocyte percentage of 80% without associated leukocytosis, maintained for 20 days. Immunophenotyping by cytometry showed an immunophenotypic pattern compatible with **type B acute lymphoblastic leukemia with 87% of blast cells.** Negative CD10 expression is characteristic of the pro-B subtype, although this is usually associated with NG2 and CD15 expression. The MPO study was negative.On the other hand, the expression of myeloid markers and CD66c is characteristic of B-ALL associated with the t(9;22) BCR-ABL1 translocation, which was negative by FISH. On the other hand, massive sequencing tests showed a **monosomy of chromosome7** associated with a deletion in the IKZF1 gene. This finding, more characteristic of acute myeloid leukemias, presented the possibility of an acute leukemia of mixed phenotype.
In addition, in the massive sequencing, genetic alterations were found in chromosome 8 and 12. As addedinformation of the study by Optical Mapping, a t(2;9)(p11.1;p24.1) and another t(12;12)(p13.31; p12.1) of uncertain significance were detected.
Treatment with PETHEMA LAL 2019 is decided, since the result of MPO- CMF has more weight in the diagnosis of B-ALL than the possibility of leukemia of mixed etiology.
The morphological smear in Hematology after treatment shows 4% undifferentiated blasts and post- chemotherapy agranulocytosis and in the CMF 0.04% of EMR B-lymphoblasts: CD19+, CD10-, CD34+, CD20-, CD73/CD304+, CD81+, CD38 weak.
Currently the patient is awaiting unrelated BMT, responding well to chemotherapy treatment.

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P2.15 LYMPHOID LINEAGE

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451 – P2.15.01

The role of toll-like receptor agonists in modulating thymocyte development in fetal thymus organ culture

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The microenvironment of the thymus represents a unique phenomenon, where even in physiological conditions there is a constitutive production of pro-inflammatory mediators, like toll-like receptor (TLR) induced cytokines and type I IFNs. This type of tonic inflammation is believed to play a crucial role in thymocyte development; nevertheless, a comprehensive understanding remains elusive.
Using the fetal thymus organ culture (FTOC) system, we aimed to determine how introducing additional exogenous toll-like receptor agonists like CpG ODN (TLR9), LPS (TLR4) and Gardiquimod (TLR7) would affect the development of different thymocyte populations. Additionally, we examined the impact of IFNα1, a product of TLR-mediated signaling, on thymocyte development. For this purpose, we harvested the thymi from E16.5 or E17.5 C57BL/6J mouse embryos, cultivated them in FTOC with various stimulants, and analyzed the samples at multiple time-points.
By using qPCR, we were able to observe several stimuli-induced alterations in gene expression, proving that the mediators are able to affect thymocyte development in the FTOC system. For analyzing functional changes in the thymus, we employed a 17-color spectral flow cytometry panel, enabling simultaneous assessment of over 40 different thymic cell populations. We saw numerous changes across various thymocyte populations, including an increase of CD4SP and CD8SP mature 1 (CD3+CD5+CD69+MHCI+) and mature 2 (CD3+CD5+CD69-MHCI+) subsets, induced by both CpG ODN and IFNα1. Additionally, CpG led to a significant rise in CD4SP cells undergoing negative selection (CD3+CD5+Foxp3-CD69+Helios+); and at the same time induced a negative trend in the Treg population (CD3+CD5+Foxp3+CD25+). What's more, when incubating thymi with anti-IFNα1 antibodies we detected trends in some T-cell populations that were opposite of IFNα1 and CpG treatment.
Our experiments provide insight into how various thymocyte populations respond to an augmented inflammatory environment induced by different TLRs. This data also suggests that manipulation of the thymic pro-inflammatory microenvironment has a potential to modulate the developmental fate of thymocytes, and thus consequently affect the peripheral T cell pool.

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2024 – P2.15.02

Urinary immune cells in ANCA-associated vasculitis: a marker of disease relapse?

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Background: Immune cells are detectable in the urine of healthy individuals, and their numbers increase during infection or inflammation, particularly during renal inflammation. ANCA-Associated Vasculitis (AAV) is an autoimmune disease that follows a relapsing-remitting disease course and commonly affects the kidneys. Measurement of urinary immune cells as a non-invasive prognostic marker has been demonstrated in another autoimmune renal disease, lupus nephritis. This work seeks to define the urine immune cell phenotype during a period of stable remission and enumerate these populations for comparison to a healthy control group.
Methods: Urine was collected from patients recruited to the RITA Ireland Vasculitis Biobank (n=51). Healthy controls were recruited from Trinity Translational Medicine Institute (n=12). Patients with positive urine cultures were excluded from analysis. Cells were isolated from the urine and stained with fluorescent conjugated antibodies for CD45, CD3, CD16, CD14 and CD15 for subsequent analysis by flow cytometry using the BD FACS Canto II. Counting beads were used to allow estimation of absolute cell counts. All samples were gated on live cells to mitigate excess autofluorescence from urine.
Results: 41 patients across 51 encounters were included. The cohort was 54% female and 46% male, with a median age of 68 years. It was found that patients with AAV in remission presented with a statistically higher number of CD45+ cells in their urine (median 18,800 cells/ml) when compared to healthy controls (median 5,540 cells/ml) (p=0.0002). Of these leukocytes, neutrophils were the most common subset in both remission patients (median 16,300 cells/ml) and healthy controls (1,665 cells/ml) (p=0.0009). T cells were significantly higher in patients in remission (median 669 cells/ml) than in healthy controls (median 111 cells/mls)(p=0.0003). Classical monocytes were the most common monocyte subset identified, with a median of 602 cells/ml in remission patients and 26.2 cells/ml in healthy controls (p=0.0032).
Conclusion: Despite being in clinical remission, AAV patients have significantly elevated numbers of T cells, monocytes and neutrophils in their urine when compared to healthy controls. This could indicate persistent subclinical renal inflammation and marks the potential for use of urinary immune cells as a non-invasive marker of disease progression in AAV.

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2175 – P2.15.03

Vacuolated lymphocytes in pleomorphic mantle cell lymphoma, case report

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Introduction: Mantle cell lymphoma (MCL) is a subtype of non-Hodgkin lymphoma derived from the malignant transformation of B lymphocytes in the mantle zone of lymph nodes. One of its variants, the pleomorphic variant, resembles diffuse large B-cell lymphoma and carries a less favorable prognosis. While cytoplasmic vacuoles are common in various conditions (infections, cancer, metabolic diseases) their occurrence in lymphoproliferative syndromes is rare.
Objectives: We report a case of pleomorphic variant MCL with cytoplasmic vacuoles to elucidate its characteristics and peculiarities to facilitate appropriate differential diagnosis.
Case description: A 65-year-old male presenting significant weight loss underwent medical examination revealing lymphadenopathy and splenomegaly. Anemia and thrombocytopenia were noted on blood count. White blood cell count was $3{,}56x10^9$/l, with a normal absolute lymphocyte count of $1{,}45x10^9$/l. The peripheral blood smear revealed vacuolated lymphocytes with a mature appearance, prompting a bone marrow (BM) aspiration. The cytomorphology of the BM showed significant infiltration of atypical lymphocytes, most of which had large vacuoles. Flow cytometry (FC) detected infiltration by 16% of large-sized and complex B cells positive for CD19, CD20, CD79b, CD5 and bright kappa light chain being negative for CD23, CD200, and CD10. BM biopsy confirmed massive infiltration by atypical lymphocytes displaying pleomorphic features and vacuolated cytoplasm with the expression of CD20, CD5, and cyclin D1. Molecular studies confirmed clonality and the presence of the CCND1/IGH t(11;14). The patient was diagnosed with pleomorphic variant mantle cell lymphoma (MCL). Treatments involving chemotherapy, ibrutinib, and splenectomy were received. Despite treatments, subsequent assessments revealed positive minimal residual disease and multi-organ lymphoma infiltration. The patient passed away six months post-diagnosis.
Conclusions: In this case, the vacuoles were a key finding that raised suspicion for an underlying lymphoproliferative disorder. They may be seen in a variety of entities, including lysosomal storage diseases and neoplastic disorders. However, there are very few cases described regarding its presence in B lymphoproliferative syndromes. Therefore, the presence of vacuoles in lymphocytes should prompt further investigation in the diagnosis and prognosis of lymphoproliferative disorders.
Clinical findings, morphology, FC, and genetic testing were necessary to reach the diagnosis of mantle cell lymphoma.

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P2.16 MAINTENANCE AND LOCAL REGULATION OF TISSUE SPECIFIC IMMUNITY

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208 – P2.16.01

The impact of T-cell responses on vascular inflammation and thrombosis

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Purpose: Pro-inflammatory T cells that recognize liver-derived apolipoprotein B-100 (ApoB100), the protein component of low-density lipoprotein particles, aggravate atherosclerosis development. However, the intrahepatic differentiation of ApoB100-specific T-cell subsets in liver patients and healthy controls was previously unknown. In addition, the pro-thrombotic function of stimulated T cells via inorganic polyphosphate (polyP) - a ubiquitously found, negatively charged biopolymer, which activates coagulation factor XII, has not been analysed so far. Thus, we investigated the contribution of hepatic T-cell responses to vascular inflammation and the contribution of T-cell stimulation to thrombotic diseases.
Methods: We analysed intrahepatic T-cell differentiation in hypercholesterolemic mice and identified ApoB100-specific T cells by activation-induced marker expression in liver disease patients and controls. To validate our findings, we applied a transient liver damage model and performed repetitive transfers of TCR-transgenic T cells into ApoB100-transgenic mice. We used a polyP-binding probe in immunofluorescence microscopy and flow cytometry for the detection of polyP on the surface of stimulated T cells and assessed their pro-thrombotic activity via chromogenic cleavage assays, real-time thrombin generation and FeCl3-induced thrombosis models.
Results: We previously showed that hypercholesterolemia promotes CD4+ T-cell stimulation intrinsically by enhanced T-cell antigen receptor signalling and extrinsically by increased liver inflammation. We found that intrahepatically differentiated T-cell subsets relocate to the atherosclerotic vasculature in adoptive transfer experiments. Consistently, increased populations of pro-inflammatory ApoB100-specific T cells were present in blood from non-alcoholic fatty liver disease patients or cardiovascular disease patients in comparison to healthy controls. However, repetitive ApoB100 recognition in the absence of inflammation promoted the generation of IL-10-secreting regulatory T type 1 cells in healthy subjects. Moreover, we revealed that stimulated T cells accumulate polyP and that plasma membrane-bound polyP on their surface activates factor XII, enhanced thrombin generation in vitro and increased the thrombus formation in a venous thrombosis model in vivo.
Conclusion: Liver damage triggers pro-inflammatory ApoB100-specific T-cell responses and stimulated T cells promote polyP-driven thrombus formation, indicating a role of antigen-specific T cells in vascular inflammation and thrombosis.

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388 – P2.16.02

Red Complex Pathobionts on Osteoclast-Like Cell Maturation

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Purpose: Osteoclasts, arising from mononuclear cells, are characterized as multinucleated giant cells. Apart from host-related factors, bacteria in periodontal disease also play a role in bone degradation by promoting the differentiation of osteoclasts from monocyte/macrophages. Cathepsin K and Tartrate-Resistant Acid Phosphatase are osteoclast markers. Osteoclasts destroy connective tissues and contribute to destruction of tooth supporting tissue. Our study investigated the impact of periodontal pathobionts from the "red complex" - *Porphyromonas gingivalis*, *Treponema denticola*, and *Tannerella forsythia* - on the maturation of osteoclasts.
Methods: To generate osteoclasts, we isolated human primary blood mononuclear cells from peripheral blood and cultured them in the presence of macrophage colony-stimulating factor and receptor activator of nuclear factor-κB ligand to induce monocyte-to-osteoclast differentiation. Throughout the differentiation process, the cells were exposed to lipopolysaccharides (LPS) obtained from pathobionts of the red complex. mRNA expression of cathepsin K and tartrate-resistant acid phosphatase were assessed on days 1, 3, 7, and 14 using qRT-PCR.
Results: The expression of cathepsin k (*CTSK*) and tartrate-resistant acid phosphatase (*TRAP*) were significantly upregulated on day 14 marketing the osteoclast maturation. Furthermore, cells stimulated with lipopolysaccharides (LPS) from *T. denticola* and the combination of pathobionts from the red complex exhibited a significant augmentation in *CTSK* and *TRAP* gene expression by day 14 compared to unstimulated cells.
Conclusion: Among red complex pathobionts, especially *T. denticola* LPS enhanced the maturation of osteoclast-like cells thereby potentially contributing to the pathogenesis of periodontal disease.

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580 – P2.16.03

CD8 regulatory T cells inhibit antigen-specific CD4 T cell responses during respiratory infection with Bordetella pertussis

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Infection with the respiratory pathogen *Bordetella pertussis*, the causative agent of whooping cough, relies on both humoral and cellular immune responses for control and elimination of the bacteria from the respiratory tract. IL-17 and IFN-γ producing CD4 T cells, as well as γδ T cells, along with antigen-specific antibody responses are required for the elimination of the pathogen. However, the role of CD8 T cells during infection is less well characterised. We found that when compared with lymph node or spleen, CD8 T cells from the respiratory tract of naïve mice had high surface expression of PD1 and LAG-3, and produced IL-10, but did not express FOXP3, suggesting that they were inducible or Tr1-type regulatory T cells. Depletion of CD8 T cells from cultures of lung and nasal tissue immune cells from mice infected with *B. pertussis* significantly enhanced antigen-specific CD4 T cell responses. Furthermore, addition of CD8 T cells purified from the lung and nose of naïve or infected mice inhibited antigen-specific IL-17 and IFN-γ production by CD4 T cells purified from *B. pertussis* infected mice. Using an antibody that depletes CD8 T cells, we demonstrated that mice lacking CD8 T cells at the time of infection had significantly lower bacterial burdens, compared with mice treated with an isotype control antibody. This suggests that in contrast to CD4 T cells which mediate protective immunity, CD8 T cells inhibit the clearance of the bacteria from lung and nasal tract. Our findings identify a previously unknown suppressive role for CD8 T cells in the control of antigen-specific CD4 T cells responses that control respiratory infection with *B. pertussis*.

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607 – P2.16.04

Deficiency of the Interleukin-36 Receptor Antagonist (DITRA) disrupts dermal immune homeostasis predisposing to severe psoriatic disease.

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Deficiency of the Interleukin-36 Receptor antagonist (DITRA) is a rare autoinflammatory disease which commonly manifests with severe recurrent episodes of Generalized Pustular Psoriasis (GPP). Loss of function mutations in the *IL36RN* gene results in unopposed IL-36 cytokine dependent signalling which orchestrates severe psoriatic inflammation. Indeed, GPP has recently been shown to be successfully treated with Anti-IL-36R monoclonal antibodies. Despite such advances, there remain some key questions concerning how loss of a functional IL-36R antagonist predisposes to GPP. These include identifying the triggers which initiate psoriatic flares and what are the impacts, if any, of such mutations on skin homeostasis.

To address these questions, we investigated the consequences of IL-36 receptor antagonist deficiency using *il36rn-/-* mice, which we, and others, have demonstrated recapitulate the severe psoriatic inflammation observed in DITRA patients. Here, we show through RNAseq analysis, that in overtly healthy mice, prior to disease onset, there is a defined pattern of altered global gene expression in the skin, indicating disrupted skin homeostasis. The most significantly altered gene encodes the chemokine, Ccl27, which is decreased in *Il36rn-/-* mouse skin, at both the mRNA and protein level. Indeed, stimulation of primary mouse keratinocytes with IL-36 cytokines *in vitro* resulted in decreased *Ccl27* expression. Altered *il36rn-/-* skin homeostasis also occurred in association with dysbiosis of the skin microbiome as detected through shotgun metagenomic sequencing. This dysbiosis was characterized by a significant outgrowth of the skin commensal bacteria, *Cutibacterium acnes*. Importantly, intradermal administration of recombinant Ccl27, prior to the induction of psoriasiform inflammation, significantly reduced the enhanced severity of disease observed in *il36rn-/-* mice demonstrating a central role for this chemokine in regulating the predisposition to increased disease severity. Furthermore, RNAseq analysis of skin biopsies from GPP patients also revealed decreased *CCL27* expression in non-lesional, as well as lesional skin, when compared to healthy skin, indicating that this chemokine may also play a key instructive role among DITRA patients.

Together these data identify a novel mechanism through which IL-36 receptor antagonist deficiency alters dermal homeostasis and predisposes to increased severity of psoriatic disease observed in DITRA patients.

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745 – P2.16.05

Ectopic lung germinal centres support functional affinity maturation despite their disordered structure

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Tertiary lymphoid structures form *de novo* in response to allergy or infection, in the inflamed sites of autoimmune diseases and even adjacent to solid cancers. However, whether these structures are functionally competent and can support antibody affinity maturation is unknown. Elucidating key differences between classic and ectopic germinal centres may inform how we can improve vaccine programs and medical interventions to enhance humoral protection.
B cells undergo vigorous selection within germinal centres to improve their B cell receptors and generate high-affinity serum antibodies for protection against repeated infections. Concurrent germinal centre reactions in both lymphoid and non-lymphoid sites represent an evolutionarily inefficient duplication of efforts; we hypothesised that the less stringent assembly and organisation of tertiary lymphoid structures will result in impaired somatic hypermutation, thereby producing more broadly reactive antibodies.
Intranasal inoculation of mice with NP-KLH in combination with an allergic adjuvant house dust mite induced synchronised germinal centre kinetics in the lung and lung-draining lymph node. This novel inoculation protocol allowed us to index-sort NP-binding germinal centre B cells or memory B cells at days 21 and 42 post-inoculation and directly compare mutation rates between the lung and lung-draining lymph node.
Single B cell receptor sequencing revealed that despite their spontaneous and disordered assembly, lung germinal centres supported the same degree of somatic hypermutation and affinity maturation as the lung-draining lymph node. This shows that ectopic germinal centres are functionally capable of supporting B cell receptor diversification to enhance local protection at peripheral non-lymphoid sites. This has implications for the optimal site of vaccination in scenarios when lymph node germinal centres are less capable of supporting humoral immunity and tertiary lung lymphoid structures form easily – for example in human infants and toddlers.

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888 – P2.16.06

The HLA-G Levels of Cord Blood- and Wharton's Jelly-Derived Mesenchymal Stem Cells from Early to Late Passages

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Mesenchymal stem cells (MSCs) are known to secrete various molecules, including soluble factors, into their surrounding environment, often referred to as the supernatant when cultured in vitro. One of the molecules that MSCs can secrete is the human leukocyte antigen-G (HLA-G). This research aimed to investigate the levels of HLA-G in human cord blood (CB) and Wharton's Jelly (WJ)-derived MSCs for their immunomodulatory function from early to late passages in cell culture supernatant.
The levels of HLA-G in the supernatant of MSCs at each passage were determined using ELISA. WJ-MSCs were passaged from P0 to P7, and supernatants were collected at each passage. When comparing the HLA-G levels between P0 passage and increasing passage numbers (P1-P7), a significant increase was observed at P5 passage ($p<0.05$). When P1 passage was compared with all other passages, HLA-G levels were significantly increased from P2 to P7 ($p<0.05$). CB-MSCs were passaged from P0 to P3, and their HLA-G levels were examined. When comparing HLA-G levels with increasing passage numbers from P0, a significant decrease was observed in P1 and P3 passages ($p<0.05$).
In conclusion, MSCs are known for their immunomodulatory properties, and HLA-G is one of the key molecules involved in this process. Higher levels of HLA-G are generally associated with stronger immunomodulatory effects. Based on the information provided, it appears that the levels of HLA-G increase with passage number in Wharton's Jelly MSCs, with a significant increase observed at P5. This suggests that P5 MSCs may have higher immunomodulatory potential compared to earlier passages.

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906 – P2.16.07

Investigating the mechanism of human peripheral helper T cells (Tph) generation.

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Purpose: Peripheral helper T (Tph) cells have been recently identified and shown to be associated with disease severity and treatment response in autoimmune diseases and cancer. Recent studies defined Tph cells as CD4+CXCR5-PD1+CXCL13+ cells. The generation of Tph cells was enhanced by the high dosage of CD28 signaling and IL2 limiting condition. How Tph cells are generated in tissue where CD28 signaling is not that high and how they maintain survival in the IL2 starvation condition are unsolved questions. Herein, we analyzed single-cell RNA-sequencing data of Tph cells from tissues of different diseases and in vitro models to investigate the cellular mechanism of Tph generation.
Methods: scRNA-seq data from tissue in breast cancer, psoriasis and rheumatoid arthritis were analyzed. Tph cells were *in vitro* generated from MACS-isolated bulk memory CD4+ T cells, FACS-isolated CD161+/CD161 cells, Th17 memory cell sin an anti-CD3-coated plate supplemented with TGF-b, IL-2 neutralizing antibodies (IL2i) and soluble anti-CD28. In some conditions, cells were supplemented with IL-7 or IL-15 to mimic the tissue milieu. After 5 days, cells were collected and phenotypically analyzed by flow cytometry.
Results: Tph cells from the tissue across diseases highly express KLRB1 (CD161). The sorted CD161+ cells were prone to give rise to Tph cells in conditions lacking CD28 signaling or limiting IL2. Tph cells carrying features of Th17 tissue-resident cells are also found in psoriasis. Sorted Th17 from memory CD4+ cells could produce CXCL13 in the presence of TGFb and IL2i. IL-2 neutralizing antibody enhanced Tph generation but dampened cell proliferation and survival. Either IL-7 or IL-15 rescued the IL-2i effect and promoted cell proliferation but only IL-7 could preserve CXCL13 production while IL-15 suppressed it.
Conclusion: Tph cells could be generated from memory CD4+ T cells. The generation of Tph from CD161+ memory cells is more efficient than CD161- ones, likely due to their differences in the dependence on CD28 and IL-2 signaling. IL-7, but not IL-15, is more likely the cytokine driving the survival of Tph cells in the IL2-limiting condition in the tissue.

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987 – P2.16.08

Tissue-specific immunity in the human fetus by γδ T cells

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Accumulating evidence indicate that γδ T cells are key players in early life immunity and physiology. We recently showed that human fetal γδ thymocytes (both the phosphoantigen-reactive Vγ9Vδ2 and nonVγ9Vδ2 subsets) are committed to either a type 1, a type 3 or a type 2-like effector fate that possess thymic-egress potential and display a wave-like pattern depending on gestation age. However, it is not clear what the tissue fate is of the different effector types in the human fetal immune system neither their potential tissue-specific roles.
Therefore, we decided to investigate fetal γδ T cell biology in different peripheral tissues by coupled single cell (sc) RNA/TCR sequencing and multiparametric flow cytometry. Interestingly, a tendency towards atomization of the previously described thymic effector clusters was observed in addition to the appearance of tissue-specific cell-states. In contrast to our previous observations in fetal thymus, Vγ9Vδ2 T cells were differentially enriched among the effector modules in the distinct peripheral tissues. Finally, sc TCR analysis showed that certain TCR sequences are more prone to be associated to particular transcriptomic profiles in peripheral tissues and revealed the existence of niche-specific CDR3 motifs.

Thus, human fetal γδ T cell cells appear to acquire tissue-specific specialization both at TCR and functional level. This knowledge provides a basis to study the role of these cells in human early life immunity and physiology.

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1086 – P2.16.09

Identification of cellular and molecular biomarkers defining end-stage lung parenchyma and lung-draining lymph nodes

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Purpose: Organ transplantation provides unique access to human tissue material and enables the application of high-end techniques to identify novel cellular and molecular biomarkers associated with disease progression and clinical outcomes.
Methods: Using SeqWell scRNA Sequencing combined with broad immune phenotyping and Luminex multiplex protein array (n=50) we transcriptionally and immunologically defined and compared immune cell subsets in the human end-stage lung parenchyma (Par.) and lung-draining lymph nodes (LN): Par. Emphysema (Emph, n=6), Par. Fibrosis (Fib, n=6), Par. PAH (n=1), Par. tum. free (tumor-free lung tissue of lung cancer patients (n=5)), LN Emph (n=4), LN Fib (n=5).
Results: We found compartment and disease-specific alterations in the transcriptional profile of immune cell subsets such as tissue-resident vs circulating T cells, monocytes, and granulocytes in Par. and LN. We also defined disease-specific cell-cell interaction networks as an important characteristic of end-stage tissue microenvironment. These data were supported by cytometry analyses. Finally, we measured differences in the concentrations of chemokines, cytokines, and growth factors, which could potentially drive end-stage lung cell alterations in a compartment- and disease-specific manner.
Conclusion: The discovery of novel disease- and compartment-associated cellular and molecular biomarkers may pave the way to understanding the end-stage disease progression paving the way to improved therapeutic and diagnostic options.

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1088 – P2.16.10

Tissue-Specific Imprinting Shapes Conventional Dendritic Cell Functionality in Tumors and Non-Malignant Tissues

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The tumor microenvironment (TME) is a complex ecosystem comprising diverse cell populations, including immune cells, that play pivotal roles in cancer development and response to therapies. Conventional dendritic cells (cDCs) are crucial antigen-presenting cells influencing adaptive immunity and tumor progression, but their adaptations within the TME remain unclear. Here, by performing an in-depth single-cell analysis, we investigated cDC subsets across human tumors, including both immunologically cold and hot malignancies, and delineating their functional states and tissue-specific plasticity. Our integrated analyses across tumors revealed four cDC phenotypes: cDC1s, cDC2s, CCR7$^+$ DCs, and CD207$^+$ DCs.
CD207$^+$ DCs, enriched in tumor tissues, exhibit a unique transcriptional profile linked to inflammatory responses and type 1 immunity. Strikingly, in immunologically hot cancers, CD207$^+$ DCs are enriched in the early stages of the disease and correlate with improved patient survival. These features were not observed in immunologically cold tumors.
We also investigated the transcriptional alterations in cDC subsets infiltrating hot and cold tumors, showing that cDC2 were the most impacted by the different tumor microenvironments. In detail, in hot tumors, their transcriptomes exhibited upregulation of genes involved in chemokine-mediated immune cell trafficking and antigen presentation, likely promoting immune cell recruitment.
Furthermore, tissue-specific imprinting influences cDC2 plasticity not only within tumors but also in non-malignant tissues, as they were found to have the greatest number of differentially expressed genes (DEGs) among DC subsets when comparing normal tissues, indicating a non-negligible role of tissue imprinting in shaping cDC2 functionality.
Collectively, our findings illuminate cDC adaptations in both tumoral and steady state conditions, particularly highlighting their functional plasticity and role in immune cell recruitment, driven by tissue imprinting and paving the way for tailored immunotherapies. Understanding the interplay between cDC2s and the TME holds promise for enhancing therapeutic strategies and advancing precision oncology, as they could emerge as fire starters for cold tumors.

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1128 – P2.16.12

PD-1 Pathway in Rheumatoid Arthritis: Applying the brakes on synovial inflammation.

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Purpose: In rheumatoid arthritis (RA), ongoing synovial inflammation causes degradation of cartilage and bone, leading to joint damage and disability. Co-stimulation signals are positive regulators of T cell activation in the RA joint. However, the role of negative regulators of T cell responses, have been less well described. PD-1 is an immune checkpoint, which functions to restore tolerance by binding PD-L1 expressed on antigen presenting cells such as dendritic cells. The role of PD-1 is well studied in the context of cancer, but less is known about the PD-1 pathway in autoimmunity.
Methods: Peripheral blood mononuclear cells (PBMC) and synovial fluid mononuclear cells (SFMC) were isolated from RA patients. Cells were stained with a panel of multicolour flow cytometry antibodies to assess cell frequency, activation, maturation, and metabolism. Samples were processed on the Cytek Aurora and analysed using FlowJo software.
Results: Firstly, we identified an accumulation of PD-1+ T cells (CD4 and CD8) in RA synovial fluid compared to RA blood. Within the inflamed joint, we also determined that PD-1+ CD4+T cells and PD-1+ CD8+ T cells do not display an exhausted phenotype; instead producing more TNF-α and significantly higher levels of IFN-γ (CD4; **$p<0.05$**) than their negative counterparts. PD-1+ T cells in the RA joint also produce significantly more proinflammatory cytokines then PD-1+ cells within the circulation, suggestive that the unique inflamed joint microenvironment may drive the accumulation of pathogenic PD-1+ T cells in the joint. Next, to determine if PD-1 signalling was likely to occur in the joint, we investigated if the ligand for PD-1 was available in the joint. Specifically, we found that PD-L1- cells were significantly more abundant in the RA joint compared to PD-L1+ antigen presenting cells **($p<0.05$**) including cDC1 **($p<0.05$**) and cDC2 **($p<0.05$**). Although PD-L1+ APCs are rare in the RA joint, they displayed significantly higher expression of co-stimulation markers CD80 and CD40 **($p<0.05$**).
Conclusions: PD-1+ T cells are enriched at the site of inflammation in RA, displaying a proinflammatory phenotype. This inhibitory pathway is unlikely to be engaged due to the lack of tissue specific PD-L1 in the joint.

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1139 – P2.16.13

Inflammatory CD1c+ CD163+ dendritic cells are enriched in the inflamed joint and contribute to rheumatoid arthritis pathology

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Purpose: Rheumatoid arthritis (RA) is a chronic autoimmune disease that develops because of a break in tolerance to otherwise harmless self-antigen. Given the important role dendritic cells (DC) have in maintaining immunological tolerance and immunogenicity, we hypothesised that DC may contribute to ongoing synovial inflammation in the joints of RA patients. While we previously identified an accumulation of cDC1 and cDC2 in the RA joint, the contribution of DC3 to synovial inflammation and joint destruction remains unknown. DC3 are a newly identified population of inflammatory DC, co-expressing the markers CD1c and CD163, thought to contribute to tissue specific inflammation. While they have been studied in the context of osteoarthritis and systemic lupus erythematosus, their role in RA is unknown.

Methods: Peripheral blood mononuclear cells (PBMC) and synovial fluid mononuclear cells (SFMC) were isolated from RA patients. Cells were stained with a panel of multicolour flow cytometry antibodies to assess cell frequency, activation, maturation, and metabolism. Samples were processed on the Cytek Aurora and analysed using FlowJo software.

Results: While circulating DC3 have been identified in SLE, the role of DC3 in RA remains unclear. Firstly, we identified an enrichment in the frequency of DC3 within the peripheral blood of RA patients compared to healthy controls **($p<0.05$)** paralleled with higher levels of CD80, PD-L1 and CD40 **($p<0.05$)** –indicative of increased DC maturation and activation in the periphery. Furthermore, upon examination at the site of inflammation – we identified a significant enrichment of DC3 in RA synovial fluid compared to peripheral blood **($p<0.05$)**. Synovial DC3 display heightened levels of CD80, CD40 and PD-L1 (**$p<0.05$**) further suggesting that inflammatory DC3 are strategically placed to reactivate synovial T cells. Importantly, DCs utilise a variety of metabolic pathways to generate energy to support their immune function. We identified that synovial DC3 significantly upregulate the expression of GLUT-1 **($p<0.05$)** concomitant with an increase in fatty acid uptake receptor CD36, – suggestive that both carbohydrate and fatty acid metabolism may support the long-term survival and function of DC3 in the RA joint.

Conclusion: DC3 are enriched in RA and may present a new therapeutic target for suppressing synovial inflammation.

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1220 – P2.16.14

The Role of Immunological Memory in Synovial Inflammation in Rheumatoid Arthritis.

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Purpose: Lifelong protective immunity requires a sufficient number of diverse naïve T cells (TN) that are ready to expand and differentiate when faced with antigenic challenge. The generation of memory T cells is also essential. Given that joint swelling arising from Rheumatoid Arthritis (RA)tends to flare in the same previously affected joints, and involvement of a formerly unaffected joint is rare, we hypothesis that local tissue memory may mediate disease flare.
Methods: Peripheral blood and synovial fluid mononuclear cells from RA patients were isolated using density gradient centrifugation and cultured overnight in the absence/presence of PMA and Ionomycin, alongside Brefeldin-A (Golgi blocker). The samples were stained with a 20-colour flow cytometry panel to assess memory T cell frequencies and their activation capacities. All samples were processed on the Cytek Aurora cytometer and analysed using FlowJo software.
Results: Upon examination of RA synovial fluid, a small but distinct population of $CD4^+$ and $CD8^+$ TN cells are present. Interestingly, following antigen stimulation $CD4^+$ synovial TN cells produced significantly higher levels of IFNγ($P<0.001$), TNFα($P<0.01$), IL-17A($P<0.01$) and GM-CSF($P<0.001$) compared to peripheral blood. Similarly, $CD8^+$ TN cells from the site of inflammation produced significantly higher levels of IFNγ($P<0.0001$) and GM-CSF($P<0.01$). Synovial TN cells demonstrated greater polyfunctionality properties compared to peripheral blood TN cells, as demonstrated by their ability to coproduce multiple proinflammatory cytokines simultaneously. This suggests that TN cells residing within the joint are primed to become more inflammatory upon stimulation compared to their peripheral blood counterparts. Furthermore, we demonstrated that this enhanced inflammatory profile is supported by altered cellular bioenergetics. The expression of GLUT1 is decreased in synovial TN cells compared to peripheral blood, concomitant with a significant increase in CD36 expression, suggestive that synovial TN cells utilise fatty acid metabolism to support their pro-inflammatory functions. These results suggest synovial TN cells are primed by inflammatory mediators within the joint microenvironment to support T cell activation and differentiation.
Conclusion: Naïve T cells present in the RA synovium are primed to respond more potently to antigenic stimulation and may utilise fatty acid metabolism to support these pathogenic functions.

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1322 – P2.16.15

PD-L1-expressing eosinophil-mediated regulation of immunity and pulmonary inflammation

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Purpose: Eosinophilia presents as a defining feature of aberrant type 2 inflammation across a range of disease states, particularly in allergic asthma. Despite extensive research, questions remain as to the functional role of eosinophils in the regulation or genesis of inflammation in tissues such as the lung. We previously reported that expression of programmed death-ligand 1 (PD-L1) on distinct cell populations acts as a checkpoint on type 2 immunity to maintain immune homeostasis in the lung. During allergic lung inflammation in mice models, we have explored the function of PD-L1 expressing eosinophil populations associated with the development of tissue inflammation and associated tissue damage.
Methods: Mice were subjected to three distinct acute or chronic models of allergic lung inflammation; *Nippostrongylus brasiliensis* infection, intranasal challenge with house dust mite (HDM: *Dermatophagoides pteronyssinus*) or Interleukin-33. Flow cytometry was used to identify distinct eosinophil populations; $CD45.2^+$ $CD11b^+$ $F4/80^-$ $SiglecF^+$. Histology and NanoString gene expression analysis were performed. Bone marrow (BM) chimera mice were generated - ΔdblGATA/WT and ΔdblGATA/PD-$L1^{-/-}$ - for eosinophil-specific targeting of PD-L1.
Results: Distinct subpopulations of eosinophil were evoked in the lungs of wildtype mice, with kinetic differences in PD-L1 expression following *Nippostrongylus brasiliensis* infection and intranasal challenge with IL-33 or HDM. HDM-induced lung sensitisation evoked marked pulmonary pathology and inflammation, with a distinct alteration in type 2 immune cells, in BM chimeras with eosinophil-specific deletion of PD-L1. Analysis of PD-L1 deficient chimeric mice revealed changed frequency in programmed cell death protein 1 (PD-1) expressing $CD4^+$ T-cell populations, including Th2 and Treg cells.
Conclusion: Allergic lung inflammation induces recruitment of distinct PD-L1 expressing inflammatory eosinophils, with functional roles in ameliorating allergic type 2 inflammatory responses in mice models. Absence of PD-L1 expressing eosinophils contribute to marked changes in PD-1^+ innate and adaptive type 2 cells and Treg cells in aberrant lung inflammation. The potential suppressor/regulatory roles for PD-$L1^+$ eosinophils recruited to the allergic lung, acting as a checkpoint in pulmonary inflammation will be described.

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1432 – P2.16.16

Unveiling the significance of cross-reactive bone marrow resident memory T cells in immune responses: Insights from SARS-CoV-2 and vaccination

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Memory T cells play a crucial role in orchestrating efficient immune responses against pathogens. Here, we present compelling evidence demonstrating the critical role of bone marrow resident memory T cells (TRM) in conferring long-term immunity. Our findings highlight the significance of bone marrow memory T cells in shaping systemic immune responses, particularly in the context of SARS-CoV-2 and vaccination. Our investigations reveal that human bone marrow professional memory T cells, despite expressing activation markers, maintain a resting state, suggesting a unique mode of long-term immune memory storage. Notably, the bone marrow harbors an enrichment of pathogen-specific $CD4^+$ memory T cells, providing sustained memory for systemic pathogens. Expanding upon this, we demonstrate the mobilization of TRM from the bone marrow into the bloodstream following secondary immune reactions to MMR (measles-mumps-rubella) vaccination. Importantly, we observe that TRM recognizing other antigens are not mobilized, unless they cross-react with the vaccine, underscoring the significance of cross-reactivity in TRM mobilization. These mobilized TRM significantly contribute to systemic immune memory, as evidenced by their representation among newly generated circulating memory T-cells post-vaccination. Furthermore, we unveil the presence and significance of preexisting SARS-CoV-2-reactive memory $CD4^+$ T cells within the bone marrow, even in individuals with no prior exposure to the virus or its vaccines. These bone marrow resident memory T cells, abundant and polyfunctional, play a crucial role in shaping systemic immune responses to SARS-CoV-2, potentially contributing to the establishment of long-lasting immunity. Collectively, our findings underscore the importance of cross-reactive bone marrow resident memory T cells in providing robust and enduring immunity against pathogens. Understanding their role enhances our comprehension of immune memory and informs the development of strategies to bolster protective immunity.

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1479 – P2.16.17

Abnormalities of skeletal muscle regeneration and macrophage polarization in Nakajo-Nishimura syndrome.

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Nakajo-Nishimura syndrome (NNS) is an autoinflammatory disease with skeletal muscle atrophy caused by homozygous point mutations in the *PSMB8* gene encoding the β5i subunit of the immunoproteasome. To elucidate the mechanism of skeletal muscle atrophy in NNS, we analyzed knock-in mice ($PSMB8^{G201V}$) in which the patient mutation was introduced and found that the efficiency of skeletal muscle regeneration was significantly lower than that in wildtype mice. The proliferative efficiency of satellite cells in $PSMB8^{G201V}$ during regeneration was also significantly lower. However, satellite cells grown in ex vivo culture showed no difference in growth rate between wildtype and $PSMB8^{G201V}$. Moreover, infiltrating macrophages during skeletal muscle regeneration showed abnormal anti-inflammatory polarization and decreased production of inflammatory cytokines in $PSMB8^{G201V}$, suggesting that they contribute to the decreased proliferation efficiency of muscle satellite cells. However, the production of inflammatory cytokines by bone marrow-derived macrophages was rather increased in $PSMB8^{G201V}$, indicating an autoinflammatory phenotype. These results suggest that $PSMB8^{G201V}$ macrophages acquire a specific phenotype during skeletal muscle regeneration and contribute to reduced efficiency of muscle regeneration.

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1642 – P2.16.18

Marine sulphated polysaccharides stimulate innate immunity to fight infection in a model of post-traumatic immunosuppression

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Ventilator-associated pneumonia (VAP) is the most frequent nosocomial infection in intensive care units, particularly affecting patients with severe traumatic brain injuries. Indeed, head injury generates a systemic inflammatory response (SIRS), which is then accompanied by a compensatory anti-inflammatory response (CARS) aiming at the restoration of immune homeostasis. If the CARS persists, it leads to systemic immunosuppression promoting secondary infections. At the cellular level, the mechanisms are a decrease in the capacity of dendritic cells and monocyte-macrophages to present antigen to effector cells, a decrease in the number and capacity of secretion of pro-inflammatory cytokines by effector cells (NK, T cells).
Among the emerging immunostimulant therapeutics, marines sulfated polysaccharides (SPs) are a relevant research axis. Current studies suggest that sulfated polysaccharides primarily exert immunomodulatory effects by promoting macrophage, T cells, and NK cell proliferation, increasing cytokine release, and regulating the immune system through feedback of anti-inflammatory cytokines. SPs from *Ulva Lucinulata* have shown their immunostimulant effects *in vitro* and *in vivo* in animals.
In our study, we investigated the immunostimulatory potential of SPs in a preclinical mouse model of secondary pulmonary infection after traumatic brain injury. We studied the effects of this compound on the main functions of innate immune cells in the lung. In our model of post-trauma immunosuppression, we were able to demonstrate that SPs improved the general condition of the mice and their resistance to infection. In the lung, SPs partially restored the number of NK cells and their IFNγ expression, as well as macrophages and their phagocytosis activity, in the context of an infectious challenge.
In conclusion, our results strongly suggest that SPs restores the ability to recruit immune cells to the lung during post-traumatic immunosuppression and protects against systemic infection. The growing interest in the effects of algal polysaccharides is a strong indication of their potential relevance as a new therapeutic modality. The fact that they target the host immune system rather than the pathogen is also a key factor in limiting the development of bacterial resistance to antibiotics.

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1649 – P2.16.19

Signaling pathways and differentially expressed genes associated with placental pathology in antiphospholipid syndrome

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Purpose: Antiphospholipid syndrome is an autoimmune thrombophilia defined by the presence of circulating antiphospholipid antibodies (aPL). Obstetric morbidity is the hallmark of APS. Heterogeneous pregnancy complications, including placental pathology, have been increasingly studied in the past years but still continue to present a formidable clinical and scientific challenge.
Methods: We performed RNA-seq analysis of the mid-pregnancy placenta from mice with well-established APS and healthy controls animals, followed by validations with morphological staining.
Results: The GO function and KEGG pathway enrichment analyses revealed that the biological functions of differentially regulated genes are highly associated with the regulation of developmental processes, cell cycle, proliferation and cellular senescence. Disturbed tissue homeostasis presumably was driven by aPL-activated signaling through p53, PI3K/AKT pathways and motor proteins, as evidenced by KEGG pathway analysis. These alterations were accompanied with metabolic adaptations, including shifts in carbon, pyrimidine and energy metabolism. Also, we evidenced differential regulation of genes involved in pathways for oxygen binding and HIF-1 hypoxia-related pathways. This observation was supported by the presence of nucleated red blood cells in a labyrinth, identified in histological sections of APS placenta, which might reflect hypoxia of the fetus. Microscopic examination of APS placenta sections also revealed vascular wall thinning and areas with fibrin depositions.
Conclusion: Altogether, we identified a number of regulatory mechanisms that separately or combine are capable of contributing to placental insufficiency. Our data indicate that altered mechanosensitive capacity of cells may define defect trophoblast functioning and survival, influenced by the levels of shear stress they encounter.

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1865 – P2.16.20

Mast cells and their adipokine receptors: a role of specific ligands in expression shift

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Purpose: Mast cells (MCs) are significant sensor and effector cells of the immune system involved in various physiological and pathological conditions. A primary function of MCs is modulating inflammatory processes since MC-derived molecules exert pro-, anti-inflammatory, and regulatory effects. Even though MCs may affect different phases of acute and chronic inflammation, emerging data indicates that they contribute to low-grade inflammatory reactions associated with obesity. This phenomenon is strongly regulated by adipokines synthesized by adipocytes and various immune cells in adipose tissue. Despite adipokines being viewed as crucial for regulating body metabolism, mounting evidence shows they are also critical for immunological mechanisms and inflammatory responses. Adipokines mediate their actions through specific receptors widely distributed in many cell and tissue types. However, limited data are available on their expression in mast cells (MCs) and, consequently, adipokine's significance in the modulation of MC activity. This study aimed to evaluate the constitutive and adipokine-induced expression of receptors for key adipokines (leptin, adiponectin, and chemerin) in MCs.
Methods: Experiments were conducted *in vitro* using freshly isolated peritoneal MCs from female albino Wistar rats. Flow cytometry evaluated adipokine receptors' constitutive and adipokine-induced expression (LEPR, ADIPOR1, ADIPOR2, and CMKLR1).
Results: Tissue MCs constitutively express receptors for leptin, i.e., LEPR, adiponectin, i.e., ADIPOR1 and ADIPOR2, and chemerin, i.e., CMKLR1. We also observed that LEPR, ADIPOR1, ADIPOR2, and CMKLR1 expression levels in MCs change in response to stimulation by their specific ligands.
Conclusion: Our findings suggest that adipokines leptin, adiponectin, and chemerin may influence the activity of tissue MCs in various processes, especially during inflammation. These observations support the thesis on the role of MCs in chronic low-grade inflammation associated with increased adiposity. This insight could open new avenues for understanding and managing inflammation-related conditions.

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1881 – P2.16.21

UTERINE NATURAL KILLER CELLS IN WOMEN WITH RECURRENT PREGNANCY LOSS AND IMPLANTATION FAILURE

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Although the functions of uterine natural killer (NK) cells are still unclear, they are thought to play an important role in the early stages of pregnancy. In this study, we aimed to investigate peripheral blood and endometrial NK cell functions in recurrent pregnancy loss. Patients who applied to Istanbul University, Istanbul Faculty of Medicine, Infertility Outpatient Clinic with recurrent pregnancy loss (n=20) and people who applied due to recurrent miscarriage but had children (n=10) constituted the control group. In cells isolated from peripheral blood and endometrium, lymphocyte subsets and NK cell cytotoxic activity (expression of perforin, granzyme and CD107a) were evaluated by flow cytometry. Uterine $CD3^+$ T lymphocyte ratio decreased significantly compared to peripheral blood in both controls and recurrent pregnancy losses ($p<0.01$, $p<0.001$, respectively). Similarly, uterine $CD19^+$ B lymphocytes were significantly lower in recurrent pregnancy losses compared to peripheral blood ($p<0.01$ $p<0.0001$, respectively). It was found that both blood and uterine NK cell ratios were significantly higher in recurrent pregnancy losses compared to the control group ($p<0.0001$). When evaluated in terms of perforin/granzyme, decreased uterine NK ratios and an increased CD107a expression were observed in both groups compared to peripheral blood ($p<0.0001$, $p<0.001$, respectively). In the control group, uterine NK CD107a expression was significantly reduced compared to peripheral blood ($p<0.05$). The altered numbers and functional properties of peripheral and uterine NK cells in recurrent pregnancy loss indicate that these cells may play a role in the pathogenesis of the disease.

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2020 – P2.16.22

Characterization of the immune cell signature in pulmonary hypertension associated with chronic lung diseases

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Rational: Chronic lung diseases (CLD), such as chronic obstructive pulmonary disease (COPD) and pulmonary fibrosis (PF), are highly heterogeneous severe conditions, which are associated with a decreased quality of life and increased mortality. The presence of pulmonary hypertension (PH) in CLD, which is caused by a progressive remodelling of the pulmonary arteries (PA), is associated with worse patient prognosis. In the idiopathic form of PH (WHO Group 1) the immune system has been shown to have an important role in this remodeling process. However, the extent of the immune involvement in PH which is associated with chronic lung diseases (Group 3) remain unclear. In this study we therefore investigate the immune cell profile of PH associated with CLD, such as COPD and PF.
Methods: PA were isolated from human end-stage PH-COPD(n = 14), PH-PF(n = 9) and control lungs from transplantations and cancer-free tissues (n = 9). The immune cell characterization was performed using several multicolour flow cytometry panels and analysed bioinformatically. Additionally, adjacent PA and lung tissues were embedded for further histological analysis.
Results: Principal component analysis (PCA) revealed an altered immune cell profile in the PA of PH-COPD and PH-PF compared to control, with the strongest separation between controls and PH-COPD. The immune composition in PH-PF were more heterogeneous and partially overlapped with the PH-COPD, indicating overlaps in the immune profile, but separated from the control in Principal Component (PC) 3. The separation of the PC1 is mainly driven by lymphocyte populations and macrophages and neutrophilic granulocytes, while the PC3 is driven by monocytes. Both CLD possessed an increased abundance of CD4 and CD8 lymphocytes and monocytes, and a decrease in neutrophil granulocytes and macrophages. Work is ongoing to determine immune cell localization and association of immune cell signatures with clinical and histological parameters, as well as single cell RNA-sequencing analysis to gain further information of immune and structural cell activation.
Conclusions: Our initial data indicates that there is an altered inflammatory profile between control and CLD-PH, and by understanding the immune profile in PA of CLD we can lay the foundations for future immunomodulatory PH treatments.

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2099 – P2.16.23

The Jak inhibitor filgotinib concentration-dependently suppresses immune responses in precision cut intestinal tissue slices ex vivo

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Purpose: The global burden of inflammatory bowel disease (IBD) is reportedly increasing worldwide. Yet, the pathogenesis of the disease remains incompletely understood. Current treatments focus on controlling the abnormal inflammatory reactions in the intestine. Among new therapeutics, the Janus kinase (JAK) pathway has gained attention, with selective inhibition by drugs like filgotinib. It has shown promise in reducing inflammation and symptoms associated with IBD. The aim of this study was to investigate the effect of the JAK1 inhibitor filgotinib in patient-derived primary intestinal tissue slices *ex vivo*.
Methods: Precision cut intestinal slices (PCIS) were prepared from ileum and colon resections of IBD and non-IBD patients and treated with the T cell mitogen Concanavalin A ± filgotinib for 24h. We assessed tissue viability (LDH-assay), cytokine release (ELISA), and PCIS morphology (H&E-staining).
Results: IBD and non-IBD-derived intestinal tissue slices maintained viability in culture and presented typical intestinal morphology (e.g. crypts, villi, immune and epithelial cells). *Ex vivo* treatment with Concanavalin A upregulated the secretion of pro-inflammatory and T cell specific cytokines such as IL-6, IL-2 and IL-17A after 24h. This induction was inhibited by filgotinib (10 and 100 µM) in a dose-dependent manner. Of note, no increase in LDH release was observed in treated PCIS, indicating no adverse effects of filgotinib on the tissue´s viability in the applied concentrations. The effects of filgotinib were comparable in both ileal and colonic intestinal tissue slices.
Conclusion: Our data highlights anti-inflammatory effects of filgotinib in primary intestinal tissue slices *ex vivo*. These effects involve modulation of various T cell-associated cytokines implicated in JAK-STAT pathways. PCIS can be used to further elucidate potential JAK1 inhibitors and their mode of action in the context of IBD treatments.

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2169 – P2.16.24

Compartmentalized immune response in sepsis

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Imbalanced immune response to infection leads to sepsis, though little is known on the compartmentalized alterations of cell numbers and microenvironment across vital organs.
We use the Peritoneal Contamination and Infection (PCI) model to track the displacement of neutrophils (PMN), Ly6Chi monocytes (Mono), DC, tissue resident macrophages (TRM), T and B cells by flow cytometry in 5 organs: peritoneum (PeC), liver, spleen, blood and bone marrow (BM).
At 6h post PCI, PMN and Mono egress from spleen and BM and infiltrate PeC and liver. In contrast, B cells abandon PeC and liver – but not BM – and migrate towards spleen. PMN and B cell, but not Mono relocation is also reflected in blood counts. In a healthy liver and blood the majority of immune cells positively correlate with each other, a dependency lost upon sepsis. Inter-organ correlations are scarce, but distinct between the conditions. During sepsis B cells in spleen negatively correlate with B cells and DC in PeC and in liver Kupffer cells positively correlate with Mono, suggesting fine-tuned intra- and interorgan mechanisms responsible for compartmentalized cell flow patterns.
From all cells, PMN and Mono in liver and BM as well as B cells in spleen (but no cell from blood) were the most informative with 73-80% accuracy in distinguishing between healthy and septic mice.
PMN and Mono migration suggest PeC and liver as focal points to fight pathogens. Bacteria spreading confirms the highest load in these 2 organs with spleen and BM being less affected. As anticipated, in vivo phagocytosis occurred predominantly in PeC and liver, but only by TRM and PMN – not Mono.
We measured a total of 200 secreted mediators and identified both a unique and a shared secreted protein signature in all 5 tissues. 8 factors including known and novel targets classify apart with up to 100% accuracy healthy from septic mice.
In conclusion, we report a highly compartmentalized immune response aligning with bacteria translocation in acute sepsis. Distinct infiltration patterns and newly formed intra- and interorgan cell dependencies suggest a complex crosstalk between immune cells and secreted mediators.

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2185 – P2.16.25

Association of autoantibodies with pregnancy outcomes

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Purpose: The impact and aetiologies of rheumatic diseases are multifactorial and mostly still unclear. Autoantibodies could be found in all autoimmune diseases such as Sjögren's disease (SJ), as well as in non-autoimmune disorders. The aim of this study was to determine the level of autoantibodies in pregnant women and explore their clinical significance.
Methods: This is a 3-year prospective study performed at the University Obstetrics-Gynecology Department of Democritus University of Thrace during the period of June 2020-June 2023. The data were collected from 79 pregnant woman and 70 controls. The testing for antinuclear autoantibodies (ANAs) and anti-dsDNA was performed by using conventional indirect immunofluorescence assay (IIF) at dilution ratios of 1/80, 1/160, 1/320, and 1/640, with a cutoff for positivity set at 1/80 for ANA and dilution ratio of 1/10 for anti-dsDNA. The ENA screen (Extractable nuclear antigens) was performed by ELISA for the following antigens: Anti-SSA(Ro), Anti-SSB(La), Anti-Sm, Anti-SmRNP, AntiJo-1, Anti- Scl-70. In case of positivity (cut-off value >1), specific ELISA kits for each autoantibody were employed. The aPL (anti-phospholipid abs) both IgG and IgM types (ACA), were measured by ELISA Statistical analysis of the data was performed using IBM Statistical Package for Social Sciences (SPSS), version 19.0 (IBM Corp., Armonk, NY, USA).
Results: ANA-positivity was significantly more frequent among pregnant (11/79) than healthy controls (3/70) (13.9% vs 4.3%, p=0.044; OR=3.61, 95% CI=0.97-13.53). Among them 7 (8.9%) had titer 1/80, 3 (3.8%) 1/160 and 1 (1.3%) 1/320. Considering the of immunofluorescence pattern of the 11 ANA positive: 5 (45.5%) had Homogeneous pattern, 4 (36.4%) had Dense Fine Speckled 70 (DFS-70) pattern, and 2 (18.2%) had fine speckled pattern. In relation to the diagnosis, ANA-positive were correlated with death fetus (2 of 5 patients, 40.0%), RSA (3 of 11 patients, 27.3%), spontaneous abortion (2 of 11 patients, 18.2%), missed abortion (2 of 29 patients, 6.9%) or other disease.
Conclusion: Our results indicate that alterations in the levels of autoantibodies are more frequent in pregnancy in comparison to controls**.** Pregnancies complicated by rheumatic diseases should be approached interdisciplinary, by teams of experienced professionals.

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2260 – P2.16.26

Ointment in the flies: augmentation and synergy of host defense peptide against AMR bacteria in epithelial and systemic infection

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Host defence peptides (HDPs) show promise in combating multidrug-resistant (MDR) bacteria; however, their use is limited by their restricted activity in physiological conditions and the development of resistance. Synthetic antibacterial and anti-biofilm peptides (SAAPs), based on HDP sequences, have been developed to bypass these limitations. The leading candidate, SAAP-148, shows promise in its direct activity against MDR bacteria, but its efficacy in robust tissue models of infection and *in vivo* remains to be explored. As HDPs are unlikely to advance clinically as monotherapies, the synergistic employment of SAAP-148 required clarification likewise. Here, we have assessed the ability of SAAP-148 to: prevent infection in 3D culture models of skin and bronchial respiratory epithelium; synergise with novel nonpeptide antibacterial agents against MDR bacteria; and deter *Staphylococcus aureus* lethality in *Drosophila melanogaster* as an *in vivo* model of infection. We demonstrated that SAAP-148 application on 3D skin and bronchial respiratory epithelial models up to 24 hours prior to infection with MDR *S. aureus* or *Pseudomonas aeruginosa* prevented the colonization of skin but not airway tissue models. In addition, SAAP-148 synergises with the nonpeptide antibiotic halicin against MDR bacteria in skin models, and the agents act additively in airway tissue models. In systemic infection of *Drosophila*, SAAP-148 and halicin synergistically inhibited the growth and lethality of *S. aureus* and *Providencia rettgeri* during early stages of infection, where neither agent alone effectively limited infection. Further, the response of infected *Drosophila* depleted of endogenous HDPs highlights the potential of SAAP-148 and halicin to synergise with native peptides against bacterial infection. Together, these results support the efficacy of SAAP-148 as a prophylactic agent against MDR epithelial infections, and as a synergistic treatment in combination with halicin against both epithelial infections *in vitro* and systemic infections *in vivo*.

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P2.17 MANIPULATION OF TOLERANCE

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588 – P2.17.02

Exploring red blood cells as a novel tolerogenic approach for factor VIII inhibitors employing immuno-dominant FVIII derived peptides presented on MHC class II

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Purpose: The primary challenge in the treatment of hemophilia A is the development of neutralizing antibodies (inhibitors) against factor VIII (FVIII). Immune tolerance induction (ITI) is the standard approach to eradicate anti-FVIII antibodies. Since ITI is efficient in only 60-80% of cases, within the EDUC8-consortium we are pioneering innovative methods to reduce the immunogenicity of biotherapeutics. Here, we utilized bioinformatics and proteomic-based peptide presentation assays to identify promiscuously presented FVIII peptides for potential use in immuno-tolerogenic approaches. To this end, we are exploring red blood cells (RBCs) as innovative antigen delivery system to modulate the immune response.
Methods: A data-set of naturally processed FVIII peptides was generated by incubating human FVIII with immature monocytes-derived DCs from HLA-typed healthy donors. Special attention was given to the identification of HLA-DP4-FVIII derived peptides, as these alleles are highly prevalent in the Caucasian population.
Results: We have developed a novel mass spectrometry-based protocol for investigating HLA-DR and HLA-DP antigen presentation using specific monoclonal antibodies. Through this method, we identified approximately 2000 HLA-DR and 1000 HLA-DP presented peptides. Notably, our analysis revealed over 100 HLA-DR and 14 HLA-DP4 presented peptides derived from FVIII. Two HLA-DR presented FVIII peptide from the A2 and C1 domain respectively were fused to a cell-penetrating peptide and incubated with RBCs. Flow cytometry demonstrated their dose-dependent binding to RBCs, which was confirmed by imaging flow cytometry. Macrophages efficiently endocytosed FVIII peptide-treated RBCs, as observed via confocal microscopy. Utilizing immunopeptidomic approaches, we established the functional presentation of RBC-derived peptides on MHC class II molecules on macrophages. Specifically, the TAT-A2 FVIII peptide exhibited efficient processing and presentation on HLA-DR molecules on macrophages. Importantly, incubation of TAT-C1 FVIII-treated RBCs loaded macrophages with a FVIII-specific T cell hybridoma led to a significant increase in IL-2 production, suggesting the potential of peptide-loaded RBCs for immune system modulation.
Conclusion: Our data provide an inventory of promiscuously presented FVIII-derived peptides which guide the development of novel tolerogenic approaches for FVIII inhibitors employing RBCs as carrier.

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746 – P2.17.03

Impact of commonly used immunosuppressants on Treg phenotype and function

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Purpose: Inborn errors of immune dysregulation are a heterogeneous group of disorders with clinical variability that affect genes involved in the regulation of the immune system. Those presenting with a phenotype caused by loss of tolerance mechanisms leading to autoimmunity, autoinflammation, lymphoproliferation, and/or severe atopy have come to be recognized as having Primary Immune Regulatory Disorders (PIRD) since 2020. Currently, high-potency nonspecific immunosuppressant treatments such as steroids or cyclosporine are being used to control these manifestations. Such treatments have multiple side effects and add many complications to the chronically affected organs in these patients, which is accentuated by the fact that these treatments must be used for long periods of time. With the aim of applying targeted therapies to control immune dysregulation in these patients and minimize side effects, new treatments are being tested. These new therapies include: JAK-STAT pathway inhibitors (Ruxolitinib), PI3K/AKT/mTOR pathway inhibitors such as sirolimus and others.
Methods: exposure of PBMCs of healthy controls to 4 commonly used immunomodulators at different concentrations (RAPA 20ng, 100ng, 500ng; Tacrolimus 5ng, 10ng, 15ng; Mycophenolic acid 5ng, 10ng, 15ng and Ruxolitinib 0.1uM, 0.3uM, 1uM) to assess the mechanistic impact of the drugs on Treg cells. Different regulatory cell populations beyond classical CD25hiCD127lowFoxP3+ Treg cells, such as Tr1, CD8+CD122+PD-1+, CD8+CD28-CD56+ cells and expression of suppression markers such as TIM-3, TIGIT, GITR are explored by multiparametric flow cytometry.
Results: preliminary results (n=2) show a different impact depending on drug, dose and study population. For Tr1 cells, ruxolitinib at 0.3uM appears to increase the cell population dramatically compared to the other drugs. For the CD8 regulatory compartment, mycophenolic acid tends to increase this cell population while rapamycin tends to decrease it. Finally, the classical Treg populations together with the expression of suppression markers do not show relevant changes.
Conclusion: Although some drugs at specific doses tend to increase or decrease certain regulatory populations, further replicates and suppression trials will be conducted to uncover the mechanism by which these commonly used immunomodulators impact on Treg function and phenotype.

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1012 – P2.17.04

In situ reprogramming of dendritic cells for immunomodulation of FoxP3+ regulatory T cells

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The purpose of this project is to investigate the tolerance mechanisms observed in mice with experimental autoimmune encephalomyelitis (EAE), a murine model for multiple sclerosis (MS), after applying a non-inflammatory mRNA coding for myelin oligodendrocyte glycoprotein (MOG). This mRNA has been developed by Krienke et al. and it is specifically taken up by CD11c+ cells where the MOG peptide is then expressed on MHCII. When applied in mice with an active EAE, there is a decrease in MOG-specific T cells and an increase in regulatory T cells in the spleen. This project focuses on the mechanisms leading to the tolerance induction as well as the cells involved.
In order to investigate the impact that the priming phase of EAE has on the tolerance induction, we will apply the mRNA in mice with an adoptive transfer EAE.
Additionally, we will analyze the cells involved in the observed tolerance induction, the mRNA will be applied in mice with an active EAE and the gene expression profile of the Tregs that infiltrate the CNS will be analyzed. To do so, a novel single cell sequencing technique called “zman sequencing” will be applied (Kirschenbaum et al., 2024). Zman sequencing allows to introduce time stamps by injecting anti-CD45 antibodies coupled to different fluorophores at different time points. When performing single cells sequencing of the CNS, it can be differentiated which cells infiltrated at which time point. This technique is especially interesting to apply in this context since it allows us to cover multiple days during the course of EAE.
Currently, we focus mostly on the establishment of this technique in EAE. We could show the exclusion of labeling of the long-term CNS-resident microglia population even during neuroinflammation at the onset, peak and recovery phase of the disease. Additionally, we could show that time stamps up to 48 hours before opening mice could still be detected by flow cytometry. These findings highlight the applicability of zman sequencing in EAE.

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1266 – P2.17.05

Induction of neonatal Fc receptor-mediated tolerance to therapeutic factor VIII in Hemophilia A

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Introduction: Development of inhibitory anti-factor VIII (FVIII) antibodies after replacement therapy with FVIII is a serious problem in hemophilia A patients.
Purpose: Foster a complete tolerance towards FVIII in Hemophilia A through the transplacental delivery of either Fc-fused FVIII, Fc-fused FVIII domains or Fc-fused immunodominant FVIII-derived peptides.
Methods: Binding of Fc-fused molecules to Fc neonatal receptor (FcRn) was evaluated at pH6 and pH7.4 by SPR and ELISA. Intracellular routing of the constructs was investigating by confocal microscopy, using human placental cells (BeWo) and human endothelial cells (HMEC-1) transfected with FcRn-EGFP. To evaluate the transplacental delivery, pregnant FVIII-KO mice were injected at day 17.5 of gestation with Fc-fused proteins. Foetuses were collected 4h later and the protein concentration was quantified by ELISA. In some mice, *in vivo* imaging on the mothers' organs, placenta, and fetuses were performed. For tolerance experiments, naïve pregnant FVIII-KO mice were injected from day 16 to 18 of gestation with different molecules, the progeny was weakly treated with FVIII. Anti-FVII antibodies were evaluated in the mice circulation.
Results: The FcRn mediates the transplacental delivery of Fc-fused FVIII (FVIII-Fc) but at insufficient levels (0.03±0.01nM) to foster FVIII-specific tolerance. The low transplacental delivery of FVIII-Fc in FVIII-KO mice is due to an impairment on the FcRn-mediated recycling of FVIII-Fc. Indeed, FVIII-Fc bound to FcRn at neutral pH, which reduced its release from FcRn in the extracellular space and favor its degradation in lysosomes. Masking the C1 and C2 domains of FVIII-Fc abrogated its binding to FcRn in neutral conditions. Accordingly, mutations in the C1 and C2 domains of FVIII-Fc prevented its binding to the FcRn at pH 7.4. Such improvement in the FcRn-mediating recycling had an impact on its transplacental delivery (the FVIII^{C1C2}Fc mutant showed a 8.7-fold increased in contrast to FVIII-Fc). In comparison with FVIII-Fc (0.03±0.01 nM), the transplacental delivery of domains-Fc (0.5-1 nM) and peptides-Fc (3-8 nM) were higher.
Conclusion: Due to their good placental crossing capacities, the FVIII^{C1C2}Fc mutant, domains-Fc and the peptides-Fc seem to be good candidates to improve the FVIII (entire, domains or peptides) delivery to the fetuses and favor induction of FVIII-specific tolerance.

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1268 – P2.17.06

Getting dynamic insights into the regulation of autoreactive germinal centers using serial intravital microscopy

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Purpose: Germinal Centers (GCs) are dynamic and transient microanatomical structures, giving rise to antibody-producing plasma cells. Upon antigen exposure, GC responses are initiated and eventually shut down again when antigen has been cleared. In autoimmune diseases, however, GCs persist. How autoreactive GCs escape the mechanisms of shutdown under ongoing inflammation is unclear. T-follicular regulatory (Tfr) cells are known regulators of GC responses, hence investigating their involvement in the persistence of autoreactive GCs is crucial. Splenic autoreactive GCs are of special interest as the spleen is critical to clearance of circulating immune complexes and apoptotic debris, and splenomegaly represents a hallmark of autoimmunity. To our knowledge, long-lasting GC dynamics in the spleen have not been investigated yet.
Method: An abdominal imaging window over the spleen was used to capture autoreactive GC dynamics over two weeks during both their emergence and their shutdown. A TLR7 agonist, R848 (Resiquimod), was administered and paused to induce the respective initiation and shutdown of autoreactive GCs. To identify and follow the same GCs during two weeks, intravital labeling of follicular dendritic cells (CD35-iFluor647) was utilized. Foxp3-GFP-DTR mice were used to visualize T-regulatory cells, and Tfr cells were identified based on their localization in GCs.
Results: The initiation of an autoimmune response caused an increased spleen weight and expansion of GCs, whereas the shutdown of an autoimmune response caused a decreased spleen weight and contraction of GCs. Serial intravital imaging of the spleen revealed different Tfr cell influx patterns during autoreactive GC initiation and shutdown, while only perturbing mouse body weight and systemic inflammation minimally.
Conclusions: Longitudinal tracking of individual autoreactive GCs allows visualization of autoimmune responses without disturbing normal physiology. This involves capturing the progression and shutdown of autoimmune responses. Serial intravital imaging of GCs in the spleen, combined with different murine transgenic reporter models is a promising new research tool to study dynamic long-lasting cellular mechanisms contributing to either the progression or shutdown of (autoreactive) immune responses.

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1317 – P2.17.07

Central Tolerance Mechanisms in a CNS1-/- Rat Model

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Central tolerance is crucial for preventing autoimmunity, with Aire playing a key role. Aire deficiency leads to autoimmune polyendocrinopathy candidiasis ectodermal dystrophy (APECED). Although CNS1's role in Aire regulation is documented in mice, its function and the impact of its deletion across species are not well understood. This study aims to elucidate the role of CNS1 in Aire expression and to characterize the autoimmune phenotype in CNS1 knockout (CNS1-/-) rats.
CNS1-/- rats were created via CRISPR/Cas9, using two guide RNAs to delete the CNS1 sequence, including NF-κB sites. These rats were used to characterize the effect of CNS1 deletion on Aire expression and the development of autoimmune phenotype by analysing the immune organs, skin, blood plasma, and adrenal glands using flow cytometry, real-time PCR, LIPS, immunefluorescence microscopy, histological examinations, and transcriptome analysis.
Our findings confirm the absence of Aire protein in immune tissues and thymic evaluations revealed altered tissue-specific protein expression, inflammatory mediation, and regulatory T-cell differentiation. CNS1-/- rats exhibited an APECED-like phenotype characterized by hair loss, nail dystrophy, and the presence of anti-interferon autoantibodies. Transcriptomic and histological analysis of the skin revealed an upsurge in inflammation cell markers and Jak-Stat pathway genes, alongside tissue damage and most hair follicles in knockout rats arrested in the telogen phase of the hair cycle, indicative of an alopecia-like condition. Additionally, blood plasma analysis suggested adrenal gland dysfunction, potentially hinting at Addison's disease due to elevated potassium and decreased corticosterone levels.
Our research confirms CNS1's vital role in modulating Aire expression across different species and unveils the CNS1-/- rat as a novel model for exploring Aire's regulatory mechanisms, central tolerance origins, and the onset of alopecia and possibly Addison's disease.

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1755 – P2.17.08

The role of thymic B cells in central T cell tolerance

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Central T cell tolerance is essential for maintaining immune homeostasis and preventing autoimmunity. Traditionally, medullary thymic epithelial cells and dendritic cells are considered the key thymic antigen presenting cells (APCs) that promote the deletion (negative selection) or Treg cell differentiation of autoreactive thymocytes. We previously showed that the thymus also harbors a distinct population of B cells expressing Aire and exhibiting characteristics akin to activated B cells such as elevated CD80 and MHCII levels. These potent APC features of thymic B cells suggest that they may play a crucial role in central T cell tolerance. To elucidate how thymic B cells contribute to shaping the T cell receptor (TCR) repertoire, we compared the TCR repertoire of mature 'conventional' CD4 thymocytes and thymic Foxp3+ Treg cells from B cell-deficient and -sufficient mice. This comparison revealed a substantial number of TCR clonotypes whose deletion or diversion to the Treg repertoire required the presence of B cells, indicating a significant contribution of thymic B cells to both modes of central tolerance. In summary, our findings suggest a more prominent role of thymic B cells in shaping the T cell repertoire than previously appreciated.

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2060 – P2.17.09

Calorie restriction as a novel therapeutic tool to modulate immune system during multiple sclerosis

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Purpose: There is a strong relationship between metabolic state and immune tolerance through a direct control exerted on immune cells by specific intracellular nutrient-energy sensors. An increased "metabolic work load" represents a novel issue linking metabolism with loss of self-immune tolerance. In this context, several dietary interventions have been shown to influence disease progression of experimental autoimmune encephalomyelitis (EAE), the experimental model of Multiple Sclerosis. Our approach aims at dissecting at the cellular level the mechanism of action of different dietary regimens, such as Free Diet (FD) and Caloric Restriction (CR), to alter disease progression and immune cell homeostasis, in relapsing remitting Multiple Sclerosis (RR-MS) subjects.
Methods: We evaluated the impact of FD and CR on the immunophenotype of different subsets of circulating immune cells and their correlation with clinical/nutritional status and patient reported outcomes (PRO) in RR-MS subjects during DMF treatment. We also investigated the effect of different dietary regimens (FD and CR) on the metabolic asset of conventional T (Tconv) cells (measurement of glycolysis and oxidative phosphorylation) from RR-MS subjects after starting first line drug treatment.
Results: We observed that CR improves the clinical/nutritional status of RR-MS subjects and modulates different immune T cell subsets. Moreover, CR is able to reduce glycolytic capacity of pro-inflammatory Tconv cells compared to FD-RR-MS subjects.
Conclusions: Overall, these data suggest that modulation of metabolic state via calorie restriction is able to improve the outcome of RR-MS and efficacy of first line drug treatment.

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2122 – P2.17.10

Impact of CCR4 ligands on Treg cells in islet autoimmunity

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Type 1 Diabetes (T1D) is an autoimmune disease defined by the selective destruction of pancreatic beta-cell cells leading to insulin deficiency. Exact mechanisms are still poorly understood, but regulatory T-cell (Treg) impairments have been shown to promote aberrant immune activation thereby triggering islet autoimmunity. Chemokine ligand (CCL) 22 and CCL17 are important mediators of T-cell trafficking with anti- and pro-inflammatory properties respectively. CCL22/CCL17 transduce signals to Tregs via the Chemokine receptor (CCR) 4, highly expressed on Tregs. CCL22-CCR4 interaction leads to Treg migration and prolonged dendritic cell (DC)-Treg crosstalk resulting in immune suppression. However, functional effects of CCL22 and CCL17 signalling on Tregs apart from migration remain incompletely understood especially in the setting of islet autoimmunity.
To approach this experimentally, our preliminary data show trends towards reduced levels of serum CCL22 in autoimmune prone non-obese diabetic (NOD) mice compared to disease-free Balb/c mice (149.22 ± 36.14 vs. 198.88 ± 72.10 pg/ml; p=0.08) while in the pancreas, both CCL22 and CCL17 levels were increased (CCL22: 2.96 ± 0.27 vs. 1.57 ± 0.50 ng/g protein; CCL17: 4.36 ± 2.96 vs. 1.55 ± 1.09 ng/g protein). This was accompanied by increased frequencies of CCR4+ Tregs in the pancreas of insulin autoantibody (IAA) positive NOD mice compared to IAA negative NODs (32.01 ± 8.29% vs. 20.66 ±5.04%; p=0.0044), suggesting a possible active recruitment of the Tregs to the pancreas via the CCL22/17-CCR4 axis.
In our next steps, we will make use of gain- and loss-of-function mouse models to specifically analyse the role of the single components of the axis in Treg ontogenesis, including Treg induction in presence/absence of DCs deriving from the models. In parallel, we will employ different mouse models for islet autoimmunity and perform in depth analysis of CCR4+ Treg phenotypes with multiparametric conventional and spectral flow cytometry technologies. These studies will provide insights into the impact of CCR4 ligands on Treg cells and their relevance for T1D development, with the overarching goal to identify critical new targets for the future development of novel Treg targeting strategies.

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P2.18 MECHANISMS OF ATOPIC DISEASE

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1668 – P2.18.01

Peripheral mast cell-derived RANKL is a prerequisite for lymphocyte egress from distant lymph nodes

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Purpose: Mast cells (MCs) are innate sentinel cells populating tissues at the interface to the environment such as skin, lung and intestine, and act as key initiators of vasoactivation and immune cell infiltration upon inflammatory insult. The receptor activator of NFκB (RANKL) is most well-known for its role in bone resorption, but recent studies have shown that it is an important immune regulator. We recently identified MCs as a prominent source of RANKL. However, the relevance of MC-derived RANKL in skin inflammation is completely unknown
Methods: Using a mouse line with a conditional RANKL knockout in connective tissue type MCs, we defined the relevance of MC-derived RANKL in DNFB-induced contact hypersensitivity, a mouse model for allergic contact dermatitis.
Results: Surprisingly, mice lacking MC-derived RANKL displayed massive lymphocyte hyperplasia in inguinal lymph nodes (LN) 24h after DNFB challenge, accompanied by profound blood lymphopenia. Despite delayed lymphocyte egress and even increased immune cell infiltration at 48h after challenge, skin inflammation remained markedly dampened in absence of MC-derived RANKL. Strikingly, MC depletion and reconstitution with RANKL deficient MCs only locally in the ear skin resembled the early inguinal LN hyperplasia, blood lymphopenia and reduced lymphocyte infiltration to inflamed skin. An i.v. administration of Sphingosine-1-phosphate (S1P) after induction of skin inflammation was able to restore timely lymphocyte egress demonstrating an organ-spanning RANKL-S1P axis.
Conclusion: In the absence of MC-derived RANKL, lymphocytes do not timely egress from LNs after the induction of skin inflammation, which leads to massive LN hyperplasia and profound blood lymphopenia. Moreover, when lymphocytes finally egress, they infiltrate the ear skin with a delay that leads, possibly because of the dys-primed inflammatory microenvironment, to diminished inflammation and ameliorated symptoms of CHS. Consequently, MC-derived RANKL is a prerequisite for lymphocyte egress from distant LN, through a mechanism involving the induction of S1P signaling.

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P2.19 MICROBIOTA

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124 – P2.19.01

Understanding the molecular recognition of Bacteroides fragilis glycosphingolipids by Natural Killer T-cell receptor

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The human gut microbiota comprises more than 50% of *Bacteroides* species that produce small diffusible molecules like sphingolipids that play a key role in modulating the host's immune responses. In particular, *Bacteroides fragilis* produces glycosphingolipids similar to α-galactosylceramides termed as 'BfaGCs' that can activate type I Natural Killer T (NKT) cells. While they share key chemical similarities with the type I NKT cell marker antigen, α-galactosylceramide (KRN7000), they possess distinctive structural features including short sphinganine chains, branching and functional groups, implying a basis for their unique immunomodulatory properties. The co-culture assay performed with bone marrow-derived dendritic cells and NKT cells in the presence of specific BfaGCs indicated that branching in their sphinganine chain is a critical determinant of NKT cell activation. As such, the strong stimulators measured by their IL-2 release were the compounds that contained the branched sphinganine chains. Our structural studies on two such CD1d-presented BfaGCs in complex with the type I NKT TCR revealed the TCR adopted a parallel docking topology atop the F'-pocket of CD1d in recognising the presented BfaGCs. Interestingly, the terminal sphinganine branching of the BfaGCs mediated unique interactions within the F'-pocket of CD1d, providing a mechanism for their differing agonistic properties. The NKT TCR recognised the CD1d presented stimulatory and non-stimulatory BfaGCs with nanomolar affinities. Thus, BfaGCs were demonstrated to be bonafide CD1d ligands that function as immunomodulatory mediators influencing the host's defence in the context of NKT cells. Together, this study highlights the structural and molecular-level paradigm of existing symbiotic relationship between the microbes producing these endogenous lipids and the host.

References:
(***** *denotes co-first authors*)
Sungwhan F. Oh*****, **Praveena T*,** Hee Bum Song, Ji-Sun Yoo, Da-Jung Jung, Deniz Erturk-Hasdemir, Yoon Soo Hwang, Changwon C. Lee, Jérôme Le Nours, Hyunsoo Kim, Jesang Lee, Richard S. Blumberg, Jamie Rossjohn, Seung Bum Park, and Dennis L. Kasper. Host immunomodulatory lipids created by symbionts from dietary amino acids ***Nature.*** 2021 Dec;600(7888):302-307.

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318 – P2.19.02

Impact of microbiota from preterm infants on the immune system and infection susceptibility

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Purpose: The neonatal period is characterized by a higher susceptibility to infection, particularly in preterm infants, for whom infection and subsequent sepsis remain significant clinical problems worldwide. Early-life host-microbe cross-talk plays a key role in the development and maturation of the mucosal and systemic immune system, exerting a major influence on lifelong immune homeostasis and disease susceptibility. However, detailed information on how age-related programming and microbiota-dependent imprinting influence an individual's immune status and lifelong susceptibility to infectious diseases is lacking in humans. Our study aims to explore the immunomodulatory effects of the preterm microbiome on the host and its potential protective role against infection.
Methods: Germ-free female mice were colonized with feces from preterm infants before mating, and their offspring were analyzed to validate the humanized mouse model and assess the impact of the preterm infant-derived microbiota on the immune status under homeostasis using high-dimensional flow cytometry. Additionally, neonatal mice were infected with enteropathogenic *Escherichia coli* (EPEC) to assess the consequences of early-life infection on immune cell subset composition within lymphoid organs and the gut.
Results: Colonization with preterm infant fecal samples induced significant alterations in $CD4^+$ T cell subset distribution, particularly in the intestine, and to a lesser extent in secondary lymphoid organs, during steady-state conditions. Three weeks after neonatal infection with EPEC, distinct preterm microbiota backgrounds exhibited differential impacts on susceptibility and recovery from gastrointestinal infection, as evidenced by differences in recovery trajectories, organ burden, and immune cell populations.
Conclusion: Overall, our results support evidence that different microbial communities have sustained effects on the neonatal immune system under homeostasis. It enhances our understanding of the complex interplay between microbiota composition and host defense in gastrointestinal infections, emphasizing the complexity of early-life immune system education. Future research should investigate specific microbial species associated with reduced infection incidence and improved outcomes in newborns, emphasizing the potential of probiotics as preventive interventions.

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901 – P2.19.03

Host glycosylation shapes intestinal microbiome and immune response in Inflammatory Bowel Disease

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Inflammatory Bowel Disease is a group of chronic debilitating disorders from the gastrointestinal tract, comprising Crohn's disease (CD) and ulcerative colitis (UC). It is caused by the interplay of the host's genetic predisposition and abnormal immune response to an altered gut microbiota driven by several environmental factors.
The currently available therapies for IBD are ineffective, being associated with several side effects. Thus, an improved understanding of the causes leading to the loss of gut microbial equilibrium (dysbiosis) and mucosal inflammatory response is crucial for a better clinical management of IBD. Cellular glycosylation displays a key role in IBD immunopathogenesis. The epithelial glycocalyx, a complex layer of glycans/sugar-chains at the cell surface, is a major interface between host and gut microbiota. In this study we aim to unveil the impact of an altered host glycocalyx in the microbiota profile and in the initiation of intestinal inflammation. For that, we have resorted to a glycoengineered mouse model, deficient in complex glycans. Colitis was induced by dextran sulfate sodium (DSS) and disease score was monitored daily. The intestinal glyco-immune profile was characterized at baseline and after disease induction. Faecal microbiota was analysed by 16S rRNA sequencing. Firstly, we observed that the increase susceptibility to severe colitis in glycoengineered mice is concomitant with a dysbiotic gut microbiota and decreased levels of SCFAs at baseline. Interestingly, the analysis of immune profile demonstrated that glycoengineered mice exhibited a proinflammatory immune response characterized by the impairment in innate immune response. Alteration in complex glycans was associated with an increased frequency of dendritic cells and increased levels of IL-6 and TNFα on colonic mucosa at steady state. Overall, our results highlight the role of glycans as essential elements that shape gut immunity and intestinal microbiome.

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992 – P2.19.04

The Microbiome-Spine Axis: Exploring Pathology, Autoimmunity, and Research Gaps through a Narrative Review

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The microbiome's relevance in human health and patient care is becoming increasingly discussed amid the rising prevalence of chronic illnesses. It is well known that the gut microbiome has a complex relationship with our immune system; helping it to mature and prevent overactivation. Microbiome research to date focuses predominantly on its relationship with the GI tract while largely ignoring any impact on the rest of the body. Our review aims to lay a foundation of knowledge to fill this gap in the literature, specifically concerning the microbiome and its relation to spinal health.
Through this narrative review, it was found that with disruptions, specific bacterial families such as Bacteroidaceae and Rikenellaceae are allowed to proliferate and are associated with increased onset of ankylosing spondylitis (AS). Dysbiosis was also seen to alter the cytokine microenvironment and subsequently increase gut wall permeability, causing immune overactivation, and improper cell function. Ultimately these changes result in a heightened state of inflammation, leading to increased susceptibility to autoimmunity and in particular AS and seronegative arthropathies.
Overall, the available literature discussing the microbiome and its relation to spine health still has significant gaps in knowledge. It was found that most studies focus solely on the bacterial composition but ignore fungal and viral components of the microbiome; which was demonstrated through their predominant usage of 16s RNA sequencing. The sheer quantity of confounding factors surrounding microbial compositions also makes for poor generalizability of results to the greater population. These limitations all serve to add constraints to a comprehensive understanding of the microbiome's true significance and underscore the severe need for standardized and organized research approaches.
In conclusion, this narrative review hopes to equip clinicians with a broad understanding of how the microbiome, and its disruption, have specific implications for spinal health; an incredibly complex and novel topic. By building on the current literature and integrating this knowledge into practice, we hope to implement more patient-specific practices in the treatment of spinal pathologies and ultimately improve and optimize patient care in a field in which the microbiome is not currently at the forefront of pathology.

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998 – P2.19.05

Involvement of gut and oral Microbiome in the development of allergy to non-specific Lipid Transfer Proteins

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Purpose: Co-sensitisation to non-specific Lipid Transfer Protein (nsLTP) from olive pollen (Ole e 7) and peach (Pru P 3) is a usually phenomenon observed in the Mediterranean basin and particularly in areas of extensive olive tree crops, without a clearly established underlying cross-reactivity between proteins. The aim of this study was to analyse other possible causes related to this phenomenon, including the interaction between microbiome and the immune system.
Methods: Gut and oral microbiome profile of 110 patients sensitised to Ole e 7 with or without sensitisation to Pru p 3 (by sIgE > 0.35 kU/L in ImmunoCAP 250) was explored, together with the expression of Toll-like receptor (TLR) pathway in oral mucosa by NanoString and circulating T-cell subpopulations by flow cytometry.
Results: Oral microbiota of monosensitised patients was characterised by a preponderance of TM7_3 and Clostridia classes, whereas that of bisensitised patients was characterised by the preponderance of an unknown genus from Actinomyces, by the Micrococcaceae family belonging genus Rothia and by an unknown genus from Gemellaceae family. Regarding gut microbiota, we found that in monosensitised patients, it was characterised by the preponderance of Betaproteobacteria class. By contrast, the gut microbiota of bisensitised patients was characterised by the preponderance of Bacteroides genus and the bacterial species *Bacteroides uniformis*. When we performed a logistic model including microbiome, immunological and TLR variables, we found that the presence of circulating Tregs $CD39^{+}$, the expression of TLR3 in oral mucosa and the preponderance of Rothia genus and Gemellacea family in oral microbiota and Bacteroides genus, Butrycimonas genus and Bilophila genus in gut microbiota were the most important variables to determine the sensitisation to Ole e 7 and Pru p 3 with an area under the curve of 0.882, an accuracy of 81.4%, a sensibility of 87.5% and a specificity of 76.3%.
Conclusions: The microbiome of patients monosensitised to Ole e 7 and patients bisensitised to Ole e 7 and Pru p 3 is different and its interaction with the immune system could be determinant to develop sensitisation to multiple nsLTPs.

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1101 – P2.19.06

IMMUNOMETABOLIC STATE IN A PORCINE MODEL AFTER SUPPLEMENTATION OF THE MIXTURE OF LACTOBACILLI ISOLATED FROM HUMAN MILK

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Purpose: The porcine model *vs*. human analogy reveals significant findings regarding the diversity of its intestinal microbiota and the presence of *lactobacilli*, which are relatively stable in both species' adult stages but fluctuate during the piglet and infant stages. In this context, previous reports show that piglets from the same litter and newborn human twins can differ concerning their intestinal microbiota, manifesting in their metabolic homeostasis. In a porcine piglet model, this study examined the immune-metabolic state after supplementation with a mixture of *lactobacilli* isolated from human milk.
Methods: We included 16 piglets *of Sus scrofa domesticus*, evaluated their body weight, and classified them as control (n = 8) without supplying a mixture of Lactobacilli and treatment (n = 8) supplying a mixture of *Lactobacilli* and treatment (n = 8) with a mixture of *Lactobacilli*. *Lactobacilli* were isolated from human milk. A mixture of three (*Lactoplantibacillus plantarum, Limosilactobacillus reuteri, and Lactobacillus fermentum*) was formulated with 109 CFU/mL and provided as a supplement at the treatment group in the experimental diet throughout 30 days. Piglet blood samples were collected on day 1 and day 30 for immune-metabolic profiling after they were euthanized and necropsied on day 30. Adipose and intestinal segments were collected, flushed with cold PBS, and fixed in 10% neutral buffered formalin. Tissue samples were then sliced into 5 µm sections and stained with hematoxylin and eosin stain to determine intestinal villus height and crypt depth morphology. Images of adipose and intestinal sections were taken using an OPTIKA microscope/LEITZ-WETZLAR photo-camera.
Results: At day 30, the piglets of the treatment group showed minor diameter adipocytes, increased serum levels of chemerin, decreased levels of glucose, insulin, triglycerides, VLDLc, CRP, and ESR, and a smaller number of monocytes circulating compared to the control group. The duodenum/jejunum/colon *villus* and height-to-Lieberkühn crypt depth ratio in the control group was reduced compared to the treatment group, while the ileum was inverse.
Conclusion: These data suggest that the mixture of *Lactoplantibacillus plantarum, Limosilactobacillus reuteri, and Lactobacillus fermentum* isolated from human milk contributes to a healthy immune-metabolic state in the piglet porcine model *Sus scrofa domesticus*.

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1330 – P2.19.08

Intestinal-derived short chain fatty acid modulates alveolar macrophage response to *Streptococcus pneumoniae*

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Lower respiratory tract infections are a leading cause of mortality worldwide. *Streptococcus pneumoniae* is a major human respiratory pathogen responsible for 30-50% of community acquired pneumonia cases and over a million deaths every year. Alveolar macrophages are resident phagocytes in the lower airways with a key role against pneumococcal infection as they promote early bacterial clearance and co-ordinate downstream inflammatory responses.
While many factors influence susceptibility to respiratory infections, the intestinal microbiota has emerged as a key regulator of respiratory immune processes. The immunological crosstalk between the intestinal and lung compartments is known as the gut-lung axis of immune regulation. The intestinal microbiota influences this crosstalk in different ways; one such mechanism is the production of immunomodulatory microbiota-derived metabolites, namely short chain fatty acids (SCFA). SCFAs are exclusively produced by the microbiota through the saccharolytic fermentation of dietary fibre in the gut. Millimolar concentrations of SCFAs are metabolised in the colon where they have potent immunomodulatory properties and reach the portal circulation at sub-millimolar levels. Intestinal dysbiosis and low SCFAs have been linked to macrophage dysfunction and susceptibility to pneumococcal pneumonia. The SCFA acetate (C2) and propionate (C3) protect against pneumococcal pneumonia by enhancing alveolar macrophage function. However, the role of the four carbon SCFA butyrate remains to be explored in this context.
Here we investigated how butyrate modulates susceptibility and alveolar macrophage function during *S. pneumoniae* infection. *In vivo* butyrate supplementation protected mice against invasive pneumococcal pneumonia. *In vitro*, butyrate increased IL-1β secretion in long-term cultures of *ex vivo* alveolar macrophage (MexAM) stimulated with the TLR2 agonist Pam3CSK4. Preliminary experiments in murine foetal liver-derived alveolar macrophage (FLAM) showed that butyrate enhanced the expression of *Nlrp3* and *Tnfa* upon *S. pneumoniae* infection or stimulation with Pam3CSK4. Collectively this evidence points towards a role of butyrate in enhancing alveolar macrophage function and increasing resistance against pneumococcal pneumonia. Our data highlights the therapeutic potential of dietary interventions and modulation of the gut microbiota as means to increase resistance to invasive pneumococcal pneumonia.

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1343 – P2.19.09

Butyric acid-producing Faecalimonas sp. NGB245 strain attenuates the symptoms of experimental autoimmune encephalomyelitis

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Purpose: The bacterial strains residing in the anaerobic environment of the colon have been shown to produce short-chain fatty acid (SCFA) essential for preserving homeostasis in the gut, and in the host more broadly. The reduction in the abundance of butyric acid (BA)-producing bacteria associated with multiple sclerosis (MS). Hence, our study aimed to isolate the bacterial strains with the high capacity to produce BA from the feces of healthy donors and to test the effects of supplementation in a mice model of MS.
Methods: By using different media in anaerobic conditions and measuring BA by HPLC we selected *Faecalimonas* sp. NGB245 strain. To overcome the sensitivity of this strict anaerobe to oxygen, the NGB245 overnight culture (NGB245-postbiotic) was used as the supplement. Myelin oligodendrocyte glycoprotein peptide/complete Freund's adjuvant/pertussis toxin-induced experimental autoimmune encephalomyelitis (EAE) in C57BL6 mice was used as a model of MS. Mice consumed NGB245-postbiotic in a 16h/day (instead of the water, ad libitum) regime during the 15 days. The control group of EAE mice consumed PYG medium enriched with cellobiose and starch, used for the NGB245 cultivation, in the same regime. The effects of NGB245 strain on EAE symptoms, immunological responses, and microbiota properties were analyzed by flow cytometry, ELISA, microscopy, and new-generation sequencing, respectively.
Results: NGB245 strain was selected for the study based on the high production of BA (15mM). The supplementation with NGB245-postbiotic alleviated daily clinical scores, maximal clinical scores, and the duration of the EAE in comparison to the control group. This was associated with the lower abundance of Th1 and Th17 cells, myeloid dendritic cells, inflammatory monocytes, macrophages, and activated microglia, but a higher level of myeloid-derived suppressor cells in the CNS of treated EAE mice. These immunomodulatory effects were associated with the higher diversity of microbiota in the colon.
Conclusion: These results support the idea of targeting the microbiota in autoimmune diseases to preserve the homeostatic functions of the immune system and microbiota and thus restrain the autoimmune process development.

This work was supported by NITRA 451-03-66/2024-03/200042, 451-03-66/2024-03/200019; by the Science Fund of the Republic of Serbia, #7744507, NextGenBiotics.

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European Journal of Immunology Clinical-Basic-Translational

1497 – P2.19.11

Examining the Role of the Microbiome-Immune Axis in the Development of Systemic Lupus Erythematosus

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Over the past decades, autoimmune disorders have drastically increased in incidence and, presently, affect one in ten people in the Western world. However, the etiology of these diseases remains unknown in most cases. Systemic lupus erythematosus (SLE) is an example of such an autoimmune illness. Despite improvements in both treatments and diagnostics, the limited understanding of SLE leads to inadequate treatment efficacy and thus morbidity and mortality remain high. Therefore, it is crucial to further explore the etiology and pathogenesis of these autoimmune disorders. One possible determining factor is the microbiome-immune axis. It has been postulated imbalanced microbiota composition and function (often called dysbiosis) lead to a loss of immunological tolerance and thus an increased risk of developing autoimmune conditions. Although no causal connection between dysbiosis and SLE has been discovered until today, multiple studies have found interesting correlations.
Using a transgenic lupus-prone mouse model called 564Igi, we investigated the effect on the autoimmune phenotype when altering the microbiome. Briefly, this was accomplished by exposing cohorts of 564Igi mice to environments with distinct sanitary differences or treating them with a cocktail of antibiotics to induce severe dysbiosis. The comprehensive collection of biological samples makes it possible to investigate whether a dysbiosis in the gut microbiome occurs before or after the development of the autoimmune condition and whether it depends on the autoimmune phenotype and the immunological response toward microbiome alterations. Preliminary results from comparative metagenomics provide novel insight into bacterial communities of the gut microbiome that correlate with an ameliorated or exacerbated autoimmune phenotype. Furthermore, flow cytometry and other immunological analyses indicate that a higher systemic response toward gut bacteria signifies better immune control of the gut microbiome and correlates with a more benign lupus phenotype. This suggests a complex interaction forming feedback and feedforward loops between the gut microbiome and the immune system, which also may play a significant role in the pathogenesis of SLE.

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1527 – P2.19.12

Gut bacteriome in mothers with and without gestational diabetes and their offspring: immunological parallels

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Purpose: Gestational diabetes mellitus (GDM) influences both the maternal and offspring microbiomes, therefore it may play a role in shaping the onset of immune-mediated diseases. We aimed to study the gut bacteriome of mothers with and without GDM and their young children and compare these results with children's allergen-specific IgE test results, skin- and respiratory-tract-associated allergy diagnoses, HLA haplotypes and presence of diabetes-associated autoantibodies (glutamic acid decarboxylase 65 antibody (GADA), islet antigen-2 autoantibodies (IA-2A) and zinc transporter 8 autoantibodies (ZnT8A)).
Methods: Stool and blood samples were collected from 53 mothers (18 with GDM and 35 without GDM) and their children (1-2 year olds) recruited from Woman's Clinic of Tartu University Hospital. Data about children's allergy diagnoses were obtained from Electronic Health Records. The composition of stool bacterial communities was profiled by 16S rDNA sequencing. Allergen-specific IgE, diabetes-associated autoantibodies and HLA haplotypes were analysed.
Results:
No differences were observed in alpha and beta diversity between mothers with and without GDM. However, we found a negative correlation between the mother's C-peptide level and alpha diversity measures. In children, no significant difference in alpha diversity was found with allergy diagnoses, maternal GDM diagnoses, HLA haplotypes or IgE. However, the microbial richness (Shannon's index) was higher in children whose mothers had diabetes-associated autoantibodies during pregnancy. Overall bacterial structure (beta diversity) revealed a difference for clustering between children with (IgE level ≥0.7 kUA/L) and without definite (IgE level <0.7 kUA/L) allergic sensitisation.
Conclusion:
The results demonstrate that alpha and beta diversity showed no significant differences between mothers with and without GDM. However, children of mothers with diabetes-associated autoantibodies exhibited higher microbial richness and distinct clustering based on allergic sensitization status.

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1970 – P2.19.13

Gut microbiota-modulated immunological profile in mouse models of eating disorders

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Purpose: Emerging studies highlight the significant impact of the gut microbiome on neuropsychiatric disorders and offer insights into its potential to influence human behavior. The intestinal microbiota modulates the central nervous system via neuroimmune and neuroendocrine pathways and thus may promote the pathophysiology of these disorders. Loss of microbial diversity and reduced abundance of beneficial microbes are frequently described in patients with eating disorders. To gain further insight, we investigated the reactivity of the immune system in mouse models of binge eating disorder (BED) and anorexia nervosa (AN) in conventional animals and animals with depleted microbiomes.
Methods: In our study, we used conventional, ATB-treated, and germ-free mouse models of BED and AN. We collected spleens of these mice to measure immune cell populations and intracellular cytokine production by flow cytometry. In addition, the levels of assorted cytokines (IL-6, IL-10, IL-12, TNFα, IFNγ, and MCP1) were determined in the serum. To assess gut barrier function, we collected colon biopsies and determined the expression of two intestinal tight junction proteins (ZO-1, occludin) and two members of the mucin protein family (MUC2, MUC13).
Results: In mice with an induced model of eating disorder (ED), the population of CD4+CD25+FoxP3+ regulatory T cells was significantly lower than in control mice. The difference was even more pronounced in mice with an ATB-depleted microbiome. Further, CD4+IFNγ+ cells were increased in mice with ED. Again, the difference was greater in ATB-treated mice. We did not detect any difference in cytokine levels in mice with ED. We observed increased expression of ZO-1 and occludin only in germ-free, but not in conventional or ATB-treated mice in the AN model compared to control mice. We also found no significant differences in mice with the induced BED model.
Conclusion: In mouse models, we have demonstrated the influence of the microbiota on changes in the representation of the population of immune cells and the production of IFNγ in the mouse spleen. These changes may lead to a long-term disturbance in the reactivity of the immune system, which is frequently observed in patients with ED, especially in patients with AN.

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2000 – P2.19.14

Influence of the microbiome on dendritic cell phenotype and function

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Purpose: The intestinal microbiome educates the immune system, in part through signaling in antigen-presenting cells including dendritic cells (DCs). Germ-free mice exhibit DC deficiency, migration impairment, and priming dysfunction, implying a relationship between microbiota and DC function. Naturally acquired, endemic *Helicobacter* species (*H.* spp.) such as *H. hepaticus* are common in many mouse colonies, whereas some facilities are *Helicobacter*-free. Although they do not cause overt disease in most immune-competent mouse strains, we hypothesized that *H.* spp. could be a source of low-level inflammation, resulting in immune reprogramming and leading to phenotypic differences between *H.* spp.-positive and *H.* spp.-negative mice.
Methods: We generated bone marrow-derived dendritic cells (BMDCs) from *H.* spp.-positive mice and from genetically identical mice rendered *H.* spp.-free through mouse rederivation. BMDCs were stimulated for 6 hours (or not) with ultra-pure lipopolysaccharide (LPS). We measured expression of molecules MHC-I and MHC II (Signal 1 of antigen presentation) and CD80, CD86 and PD-L1 (Signal 2), the "activating" epigenetic mark of trimethylation at lysine 3 of histone 4 (H3K4me3) and metabolic changes via flow cytometry. Multiplex ELISA was used to measure secreted cytokines (Signal 3) and in vitro methods were used to assay BMDC antigen presentation.
Results: Surface expression of molecules related to Signal 1 and Signal 2 of antigen presentation were increased both at steady state and after LPS stimulation in BMDCs derived from *H.* spp.-positive in comparison with *H.* spp.-free mice and this correlated with the abundance of H3K4me3. The presence or absence of *H.* spp. also altered metabolic phenotype of BMDCs upon LPS stimulation.
Conclusion: The presence of *Helicobacter* in the gut microbiome influences BMDC phenotype and may alter BMDC function. We anticipate epigenetic changes involved in BMDC phenotypic and functional changes, which will require in-depth epigenetic profiling. Future directions also include fecal microbiota transplant experiments and *Helicobacter* infection models followed by BMDC characterization using our established pipeline.

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2067 – P2.19.15

PD-L1 exaggerates obesity-induced dysbiosis

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Purpose: Obesity has grown into a major global health concern. The gut microbiome plays an important role in obesity and shaping of the immune system. However, many aspects of these interactions are only beginning to be understood. Here, we investigated the mechanisms how regulators of the immune system influence the gut microbial composition and its impact on diet-induced obesity.
Methods: Obesity was induced in mice using a high fat diet (HFD). Cell-specific function of the immune checkpoint protein programmed death-ligand 1 (PD-L1) was investigated using knockout mice as well as wild type mice treated with αPD-L1. Body weight was recorded weekly and faecal pellets were collected biweekly. We analysed the gut microbial composition by 16s rRNA sequencing. Germinal centres in the Peyer's Patches of mice were analysed using FACS analysis.
Results: The gut microbial composition of mice on HFD changed drastically in the first two weeks after onset of HFD to a more obesity-like phenotype, characterised by an increase of the *bacillota:bacteroidota* ratio. Interestingly, the early changes in microbial composition had long-lasting effects. This shift was more pronounced in wild type mice on HFD than in mice with a constitutive deletion of PD-L1. While no differences in the amount of germinal centre B cells could be observed, antibody production in the Peyer's Patches was impacted by diet and genotype.
Conclusion: Our results demonstrate that PD-L1 influences the gut microbial composition during diet-induced obesity by ameliorating the increase of the obesity-characteristic *bacillota:bacteroidota* ratio, possibly by impacting formation of tolerance. Further studies will elucidate the mechanisms how PD-L1 affects the microbial composition and development of obesity.

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2219 – P2.19.16

Mechanisms of action of microbial-derived indoles on inflammation parameters in rheumatoid arthritis

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Purpose: Rheumatoid arthritis (RA) is a chronic and progressive autoimmune disease mainly affecting the musculoskeletal system, with inflammation causing pain and destruction of joints. Recent studies reveal the role of gut microbiota in the genesis of the disease (gut-joint axis). Thereby, indoles, gut bacteria-derived metabolites of tryptophan, in particular indole-3-proioic acid (IPA), show a positive impact on the immune, nervous, gastrointestinal and cardiovascular system in various pre-clinical and clinical studies in mammals. Furthermore, the aryl hydrocarbon receptor (AHR) activated by IPA, shows a modulating effect on immune and inflammatory responses.
Methods: Mice with collagen-induced arthritis (CIA) were orally treated with IPA and antibiotics preclinical. Targeted metabolomics analyzed serum of early RA patients and untreated CIA mice. 16s RNA sequencing examined murine intestinal content and Flow cytometry presented different B cell populations in mesenteric lymph nodes.
Results: First, concentration of IPA in serum of new onset RA patients was significantly reduced compared to controls of healthy individuals. CIA mice had significantly lower IPA serum levels already on day five after immunization. Second, it was shown that IPA treatment significantly attenuated the course of CIA and restored microbial dysbiosis in the intestine. In addition, *in vivo* B cell activation- and proliferation-markers in the mesenteric lymph nodes were significantly downregulated following nutritional IPA intervention. The direct effects of IPA on B cells were further confirmed *in vitro*. Depletion of IPA producing gram-positive bacteria through targeted antibiotic treatments exacerbated arthritis scores in animal models and significantly reduced IPA serum concentration. Of note, these effects could be compensated through combined antibiotics and IPA treatment. Further, Aryl hydrocarbon receptor (AhR) specific intestinal epithelial knockout mice were used to analyse the role of AhR in the protection of IPA on arthritis scores. Thus, villin cre AhR fl/fl mice showed exacerbated arthritis scores along with significantly lower serum IPA serum concentration.
Conclusion: In conclusion, our study defines a novel approach on how gut derived indoles, specifically IPA, influences arthritis disease.

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2241 – P2.19.17

Gut microbiota protects mice from acute colitis by improving intestinal barrier function

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Purpose: Gut microbiota drives inflammation by shaping the function of intestinal barrier and stimulating the immune system of intestinal mucosa. Our aim was to analyze the mechanisms of how microbiotas from two different animal facilities influence the development of acute experimental colitis.
Methods: We induced acute colitis in conventional BALB/c mice originating from the animal facility of the Institute of Microbiology (IMIC) or the First Faculty of Medicine (FoM), and in ex-germ-free mice colonized with intestinal content from either facility. Colitis was induced by intrarectal administration of 2,4,6-trinitrobenzenesulfonic acid (TNBS) dissolved in 50 % ethanol, with (hapten-type) or without (toxic-type colitis) skin presensitization. The severity of colitis was assessed by Wallace's score and measurement of colon length. Intestinal microbiota was analyzed by 16S rRNA sequencing, gut barrier function was analyzed by a fluorescein isothiocyanate dextran (FITC-Dx) permeability assay, and immune system activity was measured by flow cytometry in cells from the spleen and mesenteric lymph nodes.
Results: IMIC mice were significantly more resistant to both types of TNBS colitis and had more bacteria of the *Verrucomicrobiae* and *Campylobacteria* classes in their feces. IMIC mice had significantly less permeable gut barrier, and significantly fewer effector memory T cells and lower percentages of IFNγ-, IL-17- and TNFα-producing $CD4^+$ T cells in both the spleen and mesenteric lymph nodes even before the colitis induction. While microbial consortia in the colonized ex-germ-free mice resembled these of the conventional mice, there were no significant differences in either sensitivity to colitis or T cells subsets.
Conclusion: We conclude that early life colonization with the *Verrucomicrobiae* and *Campylobacteria* promotes development of the gut barrier and decreases immune system reactivity, resulting in resistance to acute intestinal inflammation in mice.

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Abstracts

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P2.20 MOLECULAR MECHANISMS IN INNATE IMMUNOLOGY

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226 – P2.20.01

GBP2 engages Galectin-9 for immunity against Toxoplasma gondii

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Guanylate binding proteins (GBPs) are large interferon-inducible GTPases, executing essential host defense activities against *Toxoplasma gondii,* an invasive intracellular apicomplexan protozoan parasite of global importance. *T. gondii* establishes a parasitophorous vacuole (PV) which shields the parasite from the host's intracellular defense mechanisms. Murine GBPs (mGBPs) recognize *T. gondii* PVs and assemble into supramolecular mGBP homo- and heterocomplexes that are required for the disruption of the membrane of PVs eventually resulting in the cell-autonomous immune control of vacuole-resident pathogens. We have previously shown that mGBP2 plays an important role in *T. gondii* immune control. Here, to unravel mGBP2 functions, we report Galectin-9 (Gal9) as a critical mGBP2 interaction partner engaged for immunity to *T. gondii*. Interestingly, Gal9 also accumulates and colocalizes with mGBP2 at the *T. gondii* PV. Furthermore, we could prove the requirement of Gal9 for growth control of *T. gondii* by CRISPR/Cas9 mediated gene editing. These discoveries clearly indicate that Gal9 is a critical factor for the mGBP2 coordinated cell autonomous host defense mechanism against *T. gondii*.
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362 – P2.20.02

Stimulation of Natural Killer cell responses by different activating ligands

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Purpose: Natural Killer (NK) cells are part of the innate immune system and serve an important role in the early response against viral infections and in tumor immunosurveillance. Their activation depends on the stimulation of a broad range of receptors, which have overlapping and potentially redundant functions. Here, we aim to identify cause-effect relationships between the stimulation of distinct receptors and subsequent NK cell effector functions including cytokine secretion, cytotoxicity, and serial killing.
Methods: To stimulate human NK cells, we utilized NIH3T3 cells, an embryonic mouse fibroblast cell line that is not recognized by human NK cells. NIH3T3 cells were transfected to express defined ligands for activating NK cell receptors. To characterize different aspects of NK cell activity upon exposure to these targets, a combination of flow cytometry, ELISA, chromium-release and xCelligence killing assays is employed.
Results: We generated NIH3T3 cells stably expressing the NKp30 ligand B7H6, the 2B4 ligand CD48, the DNAM-1 ligands PVR (CD155) or Nectin-2 (CD112), the NKG2D ligand MICA, the CD16 ligand CD20 (in combination with anti-CD20 antibodies Rituximab or Obinutuzumab), or the LFA-1 ligand ICAM-1 (CD54). These cells were validated by comparing respective ligand expression levels with commonly employed human tumor cell lines. Upon incubation with pre-stimulated NK cells, target cells expressing B7H6, MICA or CD20 could effectively induce degranulation, while no significant differences were observed between the NIH3T3 wt control and remaining targets. Similar results were obtained in chromium-release assays. On the other hand, xCelligence assays revealed significantly enhanced target cell killing solely against NIH3T3-B7H6 cells.
Outlook: In the next steps of this ongoing project, we will finalize our analysis of which NK cell receptors can induce NK cell cytotoxicity. Furthermore, supernatants obtained from co-cultures of pre-stimulated NK cells with the different targets will be assessed regarding cytokine secretion by ELISA. Additionally, we will investigate NK cell adhesion to target cells and generate NIH3T3 cells expressing combinations of defined ligands.

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413 – P2.20.03

Potent induction of trained immunity by Saccharomyces cerevisiae b-glucans

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Candida albicans cell wall component b-glucan has been extensively studied for its ability to induce epigenetic and functional reprogramming of innate immune cells, a process termed trained immunity. We show that a high-complexity blend of two individual b-glucans from Saccharomyces cerevisiae possesses strong bioactivity, resulting in an enhanced trained innate immune response by human primary monocytes. The training required the Dectin-1/CR3, TLR4, and MMR receptors, as well as the Raf-1, Syk, and PI3K downstream signaling molecules. By activating multiple receptors and downstream signaling pathways, the components of this b-glucan preparation are able to act synergistically, causing a robust secondary response upon an unrelated challenge. In in-vivo murine models of melanoma and bladder cell carcinoma, pre-treatment of mice with the b-glucan preparation led to a significant reduction in tumor growth. These insights may aid in the development of future therapies based on b-glucan structures that induce an effective trained immunity response.

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416 – P2.20.04

Hyper-glycolytic monocytes in obesity have an increased oxidative stress and IL-8 response

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Obesity is associated with chronic low-grade inflammation and immune system dysfunction, which increases susceptibility to infection and serious disease outcomes. Monocytes are the precursors of the well-studied adipose tissue macrophages but little is known about functional alterations of monocytes in people with obesity. Aim of the study is to analyse the immunometabolic and functional responses of monocytes from individuals with obesity.
Monocytes were isolated from the blood of healthy human donors and people with obesity, and characterized using proteomics and analysis of cellular metabolism and immunological functions.
Monocytes from people with obesity showed a distinct proteomic profile. Activation of monocytes with lipopolysaccharide (LPS) led to the upregulation of metabolism pathways as well as phagocytosis and migration. Using the Seahorse analyzer, we found a pronounced hyper-glycolytic phenotype in resting monocytes from people with obesity compared to lean donors. After LPS activation, monocytes of people with obesity showed an enhanced glycolytic response. In addition, LPS-stimulated monocytes of people with obesity showed an increased IL-8 secretion and a prolonged production of reactive oxygen species, suggesting a dysregulated immune response.
In summary, the proteomic analysis as well as cytokine release data confirmed a dysregulated LPS-response in monocytes from people with obesity, which potentially increases the risk for infections. Targeted interventions focusing on monocytes might be beneficial and longitudinal studies investigating the dynamic changes in immunometabolism during the progression of obesity could provide valuable insights into potential targets.

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Abstracts

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469 – P2.20.05

Functional characterization of guanylate binding proteins in the host defense against chlamydia and toxoplasma infection

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Purpose: Guanylate binding proteins (GBPs) are interferon (IFN)-inducible mediators of cell-autonomous host resistance against intracellular pathogens and potent activators of canonical and non-canonical inflammasome pathways, leading to the processing of pro-inflammatory cytokines (IL-1β, IL-18) and inducing pyroptosis. The murine GBP family comprises eleven protein members (mGBP1-11) critically involved in the defense against protozoa, bacteria, and viruses. Upon infection with intracellular pathogens, GBPs are recruited to pathogen-containing compartments, significantly contributing to the immune response against *Toxoplasma gondii* and *Chlamydia trachomatis*. *In vivo* studies have revealed an increased susceptibility of mGBP2- and mGBP7-deficient mice to *T. gondii*, underscoring their importance in parasite control. In *vitro analyses* showed that mGBPs accumulate at *T. gondii* vacuoles, disrupting their integrity and restricting *Toxoplasma* replication.
Methods: To investigate the role of mGBPs in inflammasome activation and pyroptosis induced by *T. gondii* and *C. trachomatis*, we utilized mouse embryonic fibroblasts (MEFs) stably transduced with mGBP1-10. We analyzed the secretion of IL-1β and IL-18 and evaluated lactate dehydrogenase (LDH) release to assess the extent of pyroptosis after IFNγ stimulation and *T. gondii* / *C. trachomatis* infection. The activation of inflammasome components was determined by Western Blot analysis, facilitating a comprehensive examination of the molecular pathways involved.
Results: MEFs expressing mGBP1-10 exhibited increased LDH release and higher concentrations of IL-1β and IL-18 responding to *T. gondii* or *C. trachomatis* compared to control MEFs. Despite high sequence identity among mGBPs, distinct differences in effector function were observed. For instance, mGBP6-overexpressing MEFs exhibited a decreased cell death rate with high cytokine levels (hyperactivation), whereas mGBP9 led to enhanced cell death with normal cytokine release.
Conclusion: Our findings reveal the critical role of mGBPs in host defense against intracellular pathogens by modulating inflammasome pathways and inducing pyroptosis. These observations indicate that mGBPs regulate inflammasome assembly, either through direct interactions with its components or by mediating the lysis of pathogen-containing vacuoles, exposing them to the host cell cytosol. The differential effects of individual GBPs in the activation of the immune response highlight the complexity of their regulatory mechanisms and the necessity for further research to fully understand their contributions to cell-autonomous immunity.

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516 – P2.20.06

Localization of phosphatidylserine binding site in the globular domains of C1q

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Purpose: C1q is the key component of the classical pathway of the Complement system. Beside its classical function, C1q plays a crucial role in maintaining homeostasis, by partaking in the clearance of apoptotic cells. This occurs by the binding of C1q to phosphatidylserine (PS), which is externalized during apoptosis. The goal of this study was to characterize the PS binding site in the globular domains of C1q.
Methods: A quantitative ELISA was used to determine the binding activity of C1q and its globular fragments (ghA, ghB, ghC) to the physiologically relevant phospholipid 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-L-serine (POPS)**.** Fluorescence spectroscopy was used to determine the dissociation constants of the complexes formed between C1q and POPS as well as between the protein fragments and POPS. Simulation of the C1q-PS interaction was performed by molecular dynamics. The interaction was studied in the presence and absence of physiological concentration of extracellular Ca^{2+}.
Results: ELISA showed a dose-dependent interaction of C1q, ghA and ghB with POPS with significant difference in the presence and absence of Ca^{2+}. The fluorescent measurements confirmed the binding activity of C1q, ghA, ghB and also of ghC. The dissociation constant (KD) of each protein was calculated with C1q having the highest affinity (KD- 0.16 μM), followed by ghB (KD- 0.9 μM), ghA (KD- 1.2 μM) and ghC (KD- 1.5 μM). Molecular dynamics analysis supported the experimental data, revealing that amino acids in ghA and ghB interact with POPS.
Conclusion: Data from the aforementioned methods helped with the formation of a holistic view regarding the structure of the PS binding site. The three globular fragments of C1q globular domain contribute in a different way to the interaction with PS. The first contact of C1q with POPS is due to the glycosylated AA Asn-124 from ghA, followed by other residues from both ghA and ghB.
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520 – P2.20.07

Innate immune sensing of self-RNA by RIG-I-like receptors

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Melanoma Differentiation-Associated Protein 5 (MDA5) is a cytosolic pattern recognition receptor of the RIG-I-like receptor (RLR) family that senses long double-stranded RNA (dsRNA) and, upon activation, induces type I interferon (IFN). Besides its role in antiviral immunity, sensing of insufficiently modified self-RNA by MDA5 has been implicated in autoinflammatory diseases. However, presence and characteristics of endogenous MDA5 agonists under healthy conditions have not been extensively studied so far.
To address this, we utilized a transient overexpression model of MDA5 in 293T cells and showed that MDA5 responds to endogenous dsRNA in a dose-dependent manner. Using mtRNA-free cells, we found while mtRNA makes up the bulk of MDA5 agonists in total RNA, nuclear-encoded RNA is the major source for accessible MDA5 ligands within cultured cells. In line with this, we found significant levels of nuclear-encoded cytosolic dsRNA in human and murine cells by immunofluorescence staining with an anti-dsRNA antibody. To identify these cellular dsRNAs, we developed a method for targeted, sequence-independent isolation and sequencing of full-length long dsRNAs. This method will be applied to map dsRNA regions in RNA isolated from cells and tissue and will be complemented by a genome-scale CRISPR knockout screen as well as proximity-labeling based APEX-Seq.
Our findings suggest that cells express MDA5-activating dsRNAs even in non-pathological conditions, and a portion of these is accessible to MDA5. Further experiments will be needed to address the exact identity of these stimulatory RNAs, especially in comparison to ligand sources under pathological conditions, their regulation to prevent autoimmunity or a possible physiological role in priming of innate immunity.

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651 – P2.20.08

Profiling the circulating pathogen-associated molecular patterns and damage-associated molecular patterns in patients with end-stage liver disease

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Purpose: Low grade systemic inflammation is hypothesised to directly contribute to organ failure in patients with liver cirrhosis (an irreversible scarring of the liver). While elevated circulating interleukin (IL)6 and C-reactive protein (CRP) are commonly observed in patients with liver cirrhosis, it is unclear what causes this low grade systemic inflammation. Various pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) are detectable in patient samples, however, to date studies have investigated individual PAMPs and DAMPs in isolation. We hypothesise that profiling multiple PAMPs and DAMPs simultaneously may correlate better with low grade systemic inflammation and clinical outcomes in cirrhotic patients.
Methods: We profiled circulating PAMPs (lipopolysaccharide (LPS) and lipoteichoic acid (LTA)), and DAMPs (cell-free DNA (cfDNA), IL-1a, heat shock protein 70 (HSP70), cytochrome C and high mobility group box 1 (HMGB1)), in serum samples of cirrhotic patients with liver failure, undergoing assessment for liver transplant, and healthy controls. These were correlated with clinical and biochemical parameters associated with low-grade systemic inflammation and clinical outcomes.
Results: All the PAMPs and DAMPs investigated were elevated in a variable proportion of the cirrhotic patients with liver failure, in comparison to the healthy controls. When assessing all PAMPs and DAMPs in combination, the cirrhotic patients segregated into three groups. The first group showed minimum evidence of circulating PAMPs or DAMPs, and clustered together with the healthy controls. The second cluster included only cirrhotic patients who had elevated levels of two or three PAMPs or DAMPs, while the third cluster included cirrhotic patients with highly elevated levels of more than three of the assessed PAMPs or DAMPs. These patient clusters correlated with important clinical outcomes such as ascites, Charleson Comorbidity Index, and hospital length of stay.
Conclusions: Low grade systemic inflammation contributes to the transition to liver failure in patients with chronic liver disease. Our results indicate that while individual PAMPs or DAMPs are highly variable between patients, simultaneous assessment of multiple PAMPs and DAMPs can segregate patients and identify individuals as high-risk of adverse clinical outcomes.

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809 – P2.20.09

Exploring monocyte activation, migration, and terminal differentiation in inflammatory arthritis

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Purpose: Monocytes emerge as pivotal players in the pathogenesis of Rheumatoid Arthritis (RA) and Psoriatic Arthritis (PsA), where nuanced differences in activation, differentiation to DC, and function within both systemic circulation and local inflammatory sites have been observed. This study aims to delineate the divergent patterns of monocyte activation/priming, and differentiation into DCs, macrophages, and osteoclasts in RA vs PsA, offering insight into unique immunopathogenic signatures characterising the two diseases.
Methods: Monocyte subsets (Classical, Intermediate, Non-Classical) frequency and expression of activation markers and chemokine/differentiation receptors were analysed by flow cytometry in PBMCs from healthy controls (HC), RA and PsA patients. CD14+ monocytes were magnetically isolated from blood and differentiated into monocyte-derived-macrophages (Mo-MAC), -dendritic cells (Mo-DC), -osteoclasts (Mo-OC), and dendritic cell-derived osteoclasts (DC-OC) with specific cytokines, and differentiation was assessed by flow cytometry and TRAP staining.
Results: The frequencies of the three monocyte subsets showed no significant disparities between HC and RA/PsA, however an increase in activation markers CD40 and CD80 in PsA/RA *vs* HC, and CX3CR1 and CCR7 in RA>PsA>HC in classical and intermediate monocytes was observed, with a distinct co-expression profile observed for RA *vs* PsA by SPICE analysis. This hyper-inflammation in RA and PsA translated into a decrease in endocytic activity and enhanced cytokine production (TNFα and IL1β) in response to TLR stimuli. To evaluate whether this hyper-activation translated into a higher ability of monocyte differentiation into terminal cells, the frequencies of receptors implicated in monocyte differentiation were measured, demonstrating that IL4R, GMCSFR,RANK and MCSFR were higher in RA/PsA *vs* HC. In accordance, *in vitro* differentiation of CD14+ monocytes into terminal cells, demonstrated an increased differentiation of Mo-DC and Mo-MAC in RA>PsA>HC monocytes (evidenced by higher CD209 and CD64), and increased osteoclast formation and multinucleated cells in PsA>RA>HC for both Mo-OC and DC-OC in TRAP stained cells; thus suggesting cells from RA and PsA patients might prefer different routes of differentiation based on their environment.
Conclusion: Altogether, these findings indicate that differential monocyte hyper-activation and inflammation predisposes RA and PsA monocytes to selectively differentiate into terminal cells, potentially accounting for patient response to therapy.

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1051 – P2.20.11

The tuberculosis plasma milieu induces signatures of inflammation, neutrophil recruitment, and interleukin-10 family-mediated immunomodulation in monocytes

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Phenotype changes of immune cells in peripheral blood are characteristic of human tuberculosis and immunopathology seen in blood plasma plays a central role in suppressing T-cell responses in a subgroup of tuberculosis patients. In previous studies, we described changes in the phenotype of monocytes in acute tuberculosis patients and identified causative effects of the aberrant plasma milieu. Therefore, in this study, we aimed to characterize the functional implications of plasma effects on monocyte function in tuberculosis disease.
Using our established in vitro plasma-reference monocyte assay, we investigated the effect of plasma samples from patients with tuberculosis and healthy contacts (controls) on heterologous monocytes by conducting total mRNA sequencing. Affected pathways were confirmed by analyses of selected candidate proteins on plasma-treated monocytes and monocytes from tuberculosis patients and controls.
Among the transcripts induced by tuberculous plasma, we identified an enrichment of pathways involved in inflammation, recruitment of granulocytes/monocytes, and immunomodulation. Several chemokines and chemokine receptors were increased in tuberculous plasma-treated monocytes, suggesting the recruitment of granulocytes (via G-CSF, CXCL1, CXCL2, CXCL8) and monocytes. Monocytes from tuberculosis patients showed an inflammatory phenotype with increased expression of chemokine receptors (CCR1, CCR2, CCR5), as previously described. Interestingly, members of the Interleukin (IL)-10/-20 family (such as IL-10, IL-19, and IL-24), as well as IL-10 and IL-20 receptor chains were increased in tuberculosis plasma-treated monocytes.
These results suggest that monocytes and changes in the plasma milieu play a central role in tuberculosis immunopathology. Both inflammatory perturbations and immune modulation are promoted by monocytes, which may contribute to the immunosuppression of T-cell responses in patients with tuberculosis.

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1083 – P2.20.12

Dissecting structure and function of the macrophage testraspan MS4A4A

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Membrane-spanning 4A (MS4A) proteins are emerging as a new class of immune cell regulators, but the molecular mechanisms underpinning their biological functions are currently poorly characterized. In the recent years, we focused our attention on MS4A4A, which shows a pattern of expression restricted to mast cells and macrophages, both in human and mice. MS4A4A expression is induced during macrophage differentiation and is detected in tissue resident macrophages and in some body districts (CNS, colon, lungs, skin). In vitro MS4A4A expression is increased in "alternative activated" macrophages (i.e. IL-4, glucocorticoids) and is found highly expressed in some pathological settings (rheumatoid arthritis) and in tumor-associated macrophages (colon carcinoma, lung adenocarcinoma, melanoma). Here, we aim to reveal the role of MS4A4A in macrophage biology, achieving a deeper mechanistic understanding of its function in macrophages and disclosing its contribution to immune responses.
Our preliminary data showed no difference during bone marrow-derived macrophage (BMDM) generation from wild-type (WT) and Ms4a4a knock-out (KO) animals. However, MS4A4A deficiency impaired the expression of anti-inflammatory genes in BMDM after stimulation with IL-4, suggesting that this protein is involved in the acquisition of an alternative phenotype. To define MS4A4A contribution in macrophage polarization, RNA sequencing of WT and KO BMDM has been performed. To deeper investigate the biological relevance of the defect in "alternative activation" observed in Ms4a4a-KO macrophages, cytokines production, extracellular vesicles secretion and uptake and calcium fluxes upon different stimuli has been evaluated. As other tetraspan proteins, MS4A4A forms omo- and ethero-complexes. Indeed, recent publications identified several partners for MS4A4A, including MS4A4A itself, MS4A6A, MS4A7, TREM2 and Dectin-1 and they showed that MS4A4A influences macrophage functions by regulating membrane distribution and signaling properties of its partners. Interestingly, using a split-ubiquitin two-hybrid approach and mass spectrometry-based immunoprecipitation proteomics, we identified new possible partners for MS4A4A, including the γ signaling chain of Fcγ receptors, Toll-Like Receptor 2, and calcium/calmodulin-dependent 3',5'-cyclic nucleotide phosphodiesterase 1A. Furthermore, we are computationally characterizing MS4A4A structure and dynamics aiming to understand its function in macrophages, also by means of an extensive molecular docking calculations campaign with some families of endogenous and exogenous bioactive compounds.

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1260 – P2.20.13

Using Drosophila melanogaster (fruit fly) to investigate the role of two novel immune-induced peptides, IBIN and IBIN-like.

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Expanding our understanding of innate immune system mechanisms remains an important task for developing vaccines and drugs against both infectious diseases and inflammatory conditions. While mammals possess both innate and adaptive immune systems, complicating the study of the role of innate immunity, insects such as the fruit fly *Drosophila melanogaster* rely only on innate immunity. In this project, we work to determine the roles of two novel short peptides in *Drosophila*, IBIN and IBIN-like. Expression of both is highly upregulated when flies are infected with a wide range of pathogens. Our work shows at times opposing roles for these peptides in the immune response, through regulation of key immune processes, including the Toll pathway. While the short sequences of these peptides and their genes makes tracing their evolutionary histories challenging, our analysis shows that IBIN and IBIN-like are part of a gene family conserved in insects separated by approximately 100 million years of evolution.
The fruit fly model is highly suited to immunology research due to lower genetic redundancy compared to mammalian models. Despite this, a high percentage of disease relevant genes are conserved between flies and humans. This has, in the past, resulted in key immunology findings being made in the fly, including the discovery of Toll receptors, leading to the description of TLRs in mammals. *Drosophila* researchers have a wide range of genetic tools at their disposal, and fast generation times, reduced ethical constraints, and low cost of maintenance enable experiments with large numbers and therefore high power, making this an excellent model for screening work.

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1348 – P2.20.14

Investigating the molecular mechanism of tumor necrosis factor-related apoptosis-inducing ligand-induced macrophage polarization: Transcriptomic analysis of key signaling molecules

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Macrophages can be polarized into pro-inflammatory M1 and anti-inflammatory M2 macrophages. TNF-related apoptosis-inducing ligand (TRAIL), a member of the TNF superfamily, can trigger cell death or initiate survival pathways by binding to death receptors DR4 or DR5. We have recently shown that TRAIL promotes the polarization of human macrophages towards the M1 phenotype. However, the molecular mechanism of this effect is not known. In this study, to uncover the molecular mechanism, we analyzed the signaling molecules associated with M1 polarization in primary human monocyte-derived macrophages treated with TRAIL. Primary human monocyte-derived macrophages were stimulated with 200 ng/ml soluble TRAIL for 8 hours and the changes in the molecules associated with M1 polarization was analyzed by RNA-seq and qPCR. Of all M1 macrophage markers obtained from RNA-seq data, the expression rates of genes relevant to M1 polarization that reached significant expression levels were examined. It was shown that TRAIL stimulation increased the expression of STAT1, TRAF1, NFkB1, and MAPK11 in primary human macrophages. qPCR confirmation analyses showed that although the expression levels of all the signaling molecules showed an increase after TRAIL stimulation, only the increase in MAPK11 reached statistical significance. In conclusion, our study demonstrates that TRAIL stimulation leads to upregulation of MAPK11 gene expression in primary human macrophages, suggesting that TRAIL may promote M1 polarization through the MAPK signaling pathway.

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1379 – P2.20.15

Viral envelope protein fragments can modulate innate immune cell signaling via formylpeptide receptors

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Formylpeptide receptors (FPRs) comprise a small family of immune receptors that are capable of interacting with structurally diverse peptide ligands. In addition to their well-established role as pattern recognition receptors for bacterial pathogens, recent mouse studies provide clear evidence that FPRs play an important role for the outcome of influenza A and dengue virus infection. Additional interactions with peptide fragments from envelope proteins of HIV-1, Ebola- and different coronavirus strains have also been reported. Interestingly, spike protein fragments from SARS-CoV-2 were shown to directly trigger innate immune responses and cardiovascular signaling. Given that FPRs are expressed in multiple other cell types where they have a complex role in immune defense, a modulation of FPRs by breakdown products of spike protein fragments might help to explain some aspects of the COVID-19 pathophysiology. However, systematic studies that investigate the interaction between spike protein fragments, FPRs and responses of innate immune cells are lacking.
Using high-throughput calcium imaging, we first identified six regions in the spike protein ectodomain of SARS-CoV-2 that can specifically interact with FPR1, FPR2 and FPR3 in nanomolar to low micromolar concentrations. Experiments with primary human neutrophils revealed that these peptides also triggered calcium signaling and other immune responses such as chemotaxis, MMP-9 protease release and NETosis via FPRs. Interestingly, peptides from the conserved membrane proximal region in the spike protein were capable to competitively inhibit FPR1 calcium signaling, while they initiated calcium flux via the receptor subtype FPR2. Systematic structure function studies of these peptides revealed that their precise signaling pattern and resulting neutrophil responses can significantly differ depending on peptide length and strain specific amino acid composition. Moreover, the analysis of physicochemical properties revealed hidden common features in FPR ligands that are also found in envelope proteins of other viruses. In line with these predictions, tests of synthetic peptide pools from the envelope proteins of four different viruses were found to strongly activate all FPR subtypes suggesting that an activation of FPRs via viral envelope protein fragments is an underrated general mechanism of unrelated viruses modulating innate immune responses.

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1618 – P2.20.16

Itaconate drives mtRNA-mediated Type I interferon production via inhibition of succinate dehydrogenase

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Itaconate is one of the most highly upregulated metabolites in inflammatory macrophages. It has been shown to have immunomodulatory properties, although the underlying mechanisms are not fully elucidated. Here, we have investigated how itaconate regulates type I interferon production. We have found, using both pharmacological and genetic approaches, that inhibition of succinate dehydrogenase (SDH) is required for this response. SDH inhibition by itaconate leads to mitochondrial double-stranded RNA (mtRNA) release which is dependent on the mitochondrial pore VDAC1. Following this, the dsRNA sensors MDA5 and RIG-I are required for IFNβ production in response to SDH inhibition. Inhibition of SDH by itaconate therefore links TCA cycle modulation to type I Interferon production via mtRNA.

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1770 – P2.20.17

The Multifaceted Role of Lactoferrin in Covid-19

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Lactoferrin is an iron-binding glycoprotein present in most human exocrine fluids, particularly breast milk. Lactoferrin is also released from neutrophil granules and its concentration increases rapidly at the site of inflammation. Immune cells of both the innate and adaptive immune system express receptors for lactoferrin to modulate their functions in response to it. Based on these interactions, lactoferrin plays manifold roles in host defense from augmenting or calming inflammatory pathways to direct killing of pathogens. Complex biological activities of lactoferrin are determined by its ability to sequester iron and by its highly basic N-terminus, via which lactoferrin binds to a plethora of negatively charged surfaces of microorganisms, viruses, but also to mammalian cells, both normal and cancerous. Proteolytic cleavage of lactoferrin in the digestive tract generates smaller peptides, such as N-terminally derived lactoferricin. Lactoferricin shares some of the properties of lactoferrin, but also exhibits unique characteristics and functions. The focus of the presentation will be put on the potential therapeutic role of lactoferrin in COVID-19.

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1809 – P2.20.18

SHP1 knockdown enhances Toll-like Receptor 7 (TLR7) signalling in the plasmacytoid dendritic cell line CAL-1.

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Purpose: The protein tyrosine phosphatase SHP1 has been widely studied for its immunoregulatory activity in immune cells, such as in growth factor receptor, toll-like receptor (TLR), T and B cell signalling pathways. SHP1 plays a positive role in type I interferon production downstream of TLR3/4, however it acts to limit TLR4-induced expression of pro-inflammatory cytokines. Mice with mutations in the SHP1 gene, known as motheaten mice, develop severe autoimmunity and inflammation. Similarly reduced phosphorylation of SHP1 has been associated with increased T cell proliferation in autoimmune diseases such as systemic lupus erythematosus (SLE). To date, the role of SHP1 in TLR7 signalling, an antiviral signalling pathway that displays altered activity in SLE, is still unknown. This study aims to uncover the function of SHP1 within this innate immune signalling pathway.
Methods: Stable SHP1 knockdown of a plasmacytoid dendritic cell line (CAL-1) which constitutively express TLR7 were generated using a lentiviral approach to investigate the impact of SHP1 deficiency on the TLR7 pathway. CAL-1 control and SHP1 knockdown cells were stimulated with the TLR7 agonist R848, and supernatants were harvested to assess cytokine levels by ELISA. In parallel, cell lysates were profiled by Western blot analysis to examine how SHP1 affects NF-κB, MAPK and IRF activation downstream of TLR7.
Results: SHP1 knockdown in CAL-1 cells enhances the production of both pro-inflammatory cytokines and type I interferons downstream of TLR7 and enables increased activation of the major transcription factors involved in this signalling pathway.
Conclusion: These findings implicate SHP1 as a negative regulator of TLR7 signalling, suggesting the potential for future investigations into SHP1 and the increased activity of TLR7 observed in SLE.

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1815 – P2.20.19

Enhanced complement activation and MAC formation accelerates to severe COVID-19

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The complement system, a key component of innate immunity, provides the first line of defense against bacterial infection; however, it has been implicated in being a detrimental to COVID-19 patients and accelerating lung and other tissue damage. The underlying mechanism remains unclear and requires further experimental investigation. Here, we used three knock out mice strains (1. C3-/-; 2. C7-/-; and 3.CD59-/-) to evaluate the role of complement in severe COVID-19 pathogenesis. C3 deficient mice lack a key common component of all three complement activation pathways and are unable to generate C3 and C5 convertases. C7 deficient mice lack a complement protein needed for MAC formation. CD59 deficient mice lack an important inhibitor of MAC formation. In addition to use of these knock out mice, we also used anti- C5 antibody to block and evaluate the therapeutic potential of inhibiting MAC formation. We demonstrate that inhibition of complement activation (in C3-/-) and MAC formation (in C3-/-. C7-/-, and anti-C5 antibody) attenuates severe COVID-19; whereas enhancement of MAC formation (CD59-/-) accelerates severe COVID-19. The degree of MAC deposits in the alveoli, epithelial cells, and endothelial cells of $C3^{-/-}$, $C7^{-/-}$ mice, and $CD59ab^{-/-}$ mice as compared to their control mice is associated with the attenuation or acceleration of SARS-CoV-2-induced disease. However, C3 deposits in the lungs of these infected mice did not show the same trend. Further, the lack of terminal complement activation for the formation of MAC in C7 deficient mice protects endothelial function, which is associated with the attenuation of diseases and pathologic changes. In summary, these results shed light on the importance of the pathogenic role of MAC in severe COVID-19 and demonstrate the therapeutic beneficial effect of anti-MAC on severe COVID-19.

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1846 – P2.20.20

Characterisation NKG2C NK cell receptor and genotypes in relation to human cytomegalovirus infection

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Purpose: This study aims to characterize the immunogenetic profiles of HCMV infection in the healthy Malaysian population, specifically the NKG2C and NKG2A receptors on NK cells, and *HLA- E* on infected cells
Methods: Enzyme-linked immunosorbent assay (ELISA) is employed for the detection of HCMV IgG levels. *HLA-E* (01:01, 01:03and 01:114), and *NKG2C* (wild-type, and deletion-type) will be analysed using sequence-specific-primer polymerase chain reaction, while *HLA-E* (exon 3) and *NKG2A* (exon 2 and 5) sequences will be sequenced employing Sanger Sequencing.
Results: Our results currently indicate that HCMV seropositivity may be lower at 79.1% (n=67) than the previously reported 92% in 2012. Our results also indicate an almost equal distribution of NKG2C haplotypes; WT/WT at 53.70% (n=29), and WT/DEL at 46.30% (n=25). NKG2C DEL/DEL was not detected. It is also expected that HCMV seropositive individuals will have differential *HLA-E*, *NKG2C* and *NKG2A* genotypes.
Conclusion: Approximately 70-90% of Malaysians are expected to be HCMV seropositive, with them possessing a unique immunogenetic profile that can be harnessed for potential NK cell-based therapy and anti- tumour property.

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1880 – P2.20.21

The complex role of the human RNA helicase DDX3X during viral infections

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Human DDX3X is an RNA helicase with known roles in the regulation of mRNA translation, and a host factor targeted by a diverse range of viruses during infection. Although inhibition of DDX3X is explored for development of broad-spectrum antiviral drugs, DDX3X is also an immune-signalling protein in the RIG-I pathway, promoting type I interferon production and thereby exerting an anti-viral function. It is currently unknown how DDX3X's role in host mRNAs translation is impacted during viral infection and if this plays an anti-viral role separate from its immune signalling function.
Using PAR-CLIP, a technique which crosslinks and then sequences RNAs bound to immunoprecipitated proteins, we identified mRNA targets of DDX3X in uninfected and Sendai Virus-infected cells at the transcriptome level. We found that DDX3X primarily binds to the 5'-UTR and start codon in both host and Sendai Virus mRNAs, indicating a potential role in regulating translation initiation. Furthermore, Sendai Virus infection induces changes to the DDX3X-bound mRNA pool, revealing new candidates for DDX3X post-transcriptional regulation which may impact anti-viral immunity. Finally, utilising mass spectrometry, we also assessed the downstream effect of DDX3X knockdown and viral infection on the host cell proteome, linking DDX3X binding to post-transcriptional regulation of its targets. Overall, DDX3X may contribute to the dynamic of the virus-host interaction also at the level of mRNA regulation, which may be of consequence when exploring it as a potential broad-spectrum antiviral drug target.

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2113 – P2.20.22

Type I interferon regulates interleukin-1β and interleukin-18 production and secretion in human macrophages

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Inflammasomes are immune complexes that, when activated, leads to the release of pro-inflammatory cytokines IL-18 and IL-1β. Type I interferons (IFNs) are involved in fighting infections and promote the expression of IFN-stimulated genes related to inflammation. Despite the significance of these cytokines in inflammation, the relationship between type I IFNs and inflammasome regulation is not well-understood. In this study, we examined RNA-sequencing data from patients with monogenic interferonopathies and identified an increase in the expression of several inflammasome-related genes. To explore the impact of type I IFN on inflammasomes, we exposed human monocyte-derived macrophages to IFN-α. We observed an increase in *CASP1* and *GSDMD* mRNA levels over time, while *IL1B* and *NLRP3* expression did not show a direct correlation with IFN-α exposure duration. IFN-α treatment decreased the release of mature IL-1β and IL-18 in response to ATP-induced NLRP3 inflammasome activation, indicating that regulation may occur at the cytokine expression level rather than directly affecting the inflammasome itself. However, further research is needed to understand the mechanisms by which IFN-α regulates NLRP3 and other inflammasomes at both the transcriptional and post-translational levels.

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2120 – P2.20.23

NKG7 control cytolytic granule maturation and granzyme B levels in NK cells

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The tetraspanin NKG7 is preferentially expressed in cytolytic granules of NK cells and T cells. Lack of NKG7 reportedly leads to reduced cytotoxicity, and reduced control of tumors in vivo. We have previously shown that a subset of NK-cell derived extracellular vesicles (EVs) released in response to cytokines contain NKG7. The purpose of this study was therefore to investigate whether NKG7 impact EV biogenesis and function. We report here that cytotoxic granules in NK cells contain intraluminal vesicles positive for NKG7 as examined by transmission electron microscopy. Further, CRISPR-mediated knockdown of NKG7 in NK-92 cells led to accumulation of smaller-sized cytotoxic granules, accompanied with fewer intraluminal vesicles in the granules. In contrast, multivesicular bodies appeared morphologically normal. Immunogold labeling indicated a higher amount of granzyme B in granules of NK cells lacking NKG7. The data thus indicates that NKG7 may play a role in granule biogenesis, and that loss of NKG7 may result in a maturational block and build-up of granzymes. EVs released from NK cells lacking NKG7 were smaller, but induced tumor cell apoptosis at a comparable level to EVs from control NK cells, indicating that vesicles from the endosomal system also may contribute to tumor cell apoptosis. To further understand the biology of NKG7$^+$ EVs, we purified NKG7$^+$ EVs from NK-92 cells by immunocapture, and performed comparative proteomics profiling against bona fide CD63$^+$ EVs. We found that NKG7$^+$ EVs are enriched in granzymes, certain Rab proteins and proteins mediating vesicular fusion. These proteins were reduced in EVs derived from NK cells lacking NKG7, indicating that NKG7$^+$ EVs may be a separate, specialized subset of EVs, possibly mediating an alternative pathway of targeting cancer cells.

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2176 – P2.20.24

Ins and Outs of Autophagy in Measles Virus Infection

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Autophagy is a highly conserved degradation pathway used as an autonomous mechanism to combat intracellular pathogens. However, many pathogens have evolved molecular strategies to avoid or subvert autophagy for their own benefit. Over the years, we have described many very complex relationships between measles virus and autophagy, because this virus can induce autophagy through several distinct inputs: upon entry, during replication, and during its propagation. This very fine interaction has allowed us to better understand how autophagy is regulated in the context of infections, and to highlight a new paradigm on how and when autophagy can be beneficial or detrimental to a pathogen. We show that during measles virus infection, molecular regulators of autophagy can be hijacked for the benefit of the virus. Nevertheless, unexpectedly, measles virus-induced autophagy can lead to the degradation of otherwise pro-viral cellular cargoes and, at the same time, anti-bacterial cargoes facilitating the establishment of secondary infection. Thus, using mechanistic and unbiased approaches reveal new ins and outs of autophagy in microbial infection.

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2250 – P2.20.25

Glutamine metabolism is essential for cytokine-induced natural killer cell training and enhanced effector function

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Purpose: Natural Killer (NK) cells are innate, granular effector lymphocytes with important anti-viral and anti-cancer functions. There is increasing interest in NK cell therapies for cancer treatment, including cytokine-induced memory-like or "trained" NK cells which show enhanced effector functions upon restimulation. A clearer understanding of the mechanisms required for inducing and sustaining NK cell training may improve the efficacy of these cells as an immunotherapy.
Nutrient uptake is a crucial aspect of immune cell function and metabolism. The amino acid, glutamine, is an important nutrient that fuels oxidative phosphorylation (OXPHOS) in metabolically active cells via glutaminolysis which feeds into the tricarboxylic acid (TCA) cycle. The role of glutamine is beginning to be recognised in innate immune cell activation and training but remains largely unexplored in human cytokine-trained NK cells. Therefore, this project aims to investigate the role of glutamine uptake and metabolism in cytokine-induced NK cell training.
Methods: A proteomics data set comparing IL-2 stimulated and unstimulated NK cells was interrogated to determine if cytokine stimulation results in altered nutrient transporter expression. A novel click chemistry paired uptake assay was employed to quantify, by flow cytometry, SLC1A5-mediated glutamine transport in cytokine-trained NK cells. The role of glutamine uptake and metabolism during cytokine-induced NK cell training and subsequent activation was also investigated by analysing functional and metabolic readouts after treatment with inhibitors.
Results: Results demonstrate that SLC1A5, the major human glutamine transporter, is the most differentially expressed protein in IL-2 stimulated NK cells. Significant upregulation of SLC1A5-mediated amino acid transport was also observed in cytokine-trained NK cells (relative to untrained NK cells). Depletion of glutamine and inhibition of glutamine/glutamate metabolism impaired enhanced NK cell effector functions during cytokine restimulation and cytokine-induced training.
Conclusion: This data suggests that glutamine uptake, notably through SLC1A5, and subsequent metabolism in the mitochondria are essential events in sustaining the metabolic changes needed for enhanced NK cell effector functions during cytokine-induced activation and training.

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P2.21 MUCOSAL IMMUNITY

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20 – P2.21.01

Initial sensing of segmented filamentous bacteria and subsequent induction of Th17 responses first occur in Peyer's patches by privileged monocyte-derived phagocytes

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Segmented filamentous bacteria (SFB) are commensal members of the gut microbiota with unique features, including their tight attachment to the epithelial layer without triggering any pathological inflammation. SFB also strongly induce gut homeostatic Th17 responses, being crucial to the proper post-natal maturation of the intestinal barrier. However, the precise location where SFB is initially sensed by the host and the phagocytic population orchestrating Th17 priming are both questions still highly debated. LysoDCs are a subset of monocyte-derived cells with the strongest phagocytic activity in the Peyer's patches (PPs), sampling antigens especially by extending dendrites into the gut lumen through M cell-specific transcellular pores. Given their privileged location and effective functions, they had been highlighted as potential candidates in mediating SFB-induced immunity, although this relationship has never been directly approached. Here we describe higher accumulation of SFB-specific T lymphocytes and Th17 cells in PPs when compared to mesenteric lymph nodes (MLNs), both naturally after weaning (which correlates to the onset of SFB colonization) and following in vivo adoptive transfer of transgenic SFB-specific naïve CD4+ T cells. By preventing lymphocyte recirculation, we also show that although SFB-specific Th17 cells can be locally primed at both PPs and MLNs, they appear much earlier in the PPs, probably due to a more rapid response of the local antigen-presenting cells and the shorter time required for them to migrate to the interfollicular zone. Indeed, PPs imaging reveals SFB in proximity with and even engulfed by LysoDCs. Interestingly, we also observe LysoDCs in close interactions with RORgt+ proliferating SFB-specific T cells. We further describe that the profile of LysoDC maturation is strongly affected by weaning in a microbiota-dependent way, and that SFB alone can recapitulate this phenotype induced by a complex microbial community. Finally, we also demonstrate that amongst distinct phagocytes, only LysoDCs have the capacity to prime SFB-specific T cells ex vivo. We are currently working on a model for LysoDC depletion that will be crucial for elucidating their role in such complex scenario.
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23 – P2.21.02

Role of type III interferons on intestinal tissue restitution

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During inflammatory bowel diseases (IBD), the equilibrium between inflammatory cells, epithelia, and microbiome is lost, leading to inflammation, intestinal barrier breaches, and impaired epithelial regeneration. Therefore, understanding the mechanisms by which immune mediators influence tissue repair is crucial.
Here, we investigated how interferons (IFN) influence tissue repair after damage to the intestinal mucosa driven by inflammatory or physical injury.
Indeed, Interferons, which are increased during intestinal inflammation, can significantly alter the physiology of target cells. Type III IFN, or IFN-λ, are of particular interest due to their specialized ability to stimulate epithelial cells at barrier sites. Previous studies indicated elevated levels of IFN-λ and its receptor (IFNLR) in IBD patients, sparking debate over their role in colitis.
Both during dextran sulfate sodium-induced colitis or targeted irradiation damage, we found that IFN-λ signaling in intestinal epithelial cells (IECs) delays intestinal repair. The impaired tissue repair was associated with a transcriptional program inhibiting intestinal stem cell expansion. Mechanistically, IFN-λ delays epithelial cell regeneration by inducing the upregulation of ZBP1, Caspase-8 activation, and cleavage of Gasdermin C (GSDMC), both *in vivo* and in human and mouse intestinal organoids. Cleaved GSDMC drives epithelial cell death by pyroptosis and delays the re-epithelialization of the large or small intestine after colitis or irradiation, respectively.
Hallmarks of this pathway, such as ZBP1 upregulation, Casp-8, and GSDMC upregulation and cleavage, are significantly higher in IBD patients compared to controls and correlate with disease severity.
To understand the relevance of these findings during homeostasis, inflammation and repair, we mimicked injury and repair cycles in organoids grown in 2D in air-liquid interface.
In this model, IFN-λ provided the priming signal, requiring a second stimulus to mediate cell death. We identified Z-nucleic acids as this secondary signal, which increases during inflammatory damage and recovery, *in vivo* and *in vitro*.

This study unveils a novel mechanism by which IFN-λ regulates intestinal tissue repair, offering insights into how the inflammatory response affects epithelial repair in IBDs. The clinical relevance of the ZBP1/Casp-8/GSDMC pathway activation in patient samples suggests its potential as a therapeutic target for promoting tissue repair in these diseases.

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60 – P2.21.03

Modulating A-type lamins in immune and stromal cells for therapy in inflammatory bowel disease

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Maintenance of intestinal homeostasis is imperative to prevent the onset of inflammatory bowel disease (IBD). Intestinal homeostasis relies heavily on the intricate interplay among various cell types, including immune and non-immune cells. However, the precise mechanisms underlying the regulation of intestinal homeostasis by specific cell populations remain largely elusive. Here, we investigate the distinct roles of immune cells and stromal cells by modulating the expression levels of lamin A/C, a multifaceted regulator involved in structural, epigenetic, and transcriptional processes, in a murine model of IBD induced by dextran sulfate sodium salt (DSS).
We assessed the development of DSS-induced colitis in mice lacking lamin A/C ($Lmna^{-/-}$) specifically in immune cells (Vav1-Cre), T cells (CD4-Cre), select myeloid cells (LysM-Cre), and stromal cells (Col1a2-Cre), as well as in mice overexpressing lamin A/C in T cells ($Lmna^{++/++}$ CD4-Cre). Mice were subjected to one or two cycles of DSS administration to predominantly elicit innate or adaptive immune responses, respectively.
Our findings demonstrate that, compared to wild-type mice ($Lmna^{+/+}$), the absence of lamin A/C in T cells, myeloid cells, or stromal cells following a single dose of DSS confers protection against colitis. Notably, the protective effect appears more pronounced in mice lacking lamin A/C specifically in T cells when exposed to two doses of DSS, underscoring the pivotal role of lamin A/C in adaptive immunity during colitis. Conversely, lamin A/C overexpression in T cells exacerbates colitis following two doses of DSS administration. Furthermore, the absence of lamin A/C in all immune cells confers protection against colitis induced by either one or two doses of DSS.
In conclusion, the regulation of lamin A/C levels in immune cells and stromal cells emerges as a critical factor in the progression of colitis: its deficiency protects against colitis, whereas its overexpression exacerbates the disease, thereby highlighting lamin A/C modulation in specific immune and non-immune cell populations as a promising therapeutic target.
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Effects of gluten consumption on the transcriptomic landscape of Dermatitis Herpetiformis

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Dermatitis herpetiformis (DH) is a gluten-induced chronic autoimmune skin condition manifesting as itchy blisters in the extremities of the body. It is thought to develop from untreated celiac disease (CD) with both forms of the disease being gluten induced. The rash in DH resolves slowly after introduction of gluten-free diet (GFD). Although the gluten-induced transcriptomic changes of the small intestine are well studied in CD, corresponding studies have not been conducted in DH patients. Despite DH manifesting as a rash, it is though that the immune responses responsible for the skin symptoms might originate from the gut. In order to understand the skin symptoms and their relation to the intestinal symptoms, we have studied the transcriptomic landscape of DH patients during gluten challenge. Bulk 3' RNA sequencing was applied to duodenal biopsies of DH patients on GFD and after a long (3-9 months) gluten challenge, as well as to DH peripheral blood mononuclear cells (PBMCs) during a 6-day gluten challenge. In addition, T and B cell receptor sequencing is currently being carried out on the same samples, and the results will be discussed. Interestingly, the PBMCs show very little change in transcriptome during the 6-day gluten challenge, with the only gene induced by gluten on day 6 being CXCR2. The intestinal biopsies on the other hand show clear inflammatory changes, similar to the gluten-induced inflammation known from CD. In line with these changes, there is also a marked and plasma cell activation in patients consuming gluten. In this study we show, for the first time, how DH patient duodenum and PBMCs react transcriptomically to gluten challenge. Our findings indicate that the gluten induced inflammation in DH gut is similar to CD, showing that there must be other factors in play, than just the intestinal inflammation, in relaying the immune response to skin in DH patients. Although we were unable to find drastic changes in PBMCs during 6 days of gluten challenge, increase of CXCR2 expression may be closely related to skin recruitment of innate immune cells.

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383 – P2.21.06

Allergen-displaying extracellular vesicles of probiotic Escherichia coli strain O83:K24:H31 for mucosal tolerance induction in allergy

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Mucosal tolerance is a crucial regulatory mechanism that prevents unwanted immune reactivity against innocuous elements such as the colonizing microbiota and harmless environmental antigens. Failure of peripheral tolerance to such antigens leads to allergy. The processes involved are complex and depend on numerous factors, including dose, timing and context of exposure, as well as properties of the antigen (e.g. soluble or particulate nature). The exact mechanisms are not yet fully understood, but it is clear that the commensal microbiota plays a key role in the establishment of mucosal tolerance. There is therefore a strong incentive to study the details of the interaction between the immune system, the microbiota and potential allergens in the context of the mucosa. Intervention with probiotics is a promising and widely studied way of prophylaxis and therapy of allergic diseases. However, the administration of live bacteria can be problematic, particularly in newborns or in patients with immunodeficiency. Extracellular vesicles (EVs), highly resilient nanosized lipid bilayer particles released by all living cells, could provide a much safer and more controlled alternative to probiotic bacteria, retaining the probiotic properties with reduced risks. Importantly, the ability of EVs to carry proteins, lipids and nucleic acids from the releasing cells allows them to retain the strain-specific elements responsible for the probiotic effects, making it possible to examine the details of the immunological response to potential allergens delivered in a harmless context.
In our study, we transformed the probiotic strain of *Escherichia coli* O83:K24:H31 (EcO83) with the plasmid pMS470 to enable the expression of an engineered outer membrane protein A – SpyCatcher fusion protein. Vesicles expressing SpyCatcher can be decorated with SpyTag-marked proteins, enabling the modular construction of EVs with bound allergen. We obtained crude EcO83 extracellular vesicles (EcO83-EVs) by ultracentrifugation (3h, 150,000g, 4°C; Beckman Coulter 45Ti rotor) and purified them using size exclusion chromatography (IZON automated fraction collector). We tested the ability of purified EcO83-EVs with membrane-bound ovalbumin to promote mucosal tolerance after intranasal administration in the ovalbumin-induced allergic airway inflammation model in mice.

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402 – P2.21.07

Mapping of the duodenal mucosal microenvironment in irritable bowel syndrome with imaging mass cytometry

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Purpose: With a global prevalence of 4.1%, irritable bowel syndrome (IBS) has a considerable negative impact on both society and millions of individuals. The pathophysiology of IBS remains incompletely understood, but involves several factors such as low-grade intestinal inflammation, visceral hypersensitivity, changes in epithelial barrier integrity and permeability, altered gut-brain interactions, and an unfavourable composition of intestinal microbiota. There is currently no cure for IBS, but 70% of patients experience clinically significant symptom relief on a diet low in fermentable oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAPs). In this study, we will explore the duodenal intestinal immune system and barrier integrity in patients with IBS at baseline and after a 12-week strict low FODMAP diet intervention.
Methods: We have used highly multiplexed Hyperion imaging mass cytometry to investigate the cellular microenvironments in duodenal biopsies from patients (n = 46) and healthy volunteers (n = 19) at baseline and patients (n = 21) after a 12-week strict low FODMAP diet. An antibody panel consisting of 37 different markers for immunologic mapping, intestinal integrity and neuro-immune communication was used. Resulting images were segmented with the Steinbock framework in order to identify single cells. Downstream single-cell analysis for a selection of our markers was performed in R with the imcRtools R/Bioconductor and cytomapper packages.
Results: Images generated from Hyperion imaging mass cytometry were successfully segmented. Several immune cell type clusters, including CD4 and CD8 T cells, B cells, macrophages, and mast cells were identified based on marker expression and unsupervised clustering in duodenal biopsies from patients with IBS and healhty volunteers at baseline and patients with IBS at 12-week follow-up.
Conclusion: With our results from a selection of markers from our antibody panel, we have laid the foundation for further single-cell analysis of the duodenal mucosa of patients with IBS before and after 12 weeks of a strict low FODMAP diet intervention. Integration of tissue analyses and clinical symptom scores before and after treatment could provide clinically relevant intestinal biomarkers to advance the treatment strategies for patients with IBS.

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478 – P2.21.08

Understanding the anti-inflammatory effects of extracts originating in traditional Taiwanese medicine against chronic gut inflammation

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Purpose: Previous research has optimised extracts from a fern plant used within traditional Taiwanese medicine. The anti-inflammatory properties of these extracts against inflammatory bowel disease (IBD) have been identified however the underlying mechanism for this effect is yet undetermined. Here we investigated their anti-inflammatory nature within an in vitro setting, assessing impact on inflammatory responses and phenotype in macrophage cells.
Methods: The QUANTI-Blue™ assay was applied to investigate the influence compounds may have on macrophage NF-κB activation. The ability of these compounds to influence TNF-α induced cell death pathways was studied through qRT-PCR analysis, western blot, and flow cytometry. Treatment effects on M1/M2 macrophage phenotype and characteristics was also investigated through qRT-PCR, Flow Cytometry and Seahorse real-time cell metabolic analysis.
Results: Both compounds demonstrated an ability to interfere with the TNF-α – NF-κB pathway within macrophages through the QUANTI-Blue™ assay. Interference with this pathway has also been supported by these compounds dampening the expression of the pro-inflammatory protein iNOS which is under the control of the NF-κB transcription factor. To further investigate the influence on the TNF-α – NF-κB pathway in macrophages, the expression of signalling proteins involved in TNF-α induced cell death pathways was studied. qRT-PCR, western blot and flow cytometry analysis revealed the ability of both compounds to induce cell death pathways such as apoptosis and necroptosis within macrophage cells while also promoting inflammation resolution through increased efferocytosis.
Conclusions: These compounds may exert their anti-inflammatory effects through the promotion of cell death pathways while downregulating NF-κB activation within macrophage cells. We hypothesis that this feature may act to provide balance to the macrophage population within an inflamed state possibly through the upregulation of M2 macrophages capable of the efferocytosis of apoptotic bodies and necrotic cell debris. Our results so far have begun to uncover a previously uncharacterised mechanism underlying the anti-inflammatory effect of compounds originating in traditional Taiwanese medicine in the context of IBD.

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608 – P2.21.09

Intestinal TIGITnegCD38+ memory T cells proliferate, acquire an ex-Th17/Th1* pathogenic phenotype and drive inflammation in severe Crohn's disease patients with poor outcome

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Inflammatory bowel disease (IBD), including Crohn's disease (CD) and ulcerative colitis, is a T-cell-driven intestinal inflammation. The defects driving loss of T-cell regulation vary between patients and remain undefined. Previously, we have shown that, in healthy individuals, 40% of circulating $CD38^+$ ($CD62L^{neg}CD4^+$) memory T cells, which are enriched for intestinal antigen specificity, express TIGIT, an inhibitory receptor modulating dendritic cell and T-cell function. $TIGIT^+CD38^+$ memory T cells have a regulatory function while $TIGIT^{neg}$ cells are enriched in inflammatory IFN-γ-producing cells. We hypothesize that $TIGIT^{neg}CD38^+$ memory T cells have an inflammatory phenotype in a subgroup of IBD patients, driving disease. In a cohort of therapy-naïve pediatric IBD patients and age-matched healthy controls, we detected increased frequencies of circulating $TIGIT^{neg}CD38^+$ memory T cells in CD patients with a more severe disease course. As anticipated, $TIGIT^{neg}CD38^+$ memory T cells showed high expression of chemokine receptors associated with pathogenic ex-Th17 non-classical Th1, or Th1* cells, defined as high producers of IFN-γ. Cultures of stimulated memory T cells isolated from healthy peripheral blood identified IL-12 as the only IBD-related, antigen presenting cell-derived, inflammatory cytokine leading to an increase in $Th1^*TIGIT^{neg}$ cell proliferation in $CD38^+$, but not $CD38^{neg}$ cells. In agreement, IL12Rβ2 mRNA expression was higher in inflamed biopsies of CD patients compared to controls, and correlated with increased intestinal inflammation. Altogether, these results argue that, in a subgroup of CD patients, increased IL-12 signalling drives reprogramming of Th17 to a proliferative, inflammatory Th1* phenotype in $TIGIT^{neg}CD38^+$ memory T cells, leading to severe, therapy resistant disease.

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616 – P2.21.10

Enthesitis-related arthritis can be distinguished from other juvenile-idiopathic arthritis subtypes by a serum biomarker for gut-barrier dysfunction

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Introduction & Purpose: Juvenile Idiopathic Arthritis (JIA) is the most common rheumatic disease of childhood and encapsulates a heterogenous group of diseases characterised by joint inflammation persisting for at least 6 weeks. The JIA umbrella term includes subtypes with unique clinical presentations including Rheumatoid Factor positive (RF+) polyarticular-JIA, Rheumatoid Factor negative (RF-) oligoarticular/polyarticular JIA and Enthesitis-Related Arthritis (ERA). Evidence from adult inflammatory arthritis suggests that gut dysbiosis increases intestinal permeability, which may contribute to immune cell activation and subsequent joint inflammation. In this study, we aimed to explore whether increased gut permeability can also be observed in JIA.

Methods: Lipopolysaccharide Binding Protein (LBP), an acute phase protein which binds to translocated bacterial lipopolysaccharide, was used as an intestinal permeability biomarker. Concentration of LBP in venous blood serum of adolescent JIA patients (n=61) and age-matched healthy controls (HC) (n=29) was measured using a Human LBP ELISA (age range 13-23, mean age 16). JIA subtypes included were ERA (n=17), polyarticular/oligoarticular (n=31) RF- JIA, and RF+ JIA (n=14).

Results: Although there was no significant difference in serum LBP concentration between JIA patients as a whole compared to HCs, after separation by subtype, increased sera LBP concentration was found in ERA patients compared to HCs ($p=0.0262$). Further subdivision of ERA patients based on active joint count (Inactive=0, Active≥1) demonstrated this increased sera LBP was associated with active disease ($p=0.0488$ active ERA versus HCs), whilst there was no difference between inactive ERA patients and HCs.

Conclusions: This data suggests that adolescents with ERA, but not other JIA subtypes, have higher LBP levels indicating increased gut permeability. The data also suggests the increase in gut permeability in ERA is strongly driven by disease activity. This is in line with previous research showing an increased association of ERA with subclinical gut inflammation compared to other JIA subtypes.

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646 – P2.21.11

The protective effect of L. sakei CVL-001 against DSS-induced colitis through NOD2-dependent CD103 expression in dendritic cells

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Inflammatory bowel disease (IBD) is a group of disorders characterized by chronic inflammation of the gut, predominantly including crohn's disease and ulcerative colitis. These conditions frequently cause symptoms such as diarrhea, bloody stools, and weight loss, significantly impacting daily life. Recent research has shown that an imbalance of gut microorganisms, known as dysbiosis, is linked to the development of various diseases, including IBD. This finding has increased interest in microbiome-based therapies, such as probiotics. This study investigated the protective effects and mechanisms of action of the *Lactobacillus sakei* CVL-001 strain on IBD in a mouse model. In the experiment, IBD was induced in mice using DSS, and the probiotic was administered orally to evaluate its potential impact on the disease. The results showed that administration of heat-killed (H.K.) CVL-001 exhibited a protective effect against IBD, whereas its supernatant did not. H.K. CVL-001 increased the expression of the anti-inflammatory cytokine IL-10 in the colon, as well as the population of regulatory T cells and CD103+ dendritic cells (DCs), thereby reducing inflammation. Mutations in the Nod2 gene have been found to be deeply associated with IBD. Interestingly, clinical improvement was not observed in Nod2 knockout mice fed with H.K. CVL-001. Gut immune analysis also did not show increases in IL-10 expression, regulatory T cells, and CD103+ DC expression. Treatment of bone marrow-derived dendritic cells (BMDCs) with H.K. CVL-001 induced upregulation of CD103 expression, which subsequently promoted regulatory T cell differentiation in co-culture with naive T cells, a response that was absent in Nod2 knockout BMDCs. This suggests that the protective effect of H.K. CVL-001 significantly dependent on the Nod2 signaling pathway in DCs. We generated mice with depleted Nod2 signaling in DCs using the Cre-loxP system and found that administering H.K. CVL-001 to these mice did not result in protective effects or immunological changes. Conversely, injecting Nod2-intact DCs into Nod2 knockout mice restored the protective and immunological responses. This elucidates a novel role for Nod2 signaling in IBD and proposes its potential as a candidate for the treatment of IBD.

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725 – P2.21.12

Scrutinizing prime-boost immunization approaches during early-life to enhance immunity against respiratory pathogens

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Purpose: The strategic development of vaccine, incorporating diverse adjuvants, employing various routes of administration, and using optimal doses of vaccine has demonstrated efficacy in eliciting strong immune responses. The mucosal IgA antibody plays a crucial role in the initial defense, while systemic immunity relies on the significance of the IgG antibody. To optimize the desired immune response triggered by vaccination, the study aims to assess if heterologous route prime-boost immunization can induce strong and persistent humoral immune responses.
Methods: We immunized neonatal mice with a pneumococcal conjugate vaccine, Pn1-CRM_{197}, and two adjuvants by heterologous subcutaneous (s.c) priming with CAF01 followed by intranasal (i.n.) booster with mmCT, or two homologous immunizations, either s.c. or i.n.. Blood and saliva were collected at different time points post-immunizations and spleen, bone marrow (BM), cervical lymph nodes (CLNs), inguinal lymph nodes (ILNs), and lungs two or five weeks post-booster to assess anti-Pn1 antibody levels and ASCs.
Results: Compared to heterologous s.c./i.n. immunization, homologous s.c./s.c. immunization induced higher serum and lung IgG anti-Pn1, and homologous i.n./i.n. immunization induced higher serum IgA anti-Pn1 five weeks post-booster. Homologous s.c. immunization induced higher IgG anti-Pn1 antibody secreting cells (ASCs) in spleen, ILNs, and BM but lower IgA anti-Pn1 ASCs than heterologous immunization in CLNs and spleen. Notably heterologous immunization induced higher IgG and IgA anti-Pn1 ASCs in CLNs utilizing higher doses than homologous s.c./s.c. immunization. Generally, utilizing higher dose of vaccine in i.n. booster of heterologous immunization, yielded higher levels of anti-Pn1 IgG, IgA, and also higher number of anti-Pn1 ASCs.
Conclusion: Homologous s.c./s.c. immunization induces higher systemic IgG anti-Pn1 responses than heterologous s.c./i.n. immunization, which could be enhanced by increasing the vaccine dose in the i.n. booster that was still not comparable to what was induced after s.c./s.c. immunization. However, the heterologous s.c./i.n. immunization induced higher mucosal IgA anti-Pn1 responses. This study indicates that heterologous prime-boost immunization routes could be a promising early-life vaccination strategy, although further optimizations are needed.

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783 – P2.21.13

Lung dendritic cell metabolism underlies susceptibility to viral infection in diabetes

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Diabetic patients feature a life-risking susceptibility to respiratory viral infection, including influenza and SARS-CoV-2, whose mechanism remains unknown. In acquired and genetic mouse models of diabetes, induced with an acute pulmonary viral infection, we demonstrate that hyperglycemia leads to impaired costimulatory molecule expression, antigen transport and T cell priming in distinct lung dendritic cell subsets, driving a defective antiviral adaptive immune response, delayed viral clearance and enhanced mortality. Mechanistically, hyperglycemia induces an altered metabolic dendritic cell circuitry, characterized by increased glucose-to-acetyl-CoA shunting and downstream histone acetylation leading to global chromatin alterations. These, in turn, drive impaired expression of key dendritic cell effectors, including central antigen presentation-related genes. Glucose-lowering treatment, or pharmacological modulation of histone acetylation rescues dendritic cell function and antiviral immunity. Collectively, we highlight a hyperglycemia-driven metabolic-immune axis orchestrating dendritic dysfunction during pulmonary viral infection and identify metabolic checkpoints which may be therapeutically exploited in mitigating exacerbated disease in infected diabetics.

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797 – P2.21.14

IL-23 tunes inflammatory functions of human MAIT cells

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Purpose: The key role of IL-23 signaling in the pathogenesis of some chronic inflammatory diseases has been validated by the success of IL-23 blockers in the clinics. Yet cellular targets and signaling pathways affected by this cytokine remain poorly understood. In this study, we investigated the role of IL-23 signaling in human mucosal-associated invariant T (MAIT) cells.
Methods: Immune cell populations were monitored by spectral flow cytometry and isolated by cell sorting. MAIT cells were activated with an MHC related molecule 1 (MR1) tetramer presenting the potent antigen 5-OP-RU in the presence or absence of IL-23, or its structurally related cytokine IL-12. Gene expression, protein secretion and chromatin accessibility of activated MAIT cells were analyzed using bulk and single-cell RNA-sequencing, multi-analyte profiling and ATAC-sequencing, respectively.
Results: We show that human MAIT cells are the population with the highest frequency of circulating IL-23R-positive cells. Multi-omics profiling of MAIT cells at the population and single cell levels demonstrated that stimulation with IL-23 or IL-12 drives both shared and distinct functional polarization, revealing a high level of plasticity. IL-23, in particular, affected key molecules and pathways related to autoimmunity and cytotoxic functions. Integrated analysis of transcriptomic and chromatin accessibility changes identified the AP-1 family of transcription factors as key targets of IL-23 signaling in MAIT cells. CRISPR/Cas9 mediated knockdown of BATF supported the role of this AP-1 transcription factor as a regulatory node in the IL-23 pathway in this cell population.
Conclusion: High expression of IL-23 receptors and IL-23-mediated induction of autoimmune disease-related genes suggest an important role of MAIT cells in the IL-23 signaling cascade underlying the pathogenesis of chronic inflammatory diseases.

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798 – P2.21.15

Parkinson's disease-associated leucine-rich repeat kinase 2 polymorphism promotes neutrophil responses and enteric α-synuclein pathology during intestinal inflammation

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Purpose: Inflammation in the gut has been considered a trigger of Parkinson's disease (PD). However, the role of immune cells in the gut-to-brain PD pathogenesis is still unclear. In this study, we use the mice carrying mutated leucine-rich repeat kinase 2 (LRRK2), the most frequent genetic risk factor for PD, to investigate if irregulated immune responses during intestinal inflammation result in PD pathology.
Methods: LRRK2 G2019S transgenic mice, the most common LRRK2 polymorphism among PD patients, were treated with dextran sulfate sodium (DSS) to induce intestinal inflammation, the composition of immune cells and the expression of α-synuclein in the gut were examined. Neutrophils of mice were isolated and stimulated with ionomycin to study the effect of mutated LRRK2 on the function of neutrophils.
Results: LRRK2 G2019S transgenic mice showed more severe pathology and elevated α-synuclein in the colon after repeated DSS treatment. Moreover, increased expression of neutrophil extracellular traps (NETs) was also observed in the inflamed colon of LRRK2 G2019S mice. Since LRRK2 G2019S mice had a similar level of neutrophil compared to the non-transgenic mice during intestinal inflammation, the *ex vivo* study demonstrated that LRRK2 G2019S promotes the production of NETs due to the hyperactive kinase activity. The removal of NETs during DSS treatment ameliorated the inflammation and reduced enteric α-synuclein in LRRK2 G2019S mice, suggesting the disruption of intestinal homeostasis might induce the synthesis of α-synuclein in the gut.
Conclusion: This study reveals that neutrophil is likely involved in the LRRK2-mediated α-synuclein pathology during intestinal inflammation, which then initiates the gut-to-brain pathogenesis of PD.

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843 – P2.21.16

Optimization of NKTfh cell induction in vivo for improved vaccine protocols

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Purpose: Vaccines are essential tools to protect humans against infections, mainly by inducing antibody production by B cells. The activation of B cells is aided by a specialist subset of T cells, called follicular-helper T (Tfh) cells. Invariant Natural Killer T (*i*NKT) cells are memory-phenotype cells that produce copious amounts of various cytokines shortly after antigenic stimulation by e.g. the model antigen αGalCer. A subset of *i*NKT cells, called follicular helper NKT (NKTfh) cells, was detected six days after the *in vivo* stimulation that was also able to support B cell responses. However, currently not enough is known about the *in vivo* development of NKTfh cells to utilize them for vaccine development.
Methods: BALB/c wild-type and IL-10-reporter ($IL10^{GFP}$) mice were injected i.v. with 2 µg of different *i*NKT cell antigens (αGalCer, OCH, C-glycoside, DB06-1). Some mice were re-challenged on days 3-5. On day 6, splenocytes were analysed for the frequency and phenotype of *i*NKT and NKTfh (CD185+ Bcl6+) cells by flow cytometry. Their cytokine profile was assessed by intracellular cytokine staining 4 h after *in vitro* stimulation with PMA and ionomycin.
Results: Six days after αGalCer injection, about 10-18% of splenic *i*NKT cells were CD185+ and Bcl6+ and defined as NKTfh cells. When the cells were stimulated *in vitro* with PMA and ionomycin, both CD185 and Bcl-6 were downregulated and could not be used to identify NKTfh cells any longer. However, we were able to detect IL-21 production by intracellular cytokine staining in 18-20% of splenic *i*NKT cells. The majority of IL-21+ *i*NKT cells co-expressed IFNγ (25-40%) and IL-4 (12-24%) but did not express IL-10. Furthermore, the tested *i*NKT cell antigens differed in their ability to induce NKTfh cells, with a decreasing efficiency of OCH > DB06-1 > αGalCer > C-glycoside. The preparation of NKTfh and control *i*NKT cells for an RNAseq analysis is ongoing.
Conclusion: We provide novel data on the cytokine production of NKTfh cells and their antigenic requirement for *in vivo* induction, which may pave the way to utilize NKTfh cells for vaccine development.

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989 – P2.21.17

Alterations in serum IgG glycome are associated with Crohn's disease development

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Inflammatory bowel disease (IBD) is a complex chronic gut inflammatory disorder, encompassing Crohn's disease (CD) and ulcerative colitis (UC). It predominantly impacts young individuals during their most active and productive years. Despite considerable progress in IBD research, its underlying causes remain elusive and there is still no definitive cure. Evidence suggests the existence of a preclinical phase in IBD, marked by immunological changes, occurring years before symptoms manifest and diagnosis is made. A deeper understanding of this preclinical period holds promise for advancing our knowledge of disease development, designing therapeutic approaches, and enhancing the ability to predict and prevent the onset of the disease.
We have taken advantage of the unique preclinical PREDICTS cohort, containing multiple longitudinal samples many years preceding diagnosis. Analysis of preclinical serum samples by nanoLC-ESI-MS, up to 6 years before IBD diagnosis, revealed the identification of a specific glycosylation signature on circulating antibodies (IgGs) that remained stable until disease diagnosis. This glycan trait correlated with specific anti-microbial antibodies, detected in serum, that predates by years the development of CD. Also, we have demonstrated *in vitro* the effect of these particular antibodies in eliciting a pro-inflammatory response, mainly in triggering innate immune response. This was also concomitant with a significant increased expression of genes involved in pro-inflammatory signalling pathways, such as TNF and NF-κB signalling pathways. Later, we validated *in vivo* the pathogenic properties of these particular IgGs, through adoptive IgG transfer of these selected IgG in WT mice, associated with increased susceptibility to intestinal inflammation.
Overall, this work has identified a specific glycome signature in IgGs, years before CD onset, that may play a pivotal role in the initiation of inflammation many years before CD diagnosis, acting as a promising new biomarker for CD prediction.

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991 – P2.21.18

Relationships between plasma immune protein profiles, extent of inflamed tissue, and density of intestinal infiltration reveal discrete disease courses amongst therapy-naïve ulcerative colitis patients

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Ulcerative colitis (UC) is a chronic intestinal inflammation driven by inflammatory memory T helper cells (Th_M) reactivation. Heterogeneity in clinical disease and variability in response to treatment require new strategies targeting the patient's underlying immune disease. As in UC inflammation only affects the intestinal mucosa, it is debated whether peripheral blood immune responses could reliably reflect ongoing intestinal immune disease. To unequivocally establish this, we performed a deep immune characterization of therapy-naïve pediatric UC patients (n=34) and controls (n=55) by measuring 92 plasma immune proteins, analysing circulating Th_M subpopulations and performing intestinal mRNA expression and immunohistochemistry. Extensive clinical follow-up was used to relate immune responses to clinical disease severity and therapy response.
At diagnosis, 23 plasma proteins were differentially abundant in UC compared to controls. Increased protein concentrations mainly related to the IL-17 pathway (IL-17, IL-8, CCL20), tissue remodelling (MMP-10, MMP1, HGF), acute phase response (IL-6, OSM) and chemokinesis (CXCL9, CXCL10, CCL3) . Interestingly, intestinal mRNA expression of highly abundant plasma proteins similarly increased whereas no proportional changes in circulating Th_M were detected. Clustering of plasma immune profiles yielded two clusters of UC patients, UC_A with significantly increased concentrations of Th17/neutrophil-pathway-related proteins versus UC_B with lower concentrations. In UC_A, 17 proteins were increased versus UC_B. These included IL- 17A as most increased, OSM a neutrophil product previously associated with anti-TNF therapy resistance and CXCL9/CXCL11, IFN-γ-induced chemokines. Strikingly, this UC_A immune profile directly related to increased size of the affected intestinal surface and degree of infiltrating IL-$17A^+$ cells and neutrophils. Clinically, UC_A patients had more severe intestinal disease, including larger disease extent, higher clinico-pathological parameters at diagnosis and required earlier treatment intensification during >1 year follow-up, compared to UC_B patients.
These data firmly establish that peripheral blood immune protein responses reliably reflect ongoing intestinal immune disease in UC. Addition of immune profiling to routine clinical characterization at diagnosis could support tailored treatment with earlier start and more intensive immunomodulatory treatment in UC_A patients to target the more severe immune disease and avoid tissue damage.

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1003 – P2.21.19

Investigation of the role of Interleukin-11 (IL-11) in paediatric IBD patient non-responsiveness to therapeutic intervention.

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Purpose: Inflammatory bowel disease (IBD) is a chronic inflammatory disorder affecting the gastrointestinal tract which comprises two disease states, Crohn's Disease (CD) and Ulcerative Colitis (UC). While the advent of biotherapeutics has revolutionized the management of IBD, a subset of patients remain unresponsive to front-line therapeutic options. The underlying mechanisms that mediate this non-responsiveness remain unknown. This study is aimed at uncovering the role of Interleukin-11 (IL-11) signaling in IBD patient non-responsiveness to therapeutic intervention. IL-11, a member of the IL-6 cytokine family, has important roles in mediating gastrointestinal homeostasis, epithelial integrity and inflammation. Recent studies indicate a crucial involvement of IL-11 signaling pathways in the pathogenesis and progression of IBD. However, its potential impact on patient therapeutic non-responsiveness remains undefined.
Methods: We conducted a comprehensive analysis utilizing colonic biopsy samples from treatment naive paediatric IBD patients, with a subsequent two-year clinical follow-up, to determine whether an association exists between expression levels of an *IL11* gene signature and patient non-responsiveness. This was carried out in tandem with preliminary mechanistic analysis of the role of IL-11 in disease using the dextran sodium sulphate (DSS) murine model of colitis and *in vitro* primary cell culture models.
Results: Our findings reveal that an *IL11* gene signature is significantly upregulated in the colons of UC patient non-responders compared to responder patients and healthy controls. Interestingly, analysis of matrix metalloproteinase 3 (*MMP3*), which has also been previously associated with biotherapeutic non-responsiveness, showed a similarly elevated gene expression pattern among UC patients. Both *il11* and *mmp3* expression were also elevated in the inflamed intestines of mice undergoing colitis. Moreover, the induction of colitis in mice led to the acquisition of altered IL-11 responsiveness by colonic fibroblasts. These 'inflammatory' fibroblasts responded to IL-11 stimulation with significantly enhanced expression of *mmp3*.
Conclusion: These data identify a potentially novel mechanism through which IL-11 may promote IBD patient non-responsiveness to therapeutic intervention. Targeting the IL-11 pathway may improve clinical outcomes in paediatric IBD patients.

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1009 – P2.21.20

Deep immune phenotyping reveals endometrial immune modulation in women with endometriosis

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Background: Endometriosis is a chronic disease affecting 6–10% of women. Despite being a major cause of infertility and chronic pelvic pain, the aetiology remains unclear, with strong implications for altered systemic and local immunity. The endometrial mucosa is the inner lining of the uterus, containing both innate and adaptive immune cells. These cells can be found either as single cells across the stroma and epithelium or in lymphoid aggregates. We aimed to determine if the phenotypic and histological characteristics of immune cells in the endometrium across the cycle can help us reveal relevant immune modulation in endometriosis-associated subfertility, which could be detrimental to embryo implantation and pregnancy.
Methods: A 36-parameter panel for full-spectrum flow cytometry (Cytek® Aurora) was developed to study immune populations in matched endometrial and peripheral blood samples from 20 patients with surgically confirmed endometriosis, of which 4 were subfertile, and 8 non-endometriosis controls. Additionally, 3 full-thickness uterine biopsy samples were histologically examined during the different cycle phases with multiplex immune imaging (ZellScannerONE®) to study the spatial organization of single immune cells and lymphoid aggregates.
Results: We identified novel immune cell phenotypes, activation/regulatory markers, and cytokine receptors across the cycle phases. Among 861,796 endometrial CD45+ cells, CD161+ CD8+ T cells and CD11c-CD14- macrophages were increased, while early endometrial NK cells were decreased in patients with endometriosis compared to controls. Increased regulatory CD4+ T cells and decreased CCR5+ CD8+ T cells and naïve B cells were found in patients with endometriosis-associated subfertility versus fertile endometriosis patients. Systemic changes were observed in patients with endometriosis versus controls but not between subfertile and fertile endometriosis patients. Histologically, we explored differences in endometrial and myometrial immunity across the menstrual cycle and between patients and controls.
Conclusions: Findings indicate the systemic phenotype of endometriosis, while patients with endometriosis-associated subfertility only show localized immune dysregulation. Qualitative differences in endometrial and myometrial immune populations across menstrual phases and in endometriosis versus controls indicate opportunities for future research to understand potential mechanisms at the foetal-maternal interface impacting embryo implantation and immune tolerance.

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1020 – P2.21.21

The gut homing profile of B cells and T cells is altered in experimental models of arthritis.

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Gut perturbations such as dysbiosis of the gut-microbiome have been linked to autoimmune diseases including rheumatoid and juvenile idiopathic arthritis. Gut-derived signals are known to affect B and T cell development and influence their pro-inflammatory potential. Immune cells from gut-associated lymphoid tissues (GALT) enter the circulation and migrate to the intestinal mucosa. This recirculation process is orchestrated by gut-homing markers, CCR9 and integrin α4β7 (LPAM-1). It remains unknown whether alterations in gut-recirculating B and T cells impact arthritis pathology. Here, to address this gap, we profiled the gut-homing phenotype of B and T cells across peripheral and gut-associated lymphoid tissues in animal models of arthritis.
Antigen induced arthritis (AIA) was induced by subcutaneous injection of methylated bovine serum albumin (mBSA) in complete Freund's adjuvant (CFA), followed by an intra-articular injection of mBSA (n=5). Collagen induced arthritis (CIA) was induced by intra-dermal injection with type II chicken collagen in CFA (n=5). Age-matched naïve mice were included as controls (n=4 per experiment). Multi-parameter spectral flow cytometry was used to characterise the phenotype of CD45+ leucocytes from the spleen, inguinal draining lymph nodes (iLN), Peyer's patches (PP), mesenteric lymph nodes (mLN), and colonic lamina propria (cLP). One-way ANOVA was used to assess differences between groups.
In the AIA, CCR9-LPAM-1+ B cells were reduced in the PP ($p=0.0174$) but increased in the cLP ($p=0.0473$) compared to controls, with similar trends observed for CCR9-LPAM-1+ T cells ($p=0.0060$ in PPs; $p>0.005$ in cLP). Conversely, in CIA, there was an increase in the proportion of CCR9+LPAM-1- splenic B cells ($p=0.0023$) which was associated with a decrease in CCR9-LPAM-1+ B cells in both the spleen and mLN ($p=0.0485$ in spleen; $p=0.0435$ in mLN) compared to controls. In CIA, there was also an increase in CCR9-LPAM-1+ T cells in the PP and iLN compared to controls ($p=0.0235$ in PP; $p=0.0028$ in iLN).
This pilot study demonstrates that there are differences in the gut homing profiles of immune cells between arthritis and non-disease states, with features of altered gut recirculation across multiple lymphoid tissues. Our data highlights that differences in gut-homing profiles are distinct to different experimental models of arthritis.

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1183 – P2.21.22

Microbiota-related biomarkers in the serum of patients with DLBCL

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Purpose: Microbiota composition is significantly different in patients with diffuse large B-cell lymphoma (DLBCL) when compared to healthy subjects. In addition, dysbiotic microbiota often compromise the gut barrier function. Here, we focused on the levels of several serum biomarkers monitoring the gut barrier condition or inflammation and related them with therapy response.
Methods: The inclusion criteria were newly diagnosed DLBCL with subsequent immunochemotherapy. Patients in complete remission and those that remained in remission for at least three months after the treatment were assessed as responders. Patients who did not achieve complete remission or progressed within three months after treatment termination or died within this period were non-responders. The serum samples were collected in three time-points: at the diagnosis (0), after the therapy (<6m) and follow up about six months later (>6m). We analyzed the serum levels of intestinal fatty acid-binding protein (i-FABP), soluble CD14, lipopolysaccharide-binding protein (LBP), mannose-binding lectin (MBL) and insulin-like growth factor-2 (IGF-2) by commercially available ELISA kits. Data were statistically analyzed by non-parametric Mann-Whitney t-test.
Results: All biomarkers showed increased levels in non-responders when compared with responders in the time 0. However significant difference was measured only in i-FABP (p= 0.005), CD14 (p=0.002) and IGF-2 (p= 0.016). In addition, CD14, IGF-2, MBL and LBP were increased in non-responders also in the second collection (<6m) though only IGF-2 showed significant difference between non-responders and responders (p=0.017). Finally, in non-responders, higher IGF-2 and LBP (p=0.049) levels were measured in the third collection (>6m).
Conclusion: The analysis of microbiota-related biomarkers could help to improve personalized treatment of patients with the most common type of Non-Hodgkin Lymphoma – DLBCL.
The study was supported by grant nr. NU22-03-00370 from the Ministry of Health of the Czech Republic.

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1382 – P2.21.23

Spatially resolved proteogenomic uncovers a sympathetically mediated link between colitis and salivary glands pathology

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Inflammatory bowel disease (IBD) affects not only the lower gastrointestinal tract but also the distal extraintestinal organs. Up to 16% of ulcerative colitis (UC) patients exhibit upper gastrointestinal tract (UGIT) manifestations, including esophageal and oral ulcers and hyposalivation, suggesting an oral-gut organ connection that is still poorly understood. Here, we used mouse models of colitis and spatial transcriptomics (ST) of the UGIT to investigate the distal consequences of intestinal inflammation on the upper GI tract. Combining ST with a newly developed mounting method to unbiasedly interrogate the oral, esophageal and gastric mucosa, we revealed a previously unknown secretory organ in the retropharyngeal region of the murine UGIT. We propose that it is the homologue of a recently described human salivary gland, and we termed it the retropharyngeal salivary gland (RPG). We observed that the RPG is distally affected by colitis, so we aimed to investigate the effect of colitis in other SGs. Using various models of chronic or acute colitis, we observed significant atrophy and dysfunction in the submandibular and sublingual salivary glands. Colitis-induced SG pathology is characterized by an early decrease in salivary production, followed by transient ductal atrophy, decreased cellularity, accumulation of IgG+ plasma cells, and changes in secreted salivary IgA. Using the CD19iDTR mouse, we observed that B cell depletion partially rescued SG from atrophy, whereas plasma cell depletion in the CD138DTR mouse did not. Next, based on bulkRNAseq data showing early upregulation of adrenergic receptor genes on salivary glands during colitis, we explore the hypothesis that autonomic innervation may link colitis to SG pathology. We performed chemical sympathetic denervation, which completely restored SG architecture and function during colitis. These findings highlight a previously unappreciated link between intestinal inflammation and salivary gland pathology via sympathetic hyperactivation and disruption of humoral secretory immune responses in the oral cavity, which may play a role in a bidirectional oral-gut pathogenic loop.

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1444 – P2.21.24

Intraepithelial lymphogram in the diagnosis of celiac disease in adult patients: a validation cohort.

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Purpose: Celiac disease is a systemic autoimmune disorder associated with an abnormal response to gluten, highly prevalent and with a highly heterogeneous clinical presentation. There are several biomarkers that allow for its diagnosis, although there are cases where making a correct diagnosis is challenging. The analysis of duodenal intraepithelial lymphocytes (IELs) by flow cytometry (lymphogram) is becoming an increasingly useful and necessary tool for establishing a diagnosis of celiac disease and differentiating between its various forms of expression. In this research, we aim to validate the duodenal lymphogram as a diagnostic test and as a biomarker of treatment response in patients with celiac disease.
Methods: In this retrospective study, we analyzed a cohort of 780 patients, among whom 217 had active celiac disease, 195 were on a gluten-free diet, 25 had potential celiac disease and atypical forms of celiac disease, and 413 were non-celiac controls. Cut-off values for IELs were established to calculate the diagnostic accuracy of the lymphogram.
Results: 80% of confirmed celiac disease patients, whether active, potential, or atypical, exhibit a characteristic lymphogram of celiac disease (≥14% T cell receptor [TCR]-γδ IELs along with ≤4% surface-negative [sCD3-] IELs), while the remaining percentage of celiac disease patients showed a lymphogram with a partial profile (≥14% TCR-γδ IELs or ≤4% sCD3- IELs), with lower diagnostic certainty. It is worth noting that none of these patients had a non-celiac lymphogram. Quantifying the imbalance between TCR-γδ vs. sCD3- as a ratio (≥5) could be a discriminative index to rule out or suspect celiac disease at diagnosis.
Conclusion: In our cohort, the lymphogram analysis demonstrates a diagnostic accuracy of 79% sensitivity with 98% specificity, yielding a likelihood ratio of 36.2. It has been evidenced that the increase in TCR-γδ IELs serves as a reliable marker for celiac enteropathy. On the other hand, changes in the percentage of sCD3- IELs correspond to dynamic alterations during the course of celiac disease, thus proving useful as a biomarker for mucosal injury.

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1463 – P2.21.25

Segment-specific regulation of intestinal motility by colitis and the adaptive immune system

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The intestine, a precisely organized organ with multifaceted functions, consists of a complex cellular structure. This investigation delves into the motility patterns of distinct intestinal sections under homeostasis and amidst inflammatory bowel disease (IBD), with a focus on the participation of the adaptive immune system. Employing an organ bath system, we scrutinized the ileum and various segments of the colon in wild-type mice and those deficient in T and B cells, inducing IBD with dextran sulfate sodium salt (DSS).
In these intestinal segments, numerous motility parameters were finely modulated by colitis and the ensuing recovery process, significantly influenced by the adaptive immune system. This role of the immune system in motility was apparent even in healthy states.
In essence, intestinal motility in the ileum and colon is intricately regulated across segments, with the adaptive immune system assuming a crucial role. These discoveries advance our comprehension of IBD pathology, underscoring the importance of investigating gastrointestinal motility and the immune system within the realm of IBD research.

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1490 – P2.21.26

Vaccine induced memory CD8+ T cells efficiently prevent viral transmission from the respiratory tract

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Introduction: Mucosal immunization eliciting local T-cell memory has been proposed to provide better protection with improved breadth against constantly evolving respiratory viruses that are able to evade pre-existing antibodies. However, it is uncertain if T-cell targeted vaccinations are sufficient to block viral transmission, and how essential local immunity is in this context.
Methods: To study the impact of T-cell vaccination on the course of viral respiratory infection and subsequent transmission, we used a mouse model involving natural murine parainfluenza infection with a luciferase encoding virus and an adenovirus based nucleoprotein targeting vaccine.
Results and discussion: Strong mucosal CD8+ T cell immunity induced by combined intranasal and parenteral immunization rapidly shut down the incipient infection and completely inhibited contact based viral spreading. If without local T-cell memory, as in mice only received parenteral immunization, circulating memory T cells also accelerated viral clearance and reduced the intensity of inter-individual transmission. These findings underscore the importance of mucosal T cells for optimal protection of individuals as well as herd immunity, while also explaining why induction of a strong systemic T-cell response may still impact viral transmission. We are currently investigating if priming only the upper respiratory tract can exert the same extent of viral control as that following immunization of the entire respiratory tract. Preliminary data suggested that even with lower numbers of CD8+ T cells induced in the lungs, transmission of the virus to and from the immunized mice were both limited by intranasal immunization alone.

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1549 – P2.21.27

Finding Their Place: Maintenance of Tissue Resident Memory T Cells in Lung Infections

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Immune responses and immunotherapies rely on T cells. Among these, tissue resident memory T cells (TRM) located in non-lymphoid tissues such as the lungs, play a key role to fight infections. While the role of these non-circulating T_{RM} cells in infections and re-infection is well established, the aspects of their differentiation and maintenance are not fully understood. In particular, the identity of the factors that determine the fate of T_{RM} cells and at what point after activation the T_{RM} cells act. Understanding how to replenish the T_{RM} compartment could lead to strategies to promote residency of T cells to control respiratory disease. Therefore, we aim to investigate whether the differentiation of T_{RM} cells from naïve T cells is predetermined and to identify the factors that drive this process.
To achieve our objective, we used an adoptive transfer of CD45.1 T cells into CD45.2 recipients followed by a type 1 (*Influenza* H3N2 virus), type 2 (*Nippostrongylus brasiliensis*) and a type 3 (*Aspergillus fumigatus*) infection. Then, we assessed the transferred CD45.1 cells and endogenous CD45.2 cells in spleens and lungs at 1, 2, 3 and 4 weeks post-infection. At each timepoint, we used single cell RNA sequencing, to analyze gene expression and VDJ recombination of each single T cell.
Our study generated a unique dataset of 96,429 polyclonal T cells upon infection. This dataset includes various T cell types such as naïve T cells, T_{RM} cells, T_{FH} cells, and T_{EM} cells. Surprisingly, we were also able to identify a tissue resident regulatory T (Treg) and a CCL5 T cell population. We take advantage of the transcriptomic data of the CD45.1 T cells to precisely characterize T_{RM} cell development in time. We show that T_{RM} cells express specific marker genes like b*hlhe40*, *nr4a1* and *cd69*, while suppressing others like *lef1*, *klf2* and *s1pr1*, while discussing whether the TCR can reliably predict the development of TRM cells.

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1669 – P2.21.28

Impact of murine allergic airway inflammation on pIgR-mediated airway immunity and pneumococcal colonization

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Background: The transport of secretory immunoglobulin A and M (sIgA, sIgM) through the epithelial cell barrier into the mucosal lumen by the polymeric immunoglobulin receptor (pIgR) is an important mechanism of mucosal host defense in the respiratory tract. Human asthma is associated with impaired secretory immunity and increased risk for severe pneumococcal infections of the airways. The identification of immunomodulating substances that increase epithelial *Pigr* gene expression might have therapeutic implications with regard to an improved immune exclusion – and thus an augmented respiratory mucosal immunity. The aim of this project is to analyze the impact of allergic airway inflammation (AAI) on secretory immunity and pneumococcal colonization under homeostatic and immunomodulating conditions in distinctive areas of the murine upper and lower respiratory tract.
Methods: Mice exhibiting AAI were generated by i.n. treatment with house dust mite (HDM) extract. *Pigr* gene expression in lung, trachea and nasal-associated lymphoid tissue (NALT) as well as IgA levels in nasal lavage (NAL) and bronchoalveolar lavage (BAL) of AAI and non-AAI mice were determined under homeostatic conditions and in reaction to i.n. IFN-γ treatment by quantitative real-time PCR and ELISA, respectively. AAI and non-AAI mice were i.n. treated with PBS or IFN-γ and i.n. or o.p. colonized with *Streptococcus pneumoniae* serotype 19F. Bacterial burden was assessed in nasopharynx, NAL, NALT, trachea, BAL and lung.
Results: AAI mice exhibited increased *Pigr* gene expression in lung and elevated IgA levels in BAL compared to non-AAI mice. IFN-γ treatment of i.n. colonized non-AAI mice led to increased IgA levels in BAL and decreased bacterial burden in NAL. IFN-γ treatment of i.n. colonized AAI mice caused decreased IgA levels in BAL and had no effect on pneumococcal colonization. O.p. colonized AAI mice exhibited decreased bacterial burden in BAL and lung compared to non-AAI mice.
Conclusion: Murine AAI is associated with improved pIgR-mediated secretory immunity as well as antipneumococcal defense in the lower respiratory tract. Airway-associated secretory immunity can be partly modulated by IFN-γ. IFN-γ has a context-dependent effect on antipneumococcal immunity in healthy *vs*. pre-diseased individuals.

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1762 – P2.21.29

Cross-reactive T cells directed to commensal and food-derived yeasts drives cytotoxic TH1 cell responses in Crohn's disease

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Purpose: Inflammatory bowel diseases (IBD) are considered to involve a close interaction between altered intestinal microbiota, reduced epithelial barrier function and aberrant mucosal immune reactions. Dysregulated CD4+ T cell reactions against intestinal antigens are considered a causal or driving factor for IBD. However, the disease-relevant microbial species and the corresponding microbe-specific, pathogenic T cell phenotypes remain largely unknown. Most studies to date have implied a role of commensal bacteria in the etiology and maintenance of IBD. However, fungal microbes are increasingly recognized as important modulators of the human immune system. IBD patients have been reported to be more heavily colonized by *Candida* species, suggesting a possible involvement of commensal fungi in the disease pathology.
Methods and Results: Using the highly sensitive technology Antigen-Reactive T cell Enrichment (ARTE), we characterized reactive CD4+ T cells against a panel of different bacterial and fungal microbes in healthy individuals and IBD patients. Interestingly, while reactivity against the analyzed bacteria remains unchanged, we found increased T cell reactions to *Candida* species and *Saccharomyces cerevisiae* in Crohn's disease (CD) but not Ulcerative Colitis (UC) patients. Using single cell RNA and T cell receptor repertoire analysis, we found that yeast-reactive CD4+ T cells in CD display a cytotoxic T helper cell (T_H1 cell) phenotype and show selective expansion of T cell clones that are highly cross-reactive to other commensal, as well as food-derived fungal species. This suggests cross-reactive T cell selection by continuous encounter with conserved fungal antigens in the context of chronic intestinal disease.
Conclusion: We identified increased frequencies of yeast-reactive T cells in CD, as well as functional alterations of highly-crossreactive yeast-reactive T cells in a subset of CD patients. Our data highlights a role of yeasts as drivers of aberrant CD4+ T cell reactivity in patients with CD and suggests that gut-resident fungal commensals and diet-derived yeasts contribute to the chronic activation of inflammatory CD4+ T cell responses in these patients. Our data make an important contribution towards understanding the role of fungi for IBD pathogenesis. The dissection of heterogenous T helper cells against disease-relevant microbes will facilitate the development of targeted therapeutic strategies.

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1777 – P2.21.30

Genetic and inflammatory factors regulate monocyte expression of the TGFβ-activating integrin αvβ8 in IBD

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Purpose: Inflammatory bowel disease (IBD) is a chronic inflammation of the gastrointestinal (GI) tract caused by an uncontrolled immune response to normally occurring gut constituents. Transforming growth factor β (TGFβ) is a potent regulator of the immune system and a key molecule promoting immunological tolerance in the GI tract. All cells that produce TGFβ do so as an inactive complex which must be activated to function. We have previously shown that monocytes and macrophages activate TGFβ via expression of the integrin αvβ8. This is an important anti-inflammatory pathway, and in IBD expression of αvβ8 is significantly reduced on intestinal monocytes and macrophages. However, molecular mechanisms that downregulate this pathway are poorly defined. Additionally, the single nucleotide polymorphism (SNP) rs149169037 has been associated with IBD and is predicted to influence ITGB8 expression (which encodes the integrin β8 subunit). The purpose of this project is to define molecular and genetic factors that regulate expression of integrin αvβ8, and so determine important pathways that regulate immune tolerance in the intestine.
Methods: Intestinal biopsies were obtained from IBD and control patients, cultured ex vivo, and supernatants used for soluble cytokine quantification and stimulation of immune cells. These supernatants and recombinant proteins were also used to treat human blood immune cells, and integrin expression analysed by RT-qPCR and flow cytometry.
Results: Ex vivo stimulation of cells with supernatants derived from IBD patient intestinal biopsies significantly decreased αvβ8 expression on blood monocytes. Expression of αvβ8 is increased on monocytes stimulated with lipopolysaccharide (LPS) or interleukin-6 (IL-6), and decreased by IL-4, IL-33, or interferon γ (IFNγ) stimulation. Individuals homozygous for the major allele of the *ITGB8* associated polymorphism rs149169037 show high levels of αvβ8 expression on monocytes, and lower LPS-induced TNFα production. Conversely, individuals homozygous for the minor allele show low αvβ8 expression on monocytes and high LPS-induced TNFα production.
Conclusions: Cytokine signalling, microbial antigens, and genetics all contribute to αvβ8 expression on human monocytes. Identifying mechanisms that regulate αvβ8 expression to promote anti-inflammatory TGFβ signalling represents a novel approach in IBD.

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2149 – P2.21.31

Isolation of single cells from human Peyer's Patches obtained from biopsies

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Objective: Peyer's Patches (PPs) are specialized lymphoid follicles, located in the terminal ileum. They are important for the development of oral tolerance against food antigens and the protection against pathogens. To understand the involvement of different immune subsets in this process, it is important to carefully decipher immune activation in the PPs and the surrounding lamina propria. As the isolation of immune cells from human PPs presents several challenges, we established an optimized pipeline for the isolation of single cells out of PP from ileal biopsies for single cell sequencing.
Methods: Immune cells were isolated from ileal biopsies from healthy individuals obtained during routine endoscopy and after written consent. Single cells were then isolated using enzymatic digestion and mechanical force and purified using fluorescent activated cell sorting (FACS). The solution was used to prepare libraries for single cell sequencing using the Scipio Bioscience Asteria^{TM} kit and remaining cells were used for flow cytometry.
Results: Human biopsies of PP were obtained by specifically trained endoscopists during routine endoscopy from healthy individuals, characterized by no active inflammation. After mucus removal, PPs in the biopsies were identified and mechanically dissected using a stereomicroscope. The number of PPs varies between 0-3 out of 4 ileal biopsies. Afterwards, biopsies were enzymatically digested followed by mechanical force. Single cells were further purified using FACS as living immune ($CD45^+EpCAM^-7\text{-}AAD^-$) cells. Additionally, the successful identification of PPs was proven by elevated B-cell frequency compared to lamina propria (LP) samples with PPs between 30%-45% and LP <10%. The optimized isolation protocol of the biopsies led to an accelerated processing time, increased cell number as well as viability. First samples were collected and successfully processed to perform single cell sequencing.
Conclusions: With our optimized sample processing pipeline, we are able to isolate a sufficient number of single cells from the limited material of PP containing ileal biopsies to perform benchtop single cell sequencing sample and library preparation. The obtained data will be used to design targeted panels to study human PP specific immune cell subsets in a broader cohort in the context of intestinal inflammation by a multiplexed spatial approach.

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2226 – P2.21.32

Cigarette smoke-induced dysregulation of iron metabolism alters alveolar macrophage response to Streptococcus pneumoniae: implications for chronic obstructive pulmonary disease

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Chronic obstructive pulmonary disease (COPD) is an inflammatory disease characterized by emphysema and chronic bronchitis. Excess iron accumulation in the lung associates with COPD severity, with lung-resident alveolar macrophages (AMs) constituting the main iron positive cell. *Streptococcus pneumoniae* is the one of the most commonly cultured pathogens from the COPD lung and associates with exacerbation events, which contribute significantly to morbidity and mortality. While all bacteria require iron to survive and proliferate, how increased iron levels in the COPD lung microenvironment alters macrophage-*S. pneumoniae* interactions remains unclear.
To investigate this *in vitro*, foetal liver-derived alveolar macrophage-like cells (FLAMs) were treated with cigarette smoke extract (CSE) as a model of COPD, with cellular and mitochondrial iron loading confirmed through graphite furnace-atomic absorption spectrometry (GF-AAS) and confocal microscopy. Alterations in expression of factors governing host iron metabolism at the mRNA and protein level were assessed through qPCR and immunoblotting, respectively, revealing blunted expression of several factors associated with cellular and mitochondrial iron handling. The effects of CSE treatment on macrophage response to infection were assessed through ELISA (IL-6, TNF-α, lipocalin-2) and enumeration of intracellular colony forming units (CFU), revealing CSE treatment attenuates pro-inflammatory cytokine production and increases *S. pneumoniae* intracellular replication. The sensitivity of *S. pneumoniae* intracellular replication to host iron chelation was also assessed through *ex vivo* treatment of human alveolar macrophages retrieved via bronchoalveolar lavage with the iron chelator deferiprone. These results highlight the role of dysfunctional iron metabolism in promoting bacterial virulence and suggest the targeted manipulation of macrophage iron as a therapeutic avenue in COPD.

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2262 – P2.21.33

Innate immunity changes predict progression of septic shock and perturb mucosal immunity in model of iPSC derived model of lung organoid

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Background: Sepsis and septic shock are characterized by dynamic immune system changes resulting in deregulated inflammation and homeostasis failure. Circulating neutrophils, monocytes and other innate immune cells are the first responders recognizing and clearing pathogens while producing plethora of immune mediators. This prompt activation of the immune system results eventually in negative outcome, including induction of fundamental damage of mucosal tissues leading to further tissue remodulation, which can eventually dampen the process of patient's recovery after sepsis.
Objectives: This project aimed to identify novel biomarkers reflecting the dynamic of sepsis and its progression but also depict factors, which govern the damage and further remodulation of mucosal tissue impeding full recovery using morel of iPSCs derived lung organoids.
Methods: Blood samples from septic shock patients were collected within first 12 hours of ICU admission. Using flow cytometry phenotyping, proteomic and global expression analysis we have proposed several candidates correlating the early septic shock mortality. Serum proteins and cytokines were measured by ELISA or bead assays. Mucosal iPSCs derived organoids were stimulated with these mediators to characterize their potential ability to remodel the tissue.
Results: We described specific changes of monocytes subsets as well as polymorphonuclear cells associated with their altered activation. The levels of cytokines including MCP-1, IL-6, IL-8, IL-10, IL-18, as well as hepcidin, ferritin and alarmin S100A8/9 show changed production. We established hepcidin-to-ferritin ratio with high predictability for non-favorable sepsis outcome. We also characterized the role of cytokines in the sepsis-induced remodeling of mucosal tissues.
Conclusion: This project identified several valuable markers of sepsis progression, pinpointing the vulnerable patients within first 12 hours after their ICU admission. Such an approach may improve their survival of septic shock by future personalized therapy targeting the altered monocyte and polymorphonuclear functions, as well as altered tissue environment.

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2271 – P2.21.34

Adaptive T cell-mediated immunity to gluten is elicited in the gut mucosa of type 1 diabetes patients only in presence of celiac disease comorbidity

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Purpose: Type-1 Diabetes (T1D) and Celiac Disease (CeD) are two autoimmune disorders strongly associated, as they share genetic risk factors (HLA-DQ2/DQ8 genes) and immunopathogenic mechanisms. Different studies suggested an implication of wheat gluten proteins, the causative antigen of CeD, in the T1D pathogenesis. We previously showed that gluten elicit an inflammatory response in jejunal mucosa of T1D patients in *in vitro* organ culture (1). Here, we investigated the intestinal T-cell response to gluten in children with T1D, with or without CeD-comorbidity.
Methods: Thirty-three patients were enrolled (median age 10 years, range 1-24). Nineteen were T1D patients and of them, six patients had T1D and were negative for anti-tissue transglutaminase antibodies (CeD-associated autoantibodies), six had T1D and potential-CeD (positive serology and normal mucosa histology), and seven had acute-CeD (positive serology and villous atrophy) in comorbidity. The remaining fourteen had CeD and were negative for T1D. All enrolled subjects undergo the endogastroscopy and jejunal biopsy explants were processed to perform an *ex vivo* phenotypic analysis by flow-cytometry and for generation of gluten-raised T-cell lines (iTCLs). Gluten-specificity was assessed by functional IFNγ-ELISPOT in presence or absence of IL10R and TGF-β monoclonal antibodies (MoAbs) neutralizing regulatory T cells (2).
Results: No substantial differences were found in the phenotype and activation status of intestinal T cells among T1D patients with or w/o CeD-comorbidity. No IFNγ production to gluten was observed in iTCLs from T1D that were negative for CeD serology, neither in the presence of anti-IL10R and anti-TGF-β neutralizing MoAbs. By contrast, all iTCLs from T1D patients that had CeD, either potential or acute, showed a significant infiltrate of gluten-specific T cells in the gut mucosa ($p<0.05$).
Conclusions: Gluten-reactive T cells can be detected only in the intestinal mucosa of T1D patients that had CeD-comorbidity, either potential or acute disease. No IL10/TGFβ dependent regulation of T-cell response to gluten were observed in T1D mucosa in the absence of CeD. In conclusion, these data do not support the hypothesis of a role of adaptive immunity to dietary gluten in T1D pathogenesis.

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2273 – P2.21.35

Nanoconjugates of lactoferrin for treatment of viral infection

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Lactoferrin plays an important role in immune regulation and defence mechanisms against bacteria, fungi and viruses. Recently, much effort has been devoted to the development of biomedical applications of nanoparticles.
The aim of this work was to test whether lactoferrin conjugates can protect the olfactory route from in vitro and in vivo corona virus infection.
Lactoferrin modified AgNPs or AuNPs sized 5 and 30 nm were synthesized and characterised using in-house methods. Antiviral potential was tested in vitro using HCoV-229e infected human epithelial MRC-5 cells or MHV- infected NCTC cell line and a mouse model of corona infection with MHV (Murine hepatitis coronavirus). MHV-infected mice were treated with nanoconjugates by repeated instillations and further analysed for clinical signs of infection, cellular, cytokine and chemokine response in respiratory tract as well as within the olfactory tract.
Tests performed in HCoV-229e- and MHV-JHM-infected cell lines demonstrated that inhibition of HCoV-229e/MHV infection was metal and size-dependent with LF-modified 30 nm AgNPs showing the most promising anti-viral activity. During intranasal infection with MHV-JHM – only lactoferrin conjugates of 5 nm Ag/AuNPs showed significant decrease in total brain/lung titers at 7 days post infection. Lactoferrin-functionalised Au/AgNPs showed stimulation of monocyte early response withing the epithelial mucosa, thus leading to induction of local antiviral response.
Therefore, lactoferrin functionalized gold and silver nanoparticles can consist good candidates for effective anti-viral microbicide to be used in vivo due to their effectiveness at lower concentrations and induction of an anti-viral response.

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2280 – P2.21.36

Role of the cholesterol metabolizing enzyme Cyp27a1 in intestinal regeneration and tumorigenesis

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The intestinal epithelial barrier is continuously challenged by environmental stressors and therefore undergoes continuous homeostatic and damage-associated tissue renewal. However, uncontrolled regeneration leads to neoplastic transformation, which must be prevented by pathways that disengage tumour growth from regenerative processes. We have previously identified liver X receptor (LXR) as a novel pathway capable of promoting tissue repair while limiting tumorigenesis (*Das S. et al.; Under revision)*. Yet, the mechanisms underlying LXR activation in response to tissue damage remain to be determined. Using single-cell and bulk RNA sequencing datasets from murine models of intestinal damage and repair, we identified a selectively increased expression of *Cyp27a1*, a cholesterol metabolizing and oxysterol-producing enzyme, within the damaged intestinal crypts niche during regeneration. *Cyp27a1* deletion dampened intestinal regeneration in both DSS colitis and irradiation-induced damage, which was rescued by the administration of the LXR synthetic ligand GW3965. In humans, we observed an increased expression of *CYP27A1* in the terminal ileum and colon of active ulcerative colitis patients compared to those in remission, suggesting a conserved axis upon tissue injury across species. Notably, when challenged for tumorigenesis, *Cyp27a1*$^{-/-}$ mice displayed more and larger tumours, and this phenotype was rescued in GW3965-fed mice. Extending these findings to humans, we further confirmed downregulation of *CYP27A1* in the colonic epithelium of patients with colorectal cancer compared to healthy controls. Altogether, our results unveiled an intestinal stem cell niche adaptation to damage and tumorigenesis whereby Cyp27a1 upregulated in response to injury drives LXR activation in intestinal epithelial cells promoting intestinal regeneration while restraining tumorigenesis.

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P2.22 MYELOID LINEAGE

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100 – P2.22.01

Targeting Skin-resident Antigen Presenting Cells to Activate Skin-resident CD4+ Memory T cells during Staphylococcus aureus Vaccination

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Staphylococcus aureus (SA) is a leading cause of skin and soft tissue infections worldwide. The virulence and increasing numbers of antibiotic-resistant strains being reported highlights the urgent need for alternative therapies. To date, no vaccine has been approved due to the lack of efficacy reported in clinical trials. Experimental, clinical, and genetic evidence has shone light on the importance of Th17/IL-17 and Th1 in protection against SA infections. We have shown that subcutaneous (SC) infection of C57BL/6 mice induces $CD4^+$ skin-resident memory T cells (Tsrm) that readily produce IL-17 upon re-infection, similarly to what we have described in human skin. Therefore, we aimed to identify skin-resident antigen presenting cells (APCs) responsible for the activation of these Tsrm, which are the adaptive sentinels ready to fight against SA skin infections. To achieve this goal, we analyzed leukocytes present in the skin of mice infected SC with SA and sacrificed at different timepoints by single-cell RNA sequencing (scRNA-seq). By doing this we identified 14 cellular populations, comprising both novel and previously characterized cells. This analysis confirmed the infiltration of $CD4^+$ effector memory T cells at Day 14 post infection (p.i.) and the presence of $CD4^+$ Tsrm at Day 43 p.i. Regarding APCs involved in the response to SA, we identified a DC2 population as well as a population that we named "$CCR7^+$ DCs", which has been recently identified by scRNA-seq only in the context of tumors. Using an unbiased approach, we identified a chemokine/cytokine network revealing that DC2 are the principal trigger for Th17, while $CCR7^+$ DCs trigger a Th1 response. Flow cytometry has allowed us to identify these DC populations both the murine and human skin models. Future experiments will be aimed to confirm the role of these DCs in the context of cutaneous SA infection. Harnessing these DCs as potential targets for the activation of Tsrm could be a step toward novel vaccine strategies.

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261 – P2.22.02

A novel myeloid cell population: Embryonic tissue-resident neutrophils

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Purpose: Neutrophils represent the most abundant leukocyte population in the circulatory system acting as the first line of defense against pathogens. Some neutrophils infiltrate the tissue and display tissue-specific phenotypes. However, neutrophils in fetal tissues have not been described before. While studying embryonic tissue-resident macrophages, we were surprised to find a remarkable, previously unidentified Ly6G$^+$ neutrophil population in the prenatal mouse testis. This population starts to diminish after birth and disappears completely when the mouse passes puberty. The purpose is to describe and define embryonic neutrophils in testis and other tissues for the first time and to see if they share functional similarities to adult neutrophils. This study will deepen the knowledge of male reproductive health and helps to acquire tools for clinical studies in battling against male reproductive disorders like infertility and testicular cancer.
Methods: High-dimensional single-cell analyses, such as fluorescence-activated cell sorting (FACS) and single-cell-RNA-sequencing were employed to analyse the gene and protein expression of neutrophils, following the kinetics from E17.5 to adult mice. To assess the spatial localization of tissue-resident neutrophils, optical tissue-clearing techniques for whole-mount imaging was combined with advanced confocal microscopy. Several functional assays were used to compare neutrophil activity between embryonic and adult neutrophils. Neutrophil depletion models were utilized, in addition to inducible fate-mapping models, enabling tracking of neutrophils throughout the development.
Results: We noticed great heterogeneity among neutrophil populations in several embryonic tissues. Many functional abilities were similar to those observed in classical, adult circulating neutrophils: ROS production is comparable to adult bone marrow neutrophils, and embryonic neutrophils go through the same maturation steps as adult ones. However, they have inferior capacity to phagocytose particles compared to adult neutrophils *in vitro*.
Conclusion: Since many developmental complications originate from incomplete function or the lack of myeloid cells, defects in neutrophils during development may also be harmful for the developing individual. This study provides genuinely novel insight on the myeloid cell heterogeneity and formation of the immune system during embryogenesis, and our results provide foundation for advancing future treatments in immune related diseases.

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273 – P2.22.03

Establishing the role of the V-ATPase-associated heme transporter HRG1 in regulating endolysosomal function and cellular heme-iron homeostasis.

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Heme is a cofactor for essential metabolic enzymes, and constitutes the majority of iron present in the body. Heme is also emerging as a key regulator of immunity, particularly being associated with innate immune cell activation. Excess free heme causes oxidative stress, inflammatory cell death, and tissue damage. Therefore, heme levels must be tightly regulated at the cellular, tissue, and systemic levels. This is accomplished by a network of proteins that regulate heme biosynthesis, transport, and degradation.
Heme Responsive Gene-1 (HRG1, *SLC48A1*) is a 16kDa protein that is expressed throughout the endolysosomal pathway. HRG1 was identified as both a V-ATPase-interacting protein which promotes endolysosomal acidification, as well as an essential mediator of heme transport from lysosomes during erythrophagocytosis. The broader function of HRG1's role in regulating the cellular response to heme is poorly understood. This project aims to elucidate HRG1's role in regulating endolysosomal pH, trafficking, and heme-iron homeostasis, with a particular focus on macrophages.
HRG1 expression is predominantly associated with erythrophagocytic macrophages of the spleen and liver. Analysis of transcriptomic datasets demonstrates that HRG1 expression is elevated in various tissue-resident macrophage populations. Interestingly, HRG1 expression is also elevated in distinct populations of hematopoietic cells involved in erythropoiesis. This suggests that HRG1 may regulate broader tissue heme-iron homeostasis, and function to support both erythrocyte development and degradation.
Autophagy is a critical component of the immune response. Previous data from our lab shows that HRG1 stabilises the V-ATPase complex and promotes V-ATPase activity. However, overexpression of HRG1 in epithelial cells promotes the stability of various endocytosed receptors, including the transferrin receptor. This is accompanied by a concomitant decrease in the expression and activity of components of the canonical autophagy pathway, which is associated with HRG1-dependent regulation of intracellular pH levels.
Overall, our data indicates that HRG1 is expressed across various tissue-resident macrophage populations, likely contributing to the regulation of local tissue heme-iron homeostasis. At the cellular level, HRG1 modulates endolysosomal dynamics through regulating the pH of intracellular compartments.

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316 – P2.22.05

Early precursor-derived pituitary macrophages, cells connecting the immune and endocrine systems

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The endocrine system involves multiple peripheral glands and organs forming complex pathways and feedback loops throughout the body. The pituitary gland is the major endocrine regulatory organ which primary function is to produce and release several hormones. The physical location of the pituitary gland underneath the brain, though outside the protective blood-brain barrier, leads to a unique immune environment of the pituitary. Macrophages are the foremost immune cell population in the pituitary gland, but studies describing macrophage subpopulations, their ontogeny, or their role in pituitary function have not been performed. Combining single-cell transcriptomics, fate mapping, and imaging, we defined the development, diversity, niche-specific heterogeneity, and origin of the pituitary gland macrophage subsets. In this study, we created a high-density transcriptional map of pituitary resident macrophages and discovered multiple subsets that showed kinetical progression during mouse development. Our comprehensive ontogeny analysis using different genetic fate-mapping approaches, showed that pituitary gland macrophages exclusively originate from the primitive yolk sac-derived macrophages and rely on proliferation for self-maintenance. To study the spatial heterogeneity of diverse pituitary macrophage subpopulations, we utilised high-resolution 3D imaging, and discovered distinct macrophage subsets residing in different sub-tissular niches suggesting that pituitary macrophages carry out varying functional roles in the gland. Moreover, we found macrophages associated with hormone-producing cells in the anterior lobe. To determine the importance of macrophages in pituitary function, macrophage depletion experiments unveiled essential role of early macrophages in the hormonal production of the pituitary and post-pubertal expression of genes related to the sexually dimorphic processes regulated by the pituitary gland. Altogether, our study provides novel information on pituitary macrophage ontogeny and heterogeneity, their role in pituitary function and macrophage-endocrine communication.

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530 – P2.22.06

Assessing the maturation stages of PMN-MDSC to dissect the immunosuppressive mechanisms within TME

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Purpose: The impact of tumor microenvironment (TME) on cancer therapy is one of the major challenges nowadays. One of the major immunosuppressive and pro-tumoral cellular component within TME is represented by myeloid-derived suppressor cells (MDSC), in particular the polymorphonuclear (PMN) subset. PMN-MDSC are immature neutrophils that in pathological conditions (e.g. cancer, infection, and inflammation) accumulate in peripheral blood (PB) and in the inflamed tissues. In this study, we wanted to clarify the differentiation and immunosuppressive function of PMN-MDSC.
Methods: Samples used: PB and bone marrow (BM) of G-CSF mobilized healthy donor (HD-mob-PMN-MDSC) and PMN-MDSC purified from PB or BM of cancer patients (CP). After PBMC separation, PMN-MDSC were isolated by immuno-magnetic bead. Their inhibitory function was assessed on NK cell cytotoxicity.
Results: To asses the different stages and functions of PMN-MDSC, we compared PMN-MDSC isolated from PB or BM of HD and CP. We found out that PB-HD-mob-PMN-MDSC and PB-CP-PMN-MDSC showed an immunosuppressive activity. Instead, PMN-MDSC freshly isolated from BM (both HD and CP) did not show immunosuppressive functions. These data identify the BM-PMN-MDSC-like cells as the initial point of differentiation, the HD-mob-PMN-MDSC as an intermediate stage and the PB-CP-PMN-MDSC as the final stage. The short half-life of these cells (48-72 hours) is a challenge to study them *in vitro*. Thus, to prolong their viability *in vitro* (10 days), we set up a culture system with cytokines or malignant Pleural Effusion (mPE), that recreate TME. Notably, these PMN-MDSC maintained the immunosuppressive function or acquired it in culture (after 10 days), indicating that soluble factors render PMN-MDSC-like cells at early stage in fully competent PMN-MDSC, sustaining their development and function *in vitro*.
Conclusion: PMN-MDSC affect the tumor growth and immunotherapy efficacy, for this reason studying their development and inhibitory mechanisms is crucial. Our results are pivotal to clarify these processes and improve/develop new immunotherapeutic approaches.

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1078 – P2.22.07

Mast cell-specific expression of non-functional cofilin-1 leads to a mast cell-deficient mouse model fully susceptible to imiquimod-induced psoriasis, 1-fluoro-2,4-dinitrobenzene-induced contact hypersensitivity, and vaccinia virus infection

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Remodelling of the actin cytoskeleton is essential for many processes in immune cells. A large number of actin-binding proteins (ABPs) dynamically regulate actin remodelling. The ABP cofilin-1 is a member of the actin depolymerising factor (ADF)/cofilin family. Cofilin-1 is expressed in non-muscle cells including immune cells and promotes actin rearrangements by actin severing and depolymerisation. Its importance is underlined by the fact that a global knock-out of cofilin-1 in mice is embryonic lethal.
The role of cofilin-1 in mast cells *in vivo* has remained elusive. Using the Cre/loxP system, we generated mice expressing a non-functional form of cofilin-1 instead of the wild-type protein in connective tissue mast cells. These mice completely lacked connective tissue mast cells in the peritoneum and in the skin. Consequently, the knock-in mice were not sensitive to the induction of a systemic anaphylactic reaction. The immune cell compartments analysed in the spleen and lymph nodes of the knock-in mice were virtually unaffected by the absence of mast cells. Mast cell-deficient mice were normally susceptible to 1-fluoro-2,4-dinitrobenzene (DNFB)-induced contact hypersensitivity and imiquimod-induced psoriasis. In addition, clearance of vaccinia virus skin infection was normal.
Our study highlights the critical importance of the protein cofilin-1 for connective tissue mast cell development. Furthermore, it describes the generation of a c-kit-independent mouse model of mast cell deficiency without gross alterations in immune cell compartments other than mast cells. Finally, the study demonstrates that mast cells are not critically involved in the development and onset of the skin diseases CHS and psoriasis, or in the clearance of vaccinia virus infection.

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1726 – P2.22.08

Phenotypic and functional analysis of a novel macrophage population expressing a membrane-bound form of IL-18

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Purpose: Tumor-associated macrophages are heterogeneous cells. Nevertheless, up to 90% of macrophages in the peritoneal fluids of ovarian cancer patients express a membrane-bound form of IL-18 (mIL-18). These macrophages were identified as a subpopulation of unpolarized macrophages (M0) differentiated from monocytes of healthy donors. mIL-18 expression persists during IL-4-driven M2 polarization but not upon stimulation with pathogen-derived products. This work studies extensively mIL-18+ macrophages and explores their presence in tissues of cancer patients.
Methods: Monocytes were purified from PBMC of healthy donors and differentiated towards M0. mIL-18+ and mIL-18- subpopulations were sorted and studied in terms of proteomics, metabolisms, and cytomorphology. Peritoneal fluids were collected from adult cancer patients, while bone marrow (BM) aspirates from pediatric neuroblastoma (NB) patients.
Results: Proteomic analysis shows in mIL-18+, in respect of mIL-18- counterpart, an increased expression of proteins associated with pathogen recognition, activation, migration, vesicle-mediated transport, and lipidic catabolism. Moreover, Seahorse Xp reveals reduced glycolysis, mitochondrial respiration, and ATP production. Accordingly, Transmission Electron Microscopy shows round-shaped mitochondria with altered cristae. Finally, mIL-18+ macrophages have increased endocytic properties and are highly enriched in the peritoneal fluids of adults suffering from different types of cancer (e.g. ovarian, gastric), with a phenotype partially overlapped with that observed in M0. They are also present in the BM of NB patients with a higher inter-individual heterogeneity as compared to the peritoneal site. Unsupervised hierarchical clustering analysis of 166 genes characterizing mIL-18+ macrophages was carried out on the gene expression of 498 NB tumors of all stages profiled at diagnosis. Heat map visualization evidenced significantly distinct clusters of patients and genes.
Conclusions: This study provides a wide and multidisciplinary characterization of human healthy and pathological mIL-18+ macrophages and analyzes their prognostic role in neuroblastoma patients.

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1939 – P2.22.09

Development and characterization of pro-inflammatory M1 macrophage-like cell models

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More than 80 different protocols for the differentiation of monocytic cell lines into M0, M1 and M2 macrophage-like cells exist. In comparison to native cells, all differentiated cell models show considerable deviations, which significantly hampers their suitability for physiological studies. Phorbol-12-myristate-13-acetate (PMA) concentration is the most critical factor that varies strongly between different methods usually ranging from 2 to 500 nM with 100 nM being the most common concentration. Current data on marker gene expression and functional studies however indicate that lower PMA concentrations are beneficial for the polarisation into mature macrophage subtypes.
We therefore systematically compared the influence of low (10 nM) versus classic (100 nM) PMA concentration using two differentiation protocols for monocytic cell line-derived M0 and M1 macrophages on two independent cell lines (THP-1 and U-937). Immunocytochemistry experiments for the classical markers CD11b and CD14 revealed that in THP-1 cells differentiated to M0 macrophages, CD11b expression is PMA-dependent while CD14 expression is PMA-independent. In U-937, both markers showed no PMA-dependent regulation. Interestingly, the PMA-differentiation step critically influenced the subsequent M1 polarisation. CD80, an important hallmark of the M1 type, was detected in 57,86 ± 5.44% of the polarized cells when using 10 nM PMA, while 100 nM PMA resulted in only 29.95 ± 7.38% $CD80^+$ cells. To investigate the physiological relevance of our improved protocol, we examined the function of formyl peptide receptors (FPRs), a family of pattern recognition receptors important for chemotactic migration and MMP-9 release in native monocytes and macrophages. qPCR experiments revealed that FPR1, FPR2 and FPR3 expression was below the detection limit after differentiation with PMA alone, whereas we detected robust FPR1 expression in >80% in both cell lines after our improved M1 polarisation. Next, we observed a co-expression of FPRs with MMP-9 and an increased FPR-dependent matrix-metalloproteinase 9 activity that is typical for native M1 cells but absent in all standard protocols. These findings argue that lower PMA concentrations improve M1 macrophage cell models. To investigate the general effects of our optimized differentiation protocol for physiological studies, we currently perform RNAseq-experiments.

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2145 – P2.22.10

Diagnosis of FISH negative acute promyelocytic leukemia due to molecular biology techniques and optical genome mapping

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Acute promyelocytic leukemia (APL) is a subtype of acute myeloid leukemia in which malignant transformation and uncontrolled proliferation of aberrant promyelocytes occur. This subtype of leukemia cells has a very specific abnormality that affects chromosomes 15 and 17, leading to the formation of a‘fusion’gene called PML/RARα.
We present the case of a 66-year-old man who went to the emergency room for 3 weeks and developed general malaise, dysthermic sensation asthenia and weight loss Blasts were observed in the peripheral blood smear, so a bone marrow puncture was performed, confirming the presence of 67% of myeloid blasts with Auer sticks and splinters, suggesting atypical promyelocytes. An immunophenotype compatible with APL (CD117+, weak CD15, CD13+, CD11b-) is observed and treatment with all-trans retinoic acid (ATRA) is initiated.
The FISH (Fluorescent Hybridization in situ) technique was performed with 200 uncultured interphase nuclei using the XL t(15,17) Dual fusion probe and the XL RARα break apart probe in which normal signals for the RARα gene were obtained.
Despite negative results for FISH, the sample was processed for molecular study. Depending on the breakpoint of the PML gene, different possible isoforms are generated: intron 6 (BCR1), exon 6 (BCR2) and intron 3 (BCR3), while the RARα breakpoints always occur at intron 2. A positive result for BCR1 was observed.In addition, a partial amplification (exons 1-6) of the PML gene was detected in the copy number variation study, which was compatible with the gain of 15q24.
One week after the start of treatment, the bone marrow immunophenotype showed the same clone detected at diagnosis but with greater maturation (CD117 negative in up to 40% of blasts and CD15 partial gain). This sample was studied with the technique of optical genome mapping and a partial gain of the PML gene was observed and an insertion into this gene of about 100,000 pairs of bases of origin still to be determined. Our aim in successive studies is to determine whether this fragment of unknown origin is the RARα gene, and if so, why it has not been detected in FISH.

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2233 – P2.22.11

Investigation of the in vitro use of HL-60 cells induced at different maturation levels as myeloid-derived suppressor cell (MDSC)-like

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Objective: HL-60 cell line is an acute myeloid leukemia cell line and is used as a model in studies at different myeloid maturation levels. These cells are known to differentiate into granulocyte and/or monocyte/macrophage-like cells by some factors. Myeloid-derived suppressor cells (MDSC) are myeloid cells that leave the bone marrow at different maturation levels as a result of chronic inflammation and join the peripheral circulation and can perform immune regulation. Although these cells have a key role in many pathologies, there is no cell line that can be used in mechanistic studies. In this study, the effects of all-trans retionic acid (ATRA), 1a,25-dihydroxyvitaminD3 (D3), secreted factors from MDA-MB-231 or MDA-MB-468 cells on MDSC-like transformation on HL-60 cells were investigated.
Method: In order to evaluate MDSC-like chracteristics of HL-60 cells, were stimulated with ATRA and D3 for 24, 48 and 96 hours and/or were co-cultured with MDA-MB-231 and MDA-MB-468 conditioned medium (CM) for 72 hours to 8 days. Stimulated cells were evaluated by Giemsa staining for morphological changes; surface markers of CD11b, CD11c, CD14, CD15, CD16, CD33, CD40, CD62L, CD66b, CD70, CD114, TRAIL, TLR4, HLA-DR and Lox-1 were evaluated by flow cytometry; stimulated HL-60 cells on T cell proliferation were evaluated by CFSE assay; ROS (reactive oxygen species) and NO (nitric oxide) production capacities were investigated via flow cytometry. NOX2, MPO, COX2, IDO1, NOS2, ARG1, ARG2 mRNA trancription levels were evaluated by qRT-PCR.
Results and Conclusion: ROS production, NOX2 and MPO mRNA expression levels were increased in response to ATRA-stimulation. CD11b expression increased in all conditions compared to unthereated HL-60, CD14 positivity increased especially in D3-stimulated conditions. However, no difference was observed in NO production and NOS2 mRNA level in cells stimulated with ATRA and D3. CD114 and Lox-1 expression were detected on $CD11b^{high}$ in MDA-MB-231 or MDA-MB-468 CM threated HL-60 cells. Especially, LOX-1 expressio was increased in response to CM from MDA-MB-231/MDA-MB-468 on day 8. HL-60 cell lines was exhibit granulocytic (G)-MDSC-like phenotype when stimulated with MDA-MB-231/MDA-MB-468 CM. Lox-1 surface marker which is critial for G-MDSC phenotype, upregulated successfully with CM induction.

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2289 – P2.22.12

M2c-like macrophage associated immunomodulatory function on CD44/CD24 phenotype in triple negative breast cancer (TNBC) subgroups

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Objective: CD44+/CD24- breast cancer stem cell-like cells are the dominant subgroup of triple negative breast cancer(TNBC) but we have limited understanding of their immunomodulatory effect. This study aims to evaluate M2c-, M2- and M0-like monocyte/macrophage dependent immunofunctional difference on T cells in TNBC and their effect on CD44/CD24 associated phenotype.
Material and method: THP-1 (monocytic), phorbol 12-myristate 13-acetate(PMA)-Induced THP-1(M0/macrophage),M2clike THP-1(CD169+CD163+CD206dim) cells differentiation status were confirmed by NGS and flow cytometry. MDA-MB-231 or MDA-MB-468 triple negative breast cancer (TNBC) cells co-cultured with monocyte/macrophage-like cells were purified with FACS according to their CD44/CD24 expression status. To measure these myleoid cells primed TNBC cells were cocultured with PBMCs obtained from healthy donors and CD4+ or CD8+ T cell proliferation was tested with CFSE dilution by flow cytometry. CD80,CD86,PD-L1 and PD-L2 expression on cancer cells and immune cells were tested by flow cytometry for costimulation. Purified different CD44/CD24 cell subpopulation were plated for scratch assays and analyzed with ImageJ.
Results and discussion: CD44+/CD24- subpopulation collected from M2c-like THP1cells/cancer cells co-culture condition, displayed less metastatic and more immunomodulatory capacity than THP-1 and PMA-Induced THP-1 conditions. In addition,CD163,CD169 vs CD14 expression were decreased on M2c-like THP-1 cells(CD44+/CD24- cancer cells primed).CD44+CD24+ population was decreased in THP-1 derived monocytic/macrophagelike cells coculture.In co-cultures between MDA-MB-468 cell lines and THP-1 derived monocytic/macrophage-like cells, especially CD44+CD24+ population was decreased.CD4+ or CD8+ T cells proliferation were affected to monocyte/macrophage-like cells primed CD44/CD24 sub-populations.In this study,CD44/CD24 phenotype in TNBC and their effects on monocytic/macrophage cell activation polarization were evaluated.In addition, the metastatic character and T cell responses of this interaction were detected,and the first preliminary data that could help immunotherapy approaches.

Keywords: CD4, CD8, T cells, CD44, CD24 Monocyte, Macrophage

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POSTER SESSION 3

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P3.01 NEUROINFLAMMATION

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57 – P3.01.01

Clinical and electrophysiological findings of peripheral neuropathy and sensory fiber involvement in Sjögren's syndrome

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Introduction and Objective: Sjögren's syndrome (SS) is a chronic, progressive, systemic inflammatory disease characterised by lymphocytic infiltration of exocrine glands. The study's primary objectives were to determine phenotypical patterns of peripheral nervous system involvement and the frequency of peripheral and central sensory involvement. We have also aimed to show the topography and extent of peripheral and central involvement by electrophysiological methods and to examine the correlation of clinical and electrophysiological findings in patients with primary Sjögren's syndrome (pSS).
Methods: Thirty patients with Sjögren's disease were examined clinically with sensory symptoms, neurologic examination, modified Toronto Clinical Neuropathy Score and electrophysiologically with nerve conduction studies and somatosensory evoked potentials (SEP). The control group consisted of 30 healthy volunteers matched with the study group regarding age and gender.
A statistically significant difference was found in the mean median motor latency, mean tibial motor latency and mean tibial minimum F latency in the nerve conduction study parameters compared between the patient and control groups.
Results: In the patient group, polyneuropathy was detected in two patients. All of these patients had pure sensory neuropathy. Entrapment neuropathy was found in a total of 12 patients, 10 in the patient group and two in the control group. It was found that 10 of the entrapment neuropathies were carpal tunnel syndrome and two were cubital tunnel syndrome. The frequency of entrapment neuropathy was found to be higher in the patient group than in the control group. In the SEP examination, no difference was detected in P37 latency, P37-N45 amplitude and P37-TP latency between the two groups. No significant difference was observed in SEP parameters in the presence of SSA antibodies. There was no significant difference in P37 latency in the presence of SSB antibody, but the mean P37-N45 amplitude was significantly different in the patient group compared to the control group.
Conclusion: Sjögren's syndrome may have sensory involvement. It can be said that sensory involvement is due to peripheral nerve involvement and that sensory fibers in the spinal cord and spinal column are relatively spared. Electrophysiologic investigations provide a noninvasive objective measure of sensory involvement in Sjögren's syndrome.

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120 – P3.01.02

Insights from a Non-Human Primate Study: Understanding the Fluid Dynamics of Neurovascular and Immunological Factors in the Vulnerability of Substantia Nigra Dopaminergic Neurons

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Purpose: The purpose of the study was to investigate the contribution of glial cell activation and immune cell infiltration to the selective vulnerability of ventral dopaminergic neurons within the midbrain in Parkinson's disease (PD).
Methods: The study employed a non-human primate model of PD, induced by administering 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) systemically. Assessment of glial cell activation and immune cell infiltration was conducted in the midbrain of MPTP-treated monkeys, alongside the evaluation of structural characteristics of the vasculature in specific midbrain regions.
Results: MPTP-treated monkeys exhibited significant microglial and astroglial activation in the whole midbrain, without major sub-regional differences. Additionally, the ventral substantia nigra displayed a higher level of vascularization compared to other areas. Interestingly, a substantial increase in the infiltration of both T- and B-lymphocytes was observed exclusively within the ventral substantia nigra.
Conclusion: The elevated vascular density in the ventral area of the substantia nigra could play a significant role in the specific vulnerability of dopaminergic neurons in the midbrain. Additionally, the heightened infiltration of T- and B-cells, alongside other molecules or toxins, might also contribute to the increased susceptibility of dopaminergic neurons in Parkinson's disease.

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139 – P3.01.03

Peripheral Natural Killer cells profiling in Progressive Supranuclear Palsy and Parkinson's disease.

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Purpose: Recently neuropathologists have illustrated how distinct neuroimmune proteins might underlie unique mechanisms in neurodegenerative condition. Progressive supranuclear palsy (PSP) is a parkinsonism characterized by pathologic deposit of the tau protein in different brain anatomic areas and cell types. A deep analysis of immune signature of anterior cingulate cortex of PSP patients makes Natural Killer (NK) cells a highly relevant cell types to study in this context. Indeed, while in Parkinson's disease (PD), an alpha-synucleinopathy characterized by the loss of nigral dopamine neurons and the accumulation of Lewy bodies, NK cells can effectively clear α-synuclein, the impact of peripheral immune cells on PSP and the exact role of NK cells is elusive in this field. This study is focused on the phenotyping of NK cells in peripheral blood from patients diagnosed with PSP and PD for comparison.
Methods: Peripheral blood mononuclear cells(PBMCs) were extracted by Ficoll density gradient from peripheral blood of patients diagnosed with PSP (n=8) or PD (n=8) and healthy donors HD (n=8). PBMCs were stained with mAb against human canonical markers of NK cells to characterize cell subsets in the different conditions through FACS analysis. Plasma cyto-chemokines profile was also established.
Results: PSP patients showed a lower frequency of CD3-CD56+ NK cells (1,35%±0,98, p= 0,0234) with respect to healthy donors (2,97%±1,23) and PD patients (2,03%±0,86). Considering the NKs subsets distribution, a selective decrease of $CD56^{bright}CD16^{-/dim}$ cells was found in the periphery of PSP but not PD patients. Furthermore, exclusively in PSP patients, $CD56^{bright}CD16^{-/dim}$ cells displayed higher surface levels of chemokine receptor CX3CR1. On the contrary, T cell frequency was not affected both in people with PSP and PD compared to HD. Finally, a statistically significant elevation in the concentrations of CCL2/MCP-1, CXCL8/IL-8, and IL-1β is noted in plasma of PSP but not in PD *versus* healthy donors.
Conclusions: The selective plasma profile and the low frequency of $CD56^{bright}CD16^{-/dim}$ cells, putatively recruited to the sites of inflammation, highlight for the first time that peripheral NK cells could be implicated in the pathophysiology behind PSP and could have a promising value in discriminating tauopathies from other neurodegenerative processes.

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192 – P3.01.04

The role of transient receptor potential channel subfamily M member 7-kinase in a multiple sclerosis mouse model

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Purpose: The transient receptor potential channel subfamily M member 7 (TRPM7), is a cation channel with a C-terminal fused serine/threonine kinase. It is strongly linked to immune cell function, as an important signaling protein in lymphocytes. Recently, TRPM7 kinase activity was shown to be essential for the differentiation of proinflammatory T_H17 cells. Here we tested if the TRPM7 kinase is involved in the development of the autoimmune disease Multiple Sclerosis (MS.) MS is characterized by inflammation and demyelination in the central nervous system (CNS), and T_H17 cells are known to be important drivers of pathogenesis.
Methods: To investigate whether and to what extent the TRPM7 kinase plays a role in the development and progression of MS, we use an animal model of MS, the experimental autoimmune encephalomyelitis (EAE), in our established mouse line carrying a point mutation in the active site of the kinase (*Trpm7$^{R/R}$*).
Results: We were able show that the TRPM7 kinase promotes the development EAE. We found a significantly milder course of passive EAE in *Trpm7$^{R/R}$* mice compared to *Trpm7$^{+/+}$* controls with reduced incidence, score and weight-loss. We found an increased number of FoxP3$^+$ T-cells in the CNS of *Trpm7$^{R/R}$* mice exhibiting symptoms, and fewer infiltrating T_H17 cells in the CNS compared to *Trpm7$^{+/+}$* mice with and without symptoms. In addition, we found that *Trpm7$^{R/R}$* cells require IL-1β to be able to differentiate into T_H17 cells. Significantly less Trpm7$^{R/R}$ T-cells differentiated into T_H17 without IL-1β comparted to Trpm7$^{+/+}$ with and without IL-1β, respectively. And regarding the possible translation from mice to humans, pilot recordings from cerebrospinal fluid (CSF) lymphocytes, derived from MS patients show prominent TRPM7 currents.
Conclusion: Our results indicate that the TRPM7 kinase activity promotes EAE in mice. The inactivation of kinase activity may represent a promising therapeutic strategy in the treatment of MS.

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240 – P3.01.06

Anti-Caspr2 Autoantibodies and Abnormal Prostate Specific Antigen Levels: A Novel Biomarker for Prostate Cancer?

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Purpose: Contactin-associated protein 2 (Caspr2) plays a crucial role in the organization of the potassium channel Kv1 at the juxta-paranodal nodes of myelin axons. The presence of anti-Caspr2 autoantibodies (anti-Caspr2-Ab) is associated with peripheral pain hypersensitivity and disrupts Caspr2 function in the hippocampus.
Methods: We report four cases of middle-aged male individuals exhibiting high titers (1/100) of anti-Caspr2-Ab and abnormal prostate-specific antigen (PSA) levels.
Results: All patients presented with neurological symptoms, including ataxia, aphasia, memory loss, neuropathic pain, polyneuropathy, or insomnia, consistent with autoimmune encephalitis. One patient was diagnosed with prostate adenocarcinoma (PA), while the remaining three were diagnosed with benign prostatic hyperplasia (BPH).
Conclusion: Anti-Caspr2-Ab has been linked to various neoplastic conditions, with thymoma being the most common. Clinical cases exhibiting positive anti-Caspr2-Ab and elevated PSA levels, indicative of BPH or PA, are increasingly observed. Additionally, BPH has been associated with an elevated risk of developing PA. Therefore, the unexpected presence of anti-Caspr-2-Ab in middle-aged male patients presenting neurological symptoms could signify emerging prostatic pathologies, suggesting these autoantibodies as potential sentinel markers warranting close monitoring.

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241 – P3.01.07

Mycophenolate Mofetil for First-Line Treatment of Anti-NMDA Receptor Encephalitis: A Randomized Clinical Trial

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Purpose: Anti-N-methyl-D-aspartate receptor encephalitis (NMDARE) is the most prevalent and severe type of autoimmune encephalitis with heavy economic and social burden. Recommended first-line treatment for NMDARE is intravenous immunoglobulin (IVIg), high-dose glucocorticoids, or plasma exchange (PLEX), but variable responses, long term relapse risk, and side effects are serious disadvantages.
Methods: In this multicenter, open-label, randomized, controlled trial conducted in West China, we assigned patients with NMDARE, in a 1:1 ratio, to first-line treatment only (standard care) or combined first-line treatment plus long-term mycophenolate mofetil. The primary efficacy outcome was the number of patients with relapse and the difference in the time to relapse, assessed in a per-protocol analysis. Secondary outcomes were response rates, long-term profile of disease outcome, side effects, and serious adverse events.
Results: A total of 100 patients with NMDARE underwent randomization (52.0% female; mean age, 21 years [range 14 to 60]) and were followed for at least 2 years after beginning trial treatment. The mycophenolate mofetil plus group experienced few disease relapse (hazard ratio, 4.18; 95%CI, 1.49-11.65; P=0.006), better treatment response (94.12% patients achieving ≥1 point mRS score improvement within 4 weeks after randomization, P=0.03), greater prognosis (88.23% of patients having mRS score less than 2 point at 12 months after randomization vs. 63.26%; P=0.04), and favored long-term outcome profile including less physical fatigue than the first-line treatment-only group. We found no evidence of a difference between the groups in time of standard tapering prednisone, extent of IgG reduction, or treatment side effects, including infection.
Conclusion: The adjunctive of mycophenolate mofetil to first-line treatment of NMDARE resulted in a lower risk of relapsed, better outcome, and well tolerated. Future studies of large sample size are needed to further understand the quality-of-life outcomes and health economics profile of mycophenolate mofetil.

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307 – P3.01.08

Determining the Relationship between NLRP3 Inflammasome Activation and Mitophagy in a Neuronal Progenitor Cell Line Derived Alzheimer's Disease Model

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Purpose: Alzheimer's Disease (AD) is an irreversible neurodegenerative disease characterized by the formation of amyloid (αβ) plaques and neurofibrillary tangles consisting of hyperphosphorylated tau protein. Previous studies have show that aβ accumulation and tau tangles cause oxidative stress and inflammation in the brain. In our current study, we aimed to evaluate the relationship between mitophagy and NLRP3 inflammasome in a neuronal progenitor cell line derived *in vitro* AD model.
Methods: In our study, an *in vitro* AD model bearing Swedish and Indiana mutations was established by viral transduction. Transduced cells were positively selected with fluorescence-activated cell sorting. Cells underwent neuronal differentiation were characterized according to their aβ40/42 expressions and tau phosphorylation. Oxidative stress was evaluated with flow cytometry. NLRP3 inflammasome components and mitophagy markers were evaluated at the gene and protein levels with qPCR and flow cytometry, respectively.
Results: Our results indicated that in the AD model group, an increase in oxidative stress and autophagy was detected following aβ accumulation and the formation of neurofibrillary tangles, in addition to a significant increase in the autophagy marker LC3B and PINK and PARKIN proteins involved in mitophagy. Autophagy was also confirmed by confocal microscopy. However, despite the increase in NLRP3 levels, no significant increase was detected in the levels of ultimate pyroptosis markers; caspase-1, IL-1β and IL-18.
Conclusion: Mitophagic activation positively correlated with the characteristic features of AD is consistent with the literature. However, although *in vivo* studies have shown the therapeutic importance of suppressing NLRP3 and pyroptosis in AD, NLRP3 inflammasome activation was not observed in the *in vitro* model established in our study, suggesting that NLRP3-mediated pyroptosis in AD cases may not be simulated in neuronal culture due to the lack of glial cells. Moreover, our findings underline the necessity of further research on neuron-derived extravesicular NLRP3 release.

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313 – P3.01.09

Differentiation between viral and autoimmune limbic encephalitis (D-VALE): a prospective multicentre cohort study with development and validation of diagnostic model

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Purpose: We aimed to develop and validate a diagnostic prediction model to differentiate viral and autoimmune limbic encephalitis (ALE).
Methods: The model was based on data from a prospective observational multicentre study, which enrolled patients who identified autoimmune encephalitis (AE) and viral encephalitis (VE) (ChiCTR1800019762) at four large comprehensive hospitals in western China. The derivation cohort was from one center, and the external validation cohort was from the others. The demographic data, clinical features, and laboratory test results were collected and subjected to logistic regression analyses. The diagnostic model was displayed as a web-based nomogram and then modified into a novel scored prediction tool. Model performance was assessed for discrimination and calibration in both derivation and external validation cohorts.
Results: A total of 2423 individuals were recruited, and 1001 (496 VE, 505 ALE) patients were included. Based on the derivation cohort (n = 777, 389 VE, 388 ALE), the model was developed with eight variables including age at onset, acuity, fever, headache, nausea/vomiting, psychiatric or memory complaints, status epilepticus, and CSF white blood cell count. The classification threshold of a predicted probability of 0.570 showed good discrimination and calibration in both derivation cohort (area under the receiver-operating curve [AUC] 0.890; 0.868-0.913) and external validation (n=224, 107 VE, 117 ALE, AUC 0.872; 0.827-0.917) cohorts. The scored prediction tool based on this model had a total point that ranged from -4 to 10, with a cutoff value of 5 points, showing good discrimination and calibration in both derivation (AUC 0.885, 0.863-0.908) and external validation (AUC 0.868, 0.823-0.913) cohorts.
Conclusion: The D-VALE model (Differentiation between Viral and Autoimmune Limbic Encephalitis) provides a reliable and user-friendly tool for differentiating between the VE and ALE, which would benefit early diagnosis and appropriate treatment and alleviates economic burdens on both patients and society.

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315 – P3.01.10

Lipid-accumulating microglia in Alzheimer's disease

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Lipid-accumulating macrophages (LAMs) are distributed among several tissues, such as the white adipose tissue and the brain, where they accumulate lipids present in the tissue microenvironment. LAMs exhibiting impaired phagocytotic activity, excessive reactive oxygen species (ROS) production, and pro-inflammatory secretion have been described in aged and diseased brains, e.g., in neurodegenerative disorders such as Alzheimer's disease (AD). Whether these dysregulated brain LAMs promote or mitigate AD progression remains unclear. Here, we investigated the potential role of LAMs in AD using a novel transgenic mouse model showing a phenotype of reduced lipid-droplet (LD) formation in microglia (*CX3CR1*^{CreERT2}*Fit2*$^{fl/fl}$ mouse lines). Microglia impaired in LD formation showed perturbed lipid-related gene expression, reduced LD size, increased phagocytosis and efferocytosis compared to WT microglia. Furthermore, mice with Fit2 deficiency in microglia showed decreased Aβ load in the brain. Our observations constitute a step forward in understanding whether lipid accumulation in microglia affects the progression of neurodegenerative disorders.

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559 – P3.01.11

Comparison of immune response in different experimental models of retinal degeneration

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Purpose: During retinal degenerative diseases, activated microglia and macrophages represent one of the main populations supporting the local harmful inflammatory reaction and the induction of angiogenesis. Thus, focus on the modulation of immune response could provide promising innovation in the treatment of retinal pathologies. The application of sodium iodate ($NaIO_3$) is widely used approach for the induction of experimental retinal degeneration in mice and rats. The process of degeneration of retinal cells by $NaIO_3$ is well documented, but there is a limited information about the mechanism of immune response in the retina. For this reason, we analyzed the local immune reaction in the four types of induced retinal degeneration in mice at the different time points.
Methods: We compared infiltration of the retina by immune cells in the model of chronic retinal degeneration (induced by repeated intraperitoneal application of 25 mg/kg $NaIO_3$), model of acute retinal degeneration (induced by single intraperitoneal application of 40 mg/kg $NaIO_3$), direct degeneration of retina (induced by a single intravitreal application of $NaIO_3$) and a model of retinal inflammation (induced by the intravitreal application of IFN-γ, IL-17 and TNF-α). Retinas were examined on day 2, 7, 14 and 21 after induction of degeneration.
Results: Our results show that the majority of immune cells infiltrating the retina were $CD11b^+$ and $F4/80^+$ (macrophages/microglia) and $CX3CR1^+$ $P2RY12^+$ (specific combination of markers for microglia). There were differences in the range of immune cell infiltration among the experimental models at the different time points. In addition, the activation of immune reaction was imprinted in the submandibular lymph nodes (which are drainage for eyes). We have shown that the numbers of activated B-lymphocytes ($CD19^+CD40^+$ cells) and activated T-lymphocytes ($CD3^+CD25^+$ cells) were increased in the lymph nodes of mice with degenerated retina.
Conclusion: Our results provide valuable information about the mechanism of immune reaction in the retina, which should be considered in the use of experimental model of induced retinal degeneration.

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579 – P3.01.12

NADPH oxidase (NOX2)-mediated regulation of microglial NLRP3 inflammasome activation in traumatic brain injury

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Background: NADPH oxidase 2 (NOX2) is an enzyme complex responsible for phagocytic reactive oxygen species (ROS) production. Chronic NOX2 expression induces oxidative stress, drives neuroinflammation and leads to progressive cortical and hippocampal degeneration. NOX2 also acts as a priming signal for NLRP3 inflammasome activation which has been implicated in many neurodegenerative diseases such as Alzheimer's Disease, Multiple Sclerosis, and plays a pivotal role in secondary traumatic brain injury (TBI). GSK2795039 is a small molecule, brain penetrable drug that inhibits NOX2 in an NADPH competitive manner. The goal of this study was to investigate the therapeutic potential of GSK2795039 in models of microglial activation *in vitro* and to translate findings to an experimental TBI model in mice.
Methods: Immortalised Microglial (IMG) or primary microglia from p1 Wistar rat pups were pre-treated with GSK2795039 or MCC950 (NLRP3 inhibitor) and stimulated with lipopolysaccharide (LPS) and nigericin to induce NOX2/ROS and NLRP3 inflammasome activation. ROS and cell viability were measured using CM-H2DCFDA and MTT assays, respectively, while conditioned media was analysed for cytokines by ELISA, and lactate dehydrogenase (LDH) to measure pyroptosis. Protein expression of NLRP3, cleaved-Caspase-1, cleaved-IL-1β and ASC were assayed by Western immunoblot.
Results: Our *in vitro* studies demonstrated that GSK2795039 attenuated LPS/nigericin-induced microglial NOX2 activity, ROS, LDH, IL-1β and IL-18 release, as well as NLRP3 and cleaved-caspase-1 expression. *In vivo* studies using controlled cortical impact in adult male C57Bl/6J mice and multi-dimensional flow cytometry demonstrated increased infiltration of NOX2/ROS/Caspase-1/IL-1β^+ inflammatory monocytes compared to sham. Systemic administration of GSK2795039 (100mg/kg; IP) starting at 2 hours post-injury attenuated NOX2$^+$/IL-1β^+ microglia/macrophage activation. In addition, GSK2795039 reduced numbers of IL-1R$^+$CD4$^+$ and IL-1R$^+$CD8$^+$ T cells indicating that microglial-T cell crosstalk was altered by treatment. These neuroimmune changes were associated with improved motor function recovery post-TBI.
Conclusions: These translational studies indicate that GSK2795039 may be a promising therapeutic drug for mitigating the damaging effects of NOX2-mediated neuroinflammation in microglia, and peripheral immune cells, following experimental TBI in mice.

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662 – P3.01.13

The secretome of human amniotic membrane-derived stromal cells inhibits immune cell migration into a pro-inflammatory miPSC-derived neurospheroid model.

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Purpose: Neuroinflammation, a response to trauma, infection or neurodegenerative disease, involves both resident innate immune cells such as microglia but also adaptive immune cells that are actively and constantly recruited, triggering a loop that fuels the neuroinflammatory process. Mesenchymal stromal cells from the amniotic membrane and their secretome (CM-hAMSC) are capable of fostering the polarization of immune cells by triggering the acquisition of features that are typical of M2 macrophages and T regulatory cells. However, in the context of neuroinflammation, the effect CM-hAMSC on the recruitment of inflammatory immune cells into an inflammatory brain environment remains to be defined.
Methods: In this study, a murine C57BL/6 iPSC-derived neurospheroid (NSPH) cell culture model containing neurons and astrocytes was applied to study the migratory capacity of syngeneic splenocytes into NSPHs upon pro-inflammatory stimulation. Hereto, NSPHs were stimulated with IL-1β, IFN-γ and TNF-α, while splenocyte activation was induced with LPS. Following 3 or 10 days of splenocyte migration, NSPHs were fixed, embedded in OCT, cryosectioned, and subjected to immunofluorescence staining for immune infiltrate identification. Additionally, the effect of CM-hAMSC treatment was studied by two approaches: either a single administration of high-concentration or three consecutive administrations of lower concentration.
Results: Already 3 days after the initiation of NSPH/splenocyte co-cultures, activated splenocytes effectively infiltrated cytokine-primed NSPHs, which was further extended into deeper NSPH layers by day 10. Further immunocytochemical characterisation demonstrates that Iba-1+ monocytes and CD4+ T lymphocytes are the predominant migrating subpopulations, while CD19+ B lymphocytes exhibited limited invasion. NK1.1+ NK cells and CD8+ T cells displayed minimal invasion. Most remarkably, both tested approaches with CM-hAMSC treatment displayed a high reduction in the number of infiltrating immune cells, particularly evident in the innermost regions of the NSPHs being completely devoid of splenocyte infiltration.
Conclusion: Using a miPSC-derived NSPH model, we demonstrate the potency of CM-hAMSC to inhibit syngeneic immune cell migration into a brain-like environment. This positions CM-hAMSC, or still to be defined factors therein, as a potential therapeutic candidate to counteract detrimental immune cell migration into numerous brain pathologies. Consequently, our research is now extending these findings using human (iPSC-derived) cell culture models.

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685 – P3.01.14

Dietary manipulation to control immunological self-tolerance in Experimental Autoimmune Encephalomyelitis

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Background and purpose: Several studies highlighted the link between metabolic pressure and Multiple Sclerosis (MS) pathogenesis. Obesity and dyslipidemia are associated with a higher risk of onset and worse progression of MS. Excess of nutrients hyper-activates the nutrients/energy sensing mTOR pathway, leading to a decreased number of regulatory T (Treg) cells and expansion of Th17 cells, leading to an imbalanced immunotolerance. Here we investigate the impact of specific nutrient overload (lipids, carbohydrates or proteins) on immune-homeostasis and on the pathogenesis of Experimental Autoimmune Encephalomyelitis (EAE) in mice.
Methods: Mice were fed the following dietary regimens: standard diet (SD), High-fat diet (HFD), high protein diet (HPD) or high-carbohydrates diet (HCD). Mice were immunized with MOG_{35-55} peptide to induce EAE. Immune infiltration in CNS and immune asset in periphery have been analyzed by flow cytometry. A panel of Th1/Th17/Treg cytokines was dosed by Luminex both in plasma samples and in supernatant of splenocyte cultures. We tested the capacity of Treg cells to suppress the proliferation and pro-inflammatory cytokine production of conventional T cells (Tconv) in co-culture assays.
Results: HFD and HCD mice displayed a worse progression of EAE than SD and HPD groups, as indicated by clinical parameters (weight loss, clinical score), enhanced CNS immune cell infiltration (measured as $CD45^{high}CD3^{+}$ cell percentage) and increased serum pro-inflammatory cytokines. At the peripheral level, $CD4^{+}$ and $CD8^{+}$ T cells from HFD and HCD mice exhibited a more activated and proliferative profile than the other groups, with a polarization toward a pro-inflammatory profile when they were *in vitro* re-challenged with MOG_{35-55} peptide, as testified by higher levels of several pro-inflammatory cytokines (IFN-g, IL-6, Leptin) and lower levels of anti-inflammatory cytokines (IL-4, IL-10) in supernatant of splenocyte cultures. Moreover, Treg cells of not immunized mice from HFD and HCD groups displayed a reduced capacity to suppress Tconv proliferation and pro-inflammatory cytokine production in co-culture assays.
Conclusions: Metabolic overwork derived by an excess of lipids or carbohydrates induces an increased inflammatory state in periphery and an impairment of Treg cell suppressive function, leading to an exacerbated EAE progression.

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700 – P3.01.15

Uncovering the brain-homing nature of T cells at single-cell resolution in people with multiple sclerosis

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Purpose: Distinct $CD4^+$ and $CD8^+$ memory T-cell subsets have been implicated in the pathogenesis of multiple sclerosis (MS). We previously showed that Th17.1 and $CD20^{dim}$ $CD8^+$ T cells are enriched in the MS brain and are associated with a brain-homing phenotype. As these specific T-cell populations also exist in healthy individuals, we hypothesize that Th17.1 and $CD20^{dim}$ CD8+ T cells consist of different subpopulations, of which some have both pathogenic and brain-homing properties to contribute to MS.
Methods: To perform deep profiling of these T-cell subsets, we have optimized a scRNA-seq workflow using the 10x Genomics pipeline that includes analysis of several proteins by CITE-seq and T-cell receptor repertoire on enriched rare circulating subsets. Furthermore, we have set-up protocols for isolating and performing scRNA-seq on single-cell suspensions from post-mortem donor CNS compartments.
Results: From peripheral blood of MS patients prior and after treatment with natalizumab (NTZ; n=6) and healthy donors (n=3), we yielded ~15k Th17.1 cells for subsequent scRNA-seq analysis. We observed several clusters with a distinct effector memory program within Th17.1 cells, including three clusters with cytotoxicity-associated features. Interestingly, one of these clusters was defined by both *EOMES* and *PRF1* (perforin) expression and reduced in all paired post- versus pre-NTZ samples. Another cluster with a central memory profile was seemingly increased upon NTZ treatment and characterized by genes associated with functional programming of IL-17-expressing cells (*NR3C2*, *KDM6A* and *PRKCA*). From post-mortem MS brain donor material, we yielded approximately 3k T cells from brain tissue (meninges, normal-appearing white matter (NAWM) and lesion). Thus far, we found clonally expanded T-cell populations within several compartments including clonal expansion of $CD20^{dim}$ CD8+ T cells in NAWM tissue.
Conclusion: In summary, we found that Th17.1 cells consist of several subpopulations based on their RNA profile and that subset composition changes upon NTZ therapy. We also found clonally expanded populations of *ex vivo* $CD20^{dim}$ CD8 T cells from MS brain as compared to other compartments.

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702 – P3.01.16

Loss of Bmal1 in myeloid cells accelerates age-related migration of immune cells to the outer retina.

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Age is the primary risk factor for the development of Age-Related Macular Degeneration (AMD). One of the key hallmarks of AMD is an increased incidence of peripheral immune cells into the retina, which are hypothesised to have a pro-inflammatory phenotype.
Previous reports have demonstrated that loss of Brain Muscle Arnt-like protein (Bmal1) in myeloid cells results in a hyperinflammatory phenotype. Bmal1 is the master clock gene, its deletion ablates circadian rhythmic activity. As humans age, the amplitude of circadian rhythmicity lessens, and notably Bmal1 knockout mice present with an accelerated ageing phenotype.
We hypothesised that specific knockdown of Bmal1-/-in myeloid cells may provide insights into the role of inflamm-ageing of peripheral myeloid immune cells in the development of retinal degeneration.
Bmal-/-LysMCre+ and Bmal+/+LysMCre+ mice were aged to 18-months. Their retinal health was examined using Optical Coherence Tomography (OCT) at 3, 6, 12 and 18-months. Retinal tissues were prepared for protein, cryosection and flatmounts and examined using H&E staining and immunofluorescence (IF) for mononuclear phagocyte markers. Bone marrow derived macrophages (BMDMs) were isolated and examined for baseline activity and following stimulation with TLR ligands. Serum was collected for circulating cytokines and assessed using ELISA's and multiplex methods. Additionally, immune cells from patients with AMD were assayed for circadian rhythm amplitude and features of inflamm-ageing.
At the 12- and 18-month timepoints, OCT demonstrated an increase in the number of infiltrating cells in Bmal-/-LysMCre+ compared to Bmal+/+LysMCre+ controls. This was confirmed using IF staining on retinal tissue, with significantly more F4/80+ and Iba-1+ cells in the retina of Bmal-/-LysMCre+ mice. Furthermore, BMDMs isolated from Bmal-/-LysMCre+ mice had an enhanced pro-inflammatory phenotype compared to Bmal+/+LysMCre+ controls. Immune cells isolated from patients with AMD had increased levels of the DNA-damage marker γH2AX compared to aged-matched healthy patients.
The increased number of infiltrating immune cells in the Bmal-/-LysMCre+ mice confirms that deletion of Bmal1 from myeloid cells drives a clock-regulated age-dependent migration of immune cells into the retina. This model strongly supports a role for the peripheral immune system in the underlying mechanism of AMD.

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718 – P3.01.17

Early-to-mid stage idiopathic Parkinson's disease shows enhanced cytotoxicity and differentiation in CD8 T-cells in females

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Parkinson's disease (PD) is the second most common neurodegenerative disease, affecting approximately 10 million people worldwide. Neuroinflammation in the brain contributes to the pathogenesis of PD, but the potential dysregulation of peripheral immunity has not been systematically investigated for idiopathic PD (iPD). Here we showed an elevated peripheral cytotoxic immune milieu, with more terminally-differentiated effector memory (TEMRA) CD8 T, $CD8^+$ NKT cells and circulating cytotoxic molecules in fresh blood of 28 patients with early-to-mid iPD aged 60-70 years vs 24 age- and sex-matched HC, after analyzing >700 innate and adaptive immune subsets or functional features. This profile, also reflected by fewer $CD8^+FOXP3^+$ T cells, was confirmed in another subcohort. Although PD is a male-dominant disease, our observation is particularly true for female patients. Co-expression between cytotoxic molecules was selectively enhanced in CD8 TEMRA and effector memory (TEM) cells. Single-cell RNA-sequencing analysis demonstrated the accelerated differentiation within CD8 compartments, enhanced cytotoxic pathways in CD8 TEMRA and TEM cells, while CD8 central memory (TCM) and naïve cells were already more-active and transcriptionally-reprogrammed. Interestingly, the frequency of CD8 TEMRA was negatively correlated with disease duration, suggesting a contribution to PD pathogenesis. In line with our recent observation of 'younger' immune system in PD patients and rodent models with loss-of-function mutations in a specific familial PD gene, these findings suggest that specific CD8 T cell differentiation process was disturbed in early-to-mid stage iPD. Our work provides a comprehensive map of dysregulated peripheral immunity in iPD, proposing candidates not only for early diagnosis, but also for early intervention before being too late for the still-incurable ageing-associated disease.

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736 – P3.01.18

IL-34 empowers regulatory T cells with novel non-canonical function to safeguard brain barrier integrity in neuroinflammation.

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Purpose: In the pursuit of strategies to address brain damage, brain-associated regulatory T cells (Tregs) have garnered increasing attention in recent years. Beyond their established role in immunoregulation, Tregs have emerged as significant contributors in the response to brain trauma and the restoration of damaged brain tissue in neuroinflammatory diseases. Here, we report a previously undescribed, non-canonical function of Tregs in preserving the integrity of both the blood-brain barrier (BBB) and the blood-cerebrospinal fluid (CSF) barrier (BCSFB).
Methods: Using fluorescently labeled dextrans, BBB and BCSFB integrity was assessed in *in vivo* and *in vitro* settings. Furthermore, using flow cytometry, investigations into disturbances in IL-34 expression in Tregs were conducted on samples from people with multiple sclerosis (MS), Alzheimer's disease (AD), and mild cognitive impairment (MCI). In addition, the therapeutic potential of IL-34 in the experimental autoimmune encephalomyelitis (EAE) and amyloid-β precursor protein (APP) mouse model was explored.
Results: Our results have established that Tregs are crucial for the maintenance of BBB and BCSFB integrity *in vivo*. Additionally, we have identified the cytokine IL-34 as a pivotal factor in this newfound role of Tregs. Mechanistically, IL-34 influences the expression and localization of the tight junction protein ZO-1 in BBB endothelial cells and choroid plexus epithelial cells, reinforcing the integrity of the brain barriers.
Considering the established phenomenon of compromised brain barriers and the involvement of immunological elements in neurological conditions, we observed reduced IL-34 expression in Tregs derived from MS, AD, and MCI patients. Furthermore, our study unveils the potential of IL-34 therapy in restoring the integrity of brain barriers in MS and AD animal models.
Conclusion: These discoveries illuminate the intricate interplay between Tregs, IL-34, and the maintenance of brain barrier integrity, opening up novel avenues for therapeutic interventions aimed at alleviating brain barrier dysfunction in the context of neuroinflammatory disorders.

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771 – P3.01.19

Neurotransmitter acetylcholine produced by CD4 T cells drive the differentiation of regulatory CD4 T cells during gut inflammation

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Purpose: The parasympathetic nervous system extensively uses acetylcholine (ACh) as the neurotransmitter. The choline acetyltransferase (ChAT) enzyme helps the production of acetylcholine in neuronal and non-neuronal cells. How do neurotransmitter ACh produced by CD4 T cells affect differentiation and function of effector and regulatory CD4 T cells during gut inflammation and autoimmunity?
Methods: Various subsets of CD4 T cells (Th1, Th2, Th17, and Tregs) were purified from PBMC of inflammatory bowel disease (IBD) patients or non-IBD control individuals using a flow cytometry sorter. In mice, acute colitis was induced by dextran sodium sulfate (DSS) in drinking water. RT-PCR, flow cytometry, and immunofluorescence microscopy were used to detect expression of molecules.
Results: IBD patients (n=10) had significantly higher ChAT-expressing Th17 cells ($CD4^+CXCR3^-CCR6^+$ cells), whereas lower Th2 ($CD4^+CD294^+$ cells) and Tregs ($CD4^+CD25^+CD127^{low}$) as compared to the control individual (n=13). Colonic biopsies of IBD patients showed a significantly decreased ChAT expression and were strongly correlated with serum acetylcholine levels. DSS treatment with ChAT-eGFP transgenic mice showed significantly reduced $CD4^+eGFP^+$ T cells in the gut-associated lymphoid tissues (GALTs). Further, DSS-treated $ChAT^{fl/fl}CD4^{cre}$ mice (CD4 T cells deficient of ChAT) showed an increased colitic disease severity and had a significantly increased frequency of Th1 and Th17 cells and lower Th2 and Tregs in the GALTs compared to control mice. CD4 T cells showed an increased GM-CSF, IL-17A, and decreased IL-10 and IL-4 expression in the CD4-$ChAT^{-/-}$ mice compared to CD4-$ChAT^{+/+}$ mice during colitis.
Conclusion: Our results showed that autocrine production of ACh by CD4 T cells reduces the differentiation of Th1 and Th17 cells and promotes Treg differentiation, suggesting the importance of the non-neuronal cholinergic system in controlling gut inflammation and autoimmunity.

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855 – P3.01.20

Immune-related soluble factors associated with anti-LGI1 encephalitis

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Purpose: Understanding the cytokine profiles associated with anti-LGI1 encephalitis could provide valuable insights into disease pathogenesis, phenotypic variability, and new therapeutic targets. This study aimed to investigate the levels of 28 immune-related soluble factors in a cohort of patients with anti-LGI1 encephalitis.
Methods: We included 55 patients diagnosed with anti-LGI1 encephalitis. Overall, 90 samples (42 cerebrospinal fluid [CSF] and 48 sera; 34 of them CSF-serum paired) obtained during the acute phase of the disease, before immunotherapy, were included in the study. 24 serum samples and 21 CSF samples from age-matched participants with non-inflammatory neurological diseases served as controls. Levels of sCD40L, Fraktalkine, G-CSF, GM-CSF, IFNγ, IL-1α, IL-1β, IL-1RA, IL-6, IL-8, IL-10, IL-12 (p40), IL-17A, IL-18, IL-22, IP-10, MCP-1, MIG, TNFα, APRIL, BAFF, BCA1, Granzime, IL-21, IL-23, IL-35, SDF1, MIP3A, 6-KINE and Lymphotactine were quantified with a Milliplex® custom MAP Human Cytokine/Chemokine/Growth panel and analyzed with LUMINEX® xMAP100. Clinical data comprising demographic information, neurological symptoms, disease duration and severity of the disease were also collected.
Results: Our analysis revealed significant alterations in the levels of several inflammatory cytokines in patients with anti-LGI1 encephalitis. Specifically, CSF levels of Fraktalkine, IL-10, Il-6, IP-10 /CXCL10, MIG, TNFa, APRIL and IL8, and serum levels of IL-10, Il-18 and IL-1RA were significantly increased in the anti-LGI1 encephalitis cohort compared with HC. Moreover, the ratio of IL-6 level between CSF and serum was markedly elevated in LGI-1 patients with a more severe disease according to modified ranking scale (mRS). Patients with a mRS ≥3 (n=19) had a median IL-6 ratio of 1.67 pg/mL [IQR 0.50-8.20] compared to 0.37 pg/mL [IQR 0.08- 1.09] of those with mRS <3 (n=13) [p=0.0068].
Conclusion: Our findings help to elucidate the initial mechanisms underlying immune dysregulation. Whereas the increased levels of TNFa and APRIL highlight the relevance of T and B cell interaction, the higher levels of chemokines, such as CXCL10 or Fraktalkine, reinforce the significance of cell migration to the CNS in the development of the disease. These might lead to novel treatment strategies.

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922 – P3.01.21

Investigation of brain inflammation in complete Freund's' adjuvant-free experimental autoimmune encephalomyelitis

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Purpose: Experimental autoimmune encephalomyelitis (EAE) is an animal model of multiple sclerosis, which is typically induced with the use of complete Freund's adjuvant (CFA). As this adjuvant impedes the translation of the results of EAE studies to multiple sclerosis, we developed and characterized a new subtype of the EAE model induced in DA rats without CFA, using whole spinal cord homogenate (SCH) only. Despite the genetic homogeneity of the experimental animals and the controlled environmental conditions, different clinical courses of EAE were observed in the SCH-immunized DA rats: mild, moderate, severe, and lethal. This study aimed to investigate brain inflammation in correlation to clinical expression variability in this model.
Methods: Immune cells from lymph nodes draining the site of immunization were isolated in the inductive phase of the disease, and exposed to myelin basic protein (MBP), myelin oligodendrocyte glycoprotein (MOG), or β-synuclein (βSyn). Levels of IFN-γ and IL-17 in cell culture supernatants were detected by ELISA. Relevant brain structures (pons, cerebellum, hippocampus, and cortex) were isolated in the later stage of the disease (day 24-28 post-induction) and the composition of immune cells present in the structures was determined by flow cytometry. Samples obtained from moderate and severe groups were compared.
Results: Elevated levels of IFN-γ and IL-17 were detected in lymph node cell cultures exposed to MBP, MOG, or βSyn, thus implying that SCH immunization elicited T cell response against each of the antigens. Different immune cells were detected in all brain structures studied, particularly T cells, macrophages, and microglia. The distribution and number of these cells varied between structures, and in moderate vs. severe EAE. Namely, the lowest number and percentage of infiltrates and T cells were observed in the cortex. Further, a higher abundance of infiltrates, $CD4^+CD11bc^+$ and $CD8^+CD11bc^+$ macrophages was observed in severe EAE.
Conclusions: These results suggest that the heterogeneity of infiltration of brain structures in rats may be important for understanding the different clinical outcomes of EAE, making our model a perspective tool for the study of MS pathogenesis where the brain is the major target organ of autoimmunity.

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923 – P3.01.22

Porphyromonas gingivalis as an etiologic factor in neuroinflammation

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Purpose: Alzheimer's disease (AD), the most prevalent form of dementia, represents an unmet challenge in the aging population. AD patients exhibit neuroinflammation consistent with microbial infection, including microglial activation, inflammasome and complement activation as well as altered cytokine profiles. Infectious agents, such as viruses (HSV-1, HIV), bacteria (spirochetes, *Ch. pneumoniae*, and *P. gingivalis*), and fungi have been found in AD patients' brains. The presence of these germs was postulated to be an etiologic factor in AD, but robust evidence of causation has not yet been established. Herein we aimed to delineate the effect of *P. gingivalis,* a keystone pathogen in the development of chronic periodontitis, and its virulence factors on microglia cells, and whether the presence of this bacterium generates hallmark AD phenotypes.
Methods: We assessed the differences in gene expression of proinflammatory markers and cytokines between non-exposed microglia, microglia exposed to wild type *P. gingivalis*, and to mutant *P. gingivalis* (deprived of the key virulence factors gingipains) *via* quantitative PCR. Using mass cytometry, we evaluated *P. gingivalis* and its key proteases', gingipains, impact on the phenotype of microglia by profiling 36 surface and intracellular antigens.
Results: The microglia proinflammatory response to *P. gingivalis* was characterized by strong up-regulation of NOS2, COX2, IL1b and TNFa. We observed gingipains to negatively impact the microglia inflammatory response to *P. gingivalis*. Next, using mass cytometry we observed significant phenotypic shifts in microglia in response to infection with periodontopathogens. For instance, a subpopulation highly expressing CD68 and PD-L1 was four times more abundant upon infection of microglia with the wild-type strain of *P. gingivalis* than the gingipain-incompetent mutant, while almost no cells bearing this characteristic were detected in the absence of infection.
Conclusion: Infection with *P. gingivalis* promotes clustering of microglia into distinct populations, some of which may play a role in the AD pathology. *P. gingivalis'* key virulence factors, gingipains, modulate the response (including the proinflammatory one) of microglia.

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1006 – P3.01.23

Microbiota-induced intestinal Treg cells modulate neuroinflammation

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Purpose: This study aims to investigate microbial metabolites influencing the development of intestinal Treg cells, which can modulate neuroinflammation and enhance long-term recovery in experimental autoimmune encephalomyelitis (EAE), with the goal of translating these experimental findings into clinical applications.
Methods: Metabolomics analysis was performed to identify and quantify metabolites, focusing on short-chain fatty acids (SCFAs) and secondary bile acids. Seventy-five wild-type (WT) mice were divided into three groups: an EAE-induced experimental group, and two control groups—naïve mice and a technical control group receiving complete Freund's adjuvant (CFA) without antigen. Stool samples were collected before EAE induction, at disease onset, peak, and recovery phases. During peak and recovery phases, half of the mice were sacrificed, and tissues including plasma, liver, spleen, stool, ileum, cecum, colon, and central nervous system (CNS) were collected for metabolomic analysis. Additionally, fifty WT mice were divided into the same groups, and tissues were collected for flow cytometry analysis, including mesenteric lymph nodes (mLN), inguinal lymph nodes (iLN), spleen, brain, dura mater, spinal cord, colon, and liver.
Results: The latest experiment showed a significant rise in Treg cells across most organs in the experimental group compared to the naïve group. In addition to activation markers, other Treg markers such as KLRG1, ST2, and RORgt were upregulated in the EAE cohort in many organs compared to the control groups.
Conclusion: The study highlights the involvement of Treg cells and altered microbiota in EAE development. By modulating the gut microbiota to induce immunosuppressive Treg cells, this research aims to understand microbiota-induced alterations in EAE and translate these findings into potential clinical interventions. Additionally, the investigation intends to analyze and compare Treg cell responses in the gut, shedding light on the epigenetic and transcriptional profiles of different Treg cell subpopulations. This comparison will elucidate how the pathogenesis of EAE affects the Treg cell compartment in the gut and the implications of these changes for disease progression.

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1015 – P3.01.24

The Presence of Anti-NMDA Receptor and Anti-GABA-B Autoantibodies in an Elder Patient with Hallucinations

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Purpose: Autoimmune encephalitis is a group of neuroinflammatory disease characterized by neuropsyquiatric symptoms that can be caused by various autoantibodies that affect neuronal cells surface proteins their receptors or even ion channels and some of them are often associated with the presence of tumors or oncological diseases.
Methods: An 80-year-old male patient consulted his neurologist due to a four-month history of visual and sensory hallucinations without cognitive impairment. During the neurological examination, a slight loss of trunk muscle tone was detected, while the rest of the evaluation was unremarkable. Complete blood analysis was performed including autoimmunity testing, revealed the presence of Anti-NMDA receptor antibodies at a 1/100 titration and Anti-GABA-B antibodies at a 1/10 titration. Head and thoracic computed tomography scans were performed, showing frontal-predominant cortical atrophy in the brain, but no signs of neoplasms in the thorax or brain were found. Aripiprazole was initiated, and the patient continued follow-up in neurology consultations.
Conclusion: This case highlights the diagnostic and therapeutic challenges encountered in elderly patients presenting with psychiatric symptoms and the presence of autoimmune encephalitis antibodies.

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1024 – P3.01.25

Anti-MOG Antibody in a Pediatric Patient with Autism Spectrum Disorder

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Purpose: The Autism spectrum disorder is a complex neurodevelopmental pathology that affects behavior, sensory reception, and social communication in humans. When occurring alongside other neurological diseases such as autoimmune disorders, it sets a diagnostic challenge due to overlapping clinical presentations.
Methods: A 6-year-old male patient, diagnosed with a medical history at the age of 2, began experiencing recurrent exanthematous infections and hemorrhagic gingivostomatitis. Shortly after the onset of behavioral changes, including avoidance of eye contact and poor language development, he was diagnosed with autism spectrum disorder. At the age of 6, he was brought to his pediatrician by his mother due to worsening fine and gross motor skills, coordination issues, and recurrent episodes of insomnia over the past year. Physical examination revealed no significant findings other than his underlying pathology.
Results: Comprehensive blood analysis, including autoimmune testing, revealed high serum serotonin levels (752.4 mcg/l) and neuron-specific enolase levels (42.42 mcg/l). Additionally, the presence of Myelin Oligodendrocyte Glycoprotein antibodies at a titration of >1/100 was detected. Treatment commenced with a high dose of intravenous methylprednisolone at 20mg/kg administered in three sessions. After one month of follow-up, there was notable improvement in motor skills and the emergence of new cognitive abilities, such as drawing. Autoimmune tests were repeated, showing a decrease in Myelin Oligodendrocyte Glycoprotein antibody titration to 1/32.
Conclusion: This case highlights the possibility of neurological autoimmune diseases coexisting with autism spectrum disorder, thereby complicating both diagnosis and treatment in these patients.

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1049 – P3.01.26

Temporal analysis of the infiltration dynamics of pro-inflammatory cytokine producing innate and adaptive immune cells following experimental traumatic brain injury in mice

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Background: Traumatic brain injury (TBI) is the leading cause of death and disability in young adults, resulting in severe cognitive and physical disabilities in survivors. After the primary insult, secondary injury follows which is characterized by the activation of inflammatory pathways, leading to the infiltration of immune cells into the brain parenchyma. Pro-inflammatory cytokines such as interleukin(IL)-1 beta (IL-1β), tumor necrosis factor- alpha (TNF-α), IL-6, IL-17, and interferon-γ (IFNγ) significantly contribute to the pathophysiology of TBI, exacerbating neuronal damage and negatively impacting functional recovery. This preclinical study examined the primary cellular sources of pro-inflammatory cytokines following experimental TBI in mice.
Methods: Adult C57BL/6J male mice underwent moderate-level controlled cortical impact or sham surgery. Mice were humanely euthanized and brains were harvested acutely at 3 hours (h), 6h, 12h, 24h post injury, sub-acutely at 3, 10 days post-injury (DPI), or chronically at 28 DPI. Mononuclear-cells were obtained by percoll density gradient and FACS stained to identify infiltrating innate and adaptive immune cells in the brain (Neutrophils, Monocytes, Dendritic cells, T-cell subsets (CD4+, CD8+, TCRγδ+)), as well as levels of intracellular cytokine production (IL-1β, TNF-α, IL-6, IL-17 and IFN-γ).
Results: Following TBI, neutrophils rapidly infiltrated the injured brain as early as 3h post-injury, with monocytes and dendritic cells following at 6h post-injury; all innate immune cells produced IL-1β, IL-6, TNF-α. In contrast, T cell infiltration peaked at 10 DPI and persist through 28 DPI. Notably, IL-17 production was observed at 3 DPI and peaked at 10 DPI, and was mainly produced by γδ T cells. IFN-γ was primarily produced by brain infiltrating CD4+, CD8+, and NK T cells, which peaked at 10 DPI.
Conclusions: In summary, this temporal analysis revealed that IL-1β, IL-6 and TNF-α are primarily produced by innate immune cells in the acute phase post-injury, whereas IL-17 is produced by γδ T cells, and IFN-γ by CD4+, CD8+, NK T cells at chronic time points. This new preclinical study identifies targets for reducing cellular infiltration after TBI, which may be important in regulating neuroimmune responses and neurological outcomes post-TBI.

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1085 – P3.01.27

The Role of A20 in Microglia in Preventing CD8 T Cell-Mediated Neuronal Alterations

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A20 also known as Tumor-necrosis-factor-alpha-induced protein 3 (TNFAIP3) is an ubiquitin editing enzyme. It has the role of antiinflammation in mammals by negatively regulating canonical nuclear factor κB (NF-κB) signalling. Our study highlights the crucial role of A20 in maintaining brain homeostasis. In the absence of A20 in microglia (A20Δmg), there is a shift from a homeostatic to a proinflammatory state. We observed spontaneous infiltration of CD8+ T cells in the CNS that had acquired the viral response signature. These CD8+ T cells produce IFN-γ actively influence a shift in the microglial population towards an interferon response. Interestingly, the absence of these CD8+ T cells in the CNS results in no changes in A20Δmg microglial morphology or numbers, confirming the changes are due to IFN-γ secreting CD8+ T cells.

The infiltration of CD8+ T cells and activation of A20Δmg microglia altered synaptic connectivity between microglia and neurons. This increased excitatory synaptic currents in pyramidal neurons, while the density of Parvalbumin-expressing inhibitory interneurons in the cortex decreased. Notably, neurogenesis is reduced, as evidenced by a decrease in the presence of doublecortin-expressing neurons in the hippocampus. These changes in the neuronal population increase the likelihood of behavioural alterations in these animals.

The increased CD8+ T cell population in A20Δmg mice persists throughout the lifespan. Additionally, deep single-cell transcriptomic data reveal the expression of senescence signatures in the microglia population. Therefore, this research raises the question of whether the persistent inflammation in the CNS of A20Δmg mice accelerates the ageing phenotype.

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1135 – P3.01.28

The beneficial effects of N-methyl-D-aspartate receptor antagonism on experimental autoimmune encephalomyelitis in aged rats

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Purpose: Life expectancy is only slightly affected by multiple sclerosis (MS), and it is a disease that often lasts for several decades. The proportion of patients with late-onset MS has increased significantly and the mean age at diagnosis has shifted towards older age. Older age at disease onset is associated with a shorter time to disability. Immune cells, such as macrophages, microglia and lymphocytes, express *N*-methyl-D-aspartate receptors (NMDARs). Our aim was to elucidate the effects of ageing on the role of NMDARs in the pathogenesis of experimental autoimmune encephalomyelitis (EAE).
Methods: Young adult (3-month-old) and aged (24-month-old) female Dark Agouti rats were used in this study. Memantine, a non-competitive NMDAR antagonist, was administered by oral gavage for 7 consecutive days from the first day post-immunization (dpi) in the first set of experiments or from the 7th dpi in the second set of experiments. Mononuclear cells were isolated from the spinal cord or draining lymph nodes and analysed by flow cytometry. Spinal cord tissue was collected for RT-qPCR.
Results: Administration of an NMDAR antagonist during the induction phase of EAE diminished the proportion of Th1 and Th17 cells and increased the proportion of IL-10^{+} regulatory T cells in the lymph nodes draining the site of immunization in aged rats. Memantine increased the proportion of CD163^{+} and IL-10^{+} cells within CD11b^{+} cells in the draining lymph nodes of aged rats, whereas these effects were absent in young rats. Treatment with memantine from the 7th dpi resulted in a shift of microglia towards the anti-inflammatory M2 phenotype, characterized by an increase in the expression of CD163 and a decrease in the expression of MHCII molecules, with an increase in the proportion of IL10^{+} microglia and the expression level of arginase 1 in the spinal cord of aged rats at the peak of the disease.
Conclusion: NMDARs contribute significantly to the pathogenesis of EAE in aged rats in both the induction and effector phases. Our results suggest that targeting NMDARs in elderly MS patients may help to tailor treatment to the elderly MS population.

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1303 – P3.01.29

Investigation of the Effect of Complement System Components C5a and C5a Receptor C5aR1 in an in vitro Alzheimer's Disease Model

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Purpose: Along with C5aR1 (CD88), which is expressed on both myeloid and non-myeloid cells, C5a has been implicated in the pathogenesis of neurodegenerative diseases, including Alzheimer's disease (AD), but the effect of complement components on AD has not been fully elucidated. Here, we aimed to investigate the effects of C5a on the hallmarks of AD in an *in vitro* AD model established from human neuronal progenitor cells.
Methods: The cellular AD model was established by transducing the progenitor cells with a viral vector expressing familial AD mutations, Swedish and Indiana followed by neuronal differentiation. Optimal concentration of C5a was determined by MTS assay and differentiated neurons were cultured in the presence of C5a, C5aR1 inhibitor PMX53 or C5a with PMX53. C5Ar1, Aβ40, Aβ42, and amyloid precursor protein levels were measured by flow cytometry. Total and phosphorylated tau levels were assessed by Western blotting.
Results: C5Ar1 expression was significantly enhanced in the AD group in comparison with the control group while PMX53 administration has decreased its expression. C5a treatment has significantly decreased Aβ40, and Aβ42 levels compared to the AD group, though did not have a significant effect on tau phosphorylation.
Conclusion: Our results highlight the important involvement of the complement system and its components in AD and emphasize the need for further studies to be added to the literature in this regard.

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1445 – P3.01.30

Impact of parasite antigens on neuroinflammation and tissue remodelling in neurocysticercosis

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Purpose: Neurocysticercosis (NCC) is a parasitic disease in which *Taenia solium* larvae (cysticercus) invades the central nervous system and is the most prevalent cause of acquired epilepsy worldwide. The cysticercus establishes as a viable cyst evading the host response, but degenerating cysts may trigger neuroinflammation. Astrocytes are key regulators of neuroinflammation and tissue remodelling in CNS infection. The impact of monocyte-astrocyte networks activated by cysticercal antigens on neuroinflammation and tissue remodelling due to matrix metalloproteinase (MMP) secretion during NCC are ill-defined.
Methods: The effect of cysticercal antigens (total and specific recombinant antigens) was investigated in human monocyte and astrocyte primary cultures. Gene expression and secretion of cytokines, (TNF-α, IL-1b, IL-6, IFN-γ), and MMPs-1, -3, -9, -10 as well as specific tissue inhibitors of MMPs (TIMP-1 and -2) were measured by RT-PCR and ELISA/Luminex. Lastly, we optimised a novel co-culture model using conditioned medium from monocytes stimulated with NCC antigens (ComNCC) to investigate monocyte-astrocyte networks.
Results: Cysticercal antigens induce pro-inflammatory responses in monocytes, at 4h and peaking after 48h (TNF-α: 802.22 vs 13125.45pg/ml, IL-1b: 19.64 vs 233.95pg/ml, IL-6: 80.63 vs 8052.69pg/ml and IFN-γ: 84.89 vs 633.44pg/ml) ($p<0.015$). MMP secretion increased at 24h post-stimulation with antigens compared to control (MMP-1: 19.25 vs 1473.75pg/ml, MMP-9 39.75 vs 3389.55pg/ml and MMP-10: 18.42 vs 289.13pg/ml). Similarly, gene expression of inflammatory cytokines and MMPs were significantly increased ($p<0.01$). TIMP-1 and -2 secretion was constitutive, but TIMP-1 was increased markedly at 48h (control 130.13 vs 1401.56pg/ml) and TIMP-2 secretion increased three times ($p<0.025$). Finally, using our co-culture model, significant increase in IL-6, IL-8, MMP-1 and MMP-9 secretion were observed in astrocytes stimulated with ComNCC.
Conclusion: These data provide new insights into monocyte-astrocyte networks that activate neuroinflammation and tissue remodelling in NCC. Improving the understanding of neuroinflammatory responses induced by cysticercal antigens in NCC is crucial for identification of biomarkers to allow early therapeutic intervention and timely control of innate immune response.

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1452 – P3.01.31

Inflammatory and bioenergetic abnormalities in patients with ischemic stroke

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Purpose: Stroke is a major cause of mortality and remains the second leading cause of death worldwide, accounting for 55% of all neurological disabilities. These grim statistics call for targeted scientific and clinical research focused on both prevention and early diagnosis and prognosis in stroke. The aim of the present study is to identify changes in inflammatory processes and cellular bioenergetics in ischemic stroke patients, before and after therapy, and healthy controls.
Methods: PBMCs and plasma from patients with ischemic stroke, before therapy (n=9), after therapy (n=4) and healthy controls (n=9) were isolated. All patient groups underwent a clinical evaluation by assessing the severity of neurological symptoms using the National Institutes of Health Stroke Scale (NIHSS) and assessing consciousness using the Glasgow-Liege Coma Scale (GLCS). The protein levels of IL-6 in plasma were measured by ELISA. In addition, the metabolic studies of the isolated PBMCs were performed using a Seahorse XFp analyzer.
Results: The data revealed that the patients with ischemic stroke before therapy have significantly increased IL-6 levels and decreased spare respiratory capacity compared to the control group. On the other hand, patients with ischemic stroke after therapy have significantly decreased IL-6 levels and increased spare respiratory capacity compared to the patients with ischemic stroke before therapy.
Conclusion: A better understanding of the inflammatory processes and mitochondrial function may reveal new targets for neuroprotection, therapeutic monitoring, and prediction of the disease severity.

This study is financed by the European Union-NextGenerationEU, through the National Recovery and Resilience Plan of the Republic of Bulgaria, project № BG-RRP-2.004-0007-C01

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1462 – P3.01.32

Consequtive occurrence of relapsing polychondritis and mononeuritis multiplex leading to diagnosis of Sjogren's syndrome

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Background: Relapsing polychondritis (RPC) is a rare immune-related disorder characterized by recurrent inflammation of the cartilaginous tissue. RPC is associated with other diseases in about one third of patients, most notably with connective tissue diseases and vasculitides.
Case presentation: A 43-year old female presented with left retro orbital pain and double vision. Examination of ocular motility proved the diagnosis of left abducens nerve palsy. Thirteen days later, the same symptoms appeared on the right side. Otherwise neurological and MRI findings were normal. Laboratory findings showed increased inflammatory markers and high/medium concentration of anti-Ro/SSA/La/SSB antibodies respectively. She was found to have symptoms of dry eye without symptoms of dry mouth, with positive tests for dry eye, low unstimulated whole saliva flow rate and salivary gland involvement by dynamic scintigraphy. Finally, she was diagnosed with Sjogren's syndrome (SS) according to ACR/EULAR classification criteria (with total score of 5) accompanied with bilateral abducens nerve palsy, indicative of mononeuritis multiplex (MNM) type of cranial nerve lesion. Ten days later her left ear auricle became swollen and tender. During the three previous years she experienced repetitive swelling and redness of her nose and both auricle. These episodes were treated with oral corticosteroids with favorable response. Based on these parameters she met modified Damiani criteria for the diagnosis of RPC. She received pulses of methylprednisolone followed by oral prednisone and manifestations of both RPC and MNM gradually improved and resolved within two months.
Conclusion:
Herein we report a rare association of RPC as initial clinical presentation followed by MNM that led to the diagnosis of SS in a patient who was then treated successfully with steroids. Physicians should be aware that although rare, immune related diseases can coexist or even present one after another.

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1476 – P3.01.33

Inflammatory and autophagy markers as a discriminative tool of bacterial and viral infections in the central nervous system

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Purpose: The aim of our study was to evaluate the discriminative value of gene and protein expression levels of inflammatory marker (YKL-40) and autophagy-related proteins (LAMPs) in patients with central nervous system (CNS) infections.
Methods: Thirty hospitalized patients with CNS infections and undefined etiology, and 6 healthy subjects as a control group, were included in the study. The cerebrospinal fluid (CSF) of suddenly deceased healthy individuals was isolated. Total RNA was extracted from white blood cells. Gene expression of YKL-40, LAMP1 and LAMP2 was determined by qPCR. Plasma and CSF levels were examined by ELISA.
Results: Our results showed that mRNA levels of YKL-40 were significantly downregulated in patients compared to controls. Plasma YKL-40 concentrations were higher than in the control group. The CSF glycoprotein levels were significantly higher compared to plasma levels. Decrease in LAMPs expression in patients compared to controls was observed. Significantly higher plasma and CSF LAMP-1 levels in patients in comparison with the control group were found. Low plasma LAMP-2 concentrations were detected while CSF levels were higher in patients compared to controls. In addition, we separated patients depending on the etiological agent - bacterial or viral. We found significant difference between plasma levels of YKL-40 in the control group and in both disease subgroups. Higher CSF YKL-40 levels compared to plasma levels in patients with viral infection were detected. Concentrations of the glycoprotein were higher in patients with bacterial infections compared to viral ones.
We revealed that LAMP1 plasma levels significantly increased in patients with viral CNS infections in comparison with regard to healthy individuals without any difference with bacterial CNS infections.
Conclusion: We suggest that decreased mRNA expression of YKL-40 and higher protein levels result from post-translational regulation of the *YKL-40* gene. We could speculate that YKL-40 and LAMP1 levels might serve as a fast discriminative tool to determine bacterial and viral infections in CNS infections.

Acknowledgments: The study was financed by MU-Plovdiv project № 02/2022 (for ELISA kits) and European Union-NextGenerationEU, through the National Recovery and Resilience Plan of the Republic of Bulgaria, project № BG-RRP-2.004-0007-C01 **(**for qPCR reagents)

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1487 – P3.01.34

Neutrophil Heterogeneity and Susceptibility of NETs Formation in Patients Suffering from Autoimmune Neurological Disorders

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Neutrophils defend against pathogens via several mechanisms such as degranulation, phagocytosis, cytokine release, and forming neutrophil extracellular traps (NETs). NETosis, ejecting DNA and antibacterial proteins, is key to their defense. While traditionally associated with acute inflammation due to their rapid response and short lifespan, neutrophils also contribute to chronic inflammatory processes, including autoimmune neurological disorders (ANDs). These reactive neutrophils may tend to develop dysregulated NETosis, which can sustain long-term inflammation. A pivotal aspect of their contribution in ANDs lies in their ability to modulate the integrity of the blood-brain barrier and enhance antigen spreading, especially during reactive states.
This study focuses on neutrophils' role in the ANDs, such as neuromyelitis optica spectrum disorders (NMOSD), multiple sclerosis (MS), and myelin oligodendrocyte glycoprotein-antibodies associated disorder (MOGAD). We hypothesize that patients with ANDs will have different signatures in the neutrophil compartment during the remissions compared with relapses in each disorder and in intergroup comparison (NMOSD vs MS vs MOGAD).
The proportion of particular neutrophil subsets will be determined based on the presence of cell surface markers (CD14, CD15, CD16, CD62L, CD64, CD49d, CD10, CD11b, CD11c, CD101, CD35, CD71) by flow cytometry. To examine the functional properties of neutrophils, phagocytosis will be quantified using flow cytometry, while NETosis will be observed and measured through fluorescent time-lapse microscopy.
Patients suffering from ANDs have altered proportional and functional characteristics of neutrophils, with a notable rise in immature $CD15^{+}CD16^{-}$ neutrophils. Despite comparable phagocytic activity to healthy individuals, AND patients' neutrophils display a heightened propensity for NETosis during relapses, together with increased pro-inflammatory markers expression. This shift is most pronounced in patients with MS, where a substantial increase in reactive neutrophils, inclined towards NET formation together with elevation in the expression of CD64, underscoring the heightened inflammatory response. Conversely, the various treatments administered for ANDs appear to have a minimal effect on the specific proportional characteristics of neutrophil subsets.
Patients suffering from ANDs have altered proportional and functional characteristics suggesting that neutrophils could contribute to the disease onset and progression.

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1540 – P3.01.35

Correlation between inflammatory and bioenergetic parameters abnormalities in Autism Spectrum Disorder– a possible clue to cellular dysfunction?

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Purpose: The aim is to evaluate cellular metabolic status and inflammation-related molecules such as IL-1β, IL-9 and YKL-40 in Autism Spectrum Disorder (ASD) children with and without regression compared to healthy controls.
Methods: Children with ASD (n=56) and typically developing children (TDC, n=12) aged 2 to 11 years were studied. The diagnosis ASD was set based on generally accepted clinical assessment scales (ADOS) and on the ASD diagnostic criteria according to DSM 5. Mitochondrial activity was examined in peripheral blood mononuclear cells (PBMCs) isolated from children with ASD and from the control group, using a metabolic analyzer (Seahorse XFp). Expression (qPCR) and protein levels (ELISA) of IL-1β, IL-9, and YKL-40 were investigated in parallel.
Results: Our results showed that PBMCs of the ASD subgroup of regressed patients (ASD R(+)) had a specific pattern of mitochondrial activity with significantly increased maximal respiration, spare respiratory capacity and proton leak compared to the non-regressed group (ASD R(-)) and TDC. The observed unique phenotype of ASD R(+) is most likely due to an adaptive response and reflects an alteration in pathways affecting mitochondrial function. Furthermore, we found an imbalance in the studied proinflammatory molecules and increased levels in ASD R(-) proving the involvement of inflammatory changes.
Conclusion: The results of this study provide new evidence for specific bioenergetic profile of immune cells and elevated inflammation-related molecules in ASD. For the first time, data on a unique metabolic profile in ASD R(+) and its comparison with a random group of children of similar age and sex are provided. In addition, we report novel evidence for increased levels of proinflammatory molecules in a subset of ASD R(-) patients compared to ASD R(+). Therefore, targeting mitochondrial and immune regulation could reduce bioenergetic and inflammatory disorders, and might help the development of new therapeutic strategies in ASD.

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1542 – P3.01.36

Relationship between miRNAs and immune -bioenergetic dysfunction in Autism Spectrum Disorder

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Purpose: The aim of the current study was to investigate the involvement of a panel of inflammatory and mitochondrial activity-related miRNA with the relation to the immune and bioenergetic alterations in Autism Spectrum Disorder (ASD).
Methods: Thirty children with ASD and typically developing children (TDC, n=7) were included in the study. Expression levels of a pannel of five miRNA (miR-101, miR-130, miR-143, miR-181, miR-320) and COX-2, surving as an inflammatory marker, were measured in white blood cells (WBC). Mitochondrial activity in peripheral blood mononuclear cells (PBMCs) isolated from both ASD and TDC was examined using a Seahorse XFp (Agilent) analyzer.
Results: The panel of 5 poorly investigated in ASD, miRNAs, showed differential expression between patients and controls. Among the five investigated miRNA, a significant down-regulation was observed in miR-130, miR-101, miR-143 in the ASD. Patients group had increased spare respiratory capacity and expression levels of COX-2 compared to controls. In addition, a correlation between miRNA regulation and mitochondrial bioenergetics/COX-2 levels was also observed.
Conclusion: These results underline the existance of a relationship between miRNA, COX-2 and mitochondrial function in the ASD. Moreover, the observed miRNA disregulation might be a part of mechanisms leading to mitochondrial and immune dysfunction in ASD.

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1674 – P3.01.37

Plant-derived cannabinoids: Regulators of NLRP3 inflammasome signaling in immune cells with relevance to Multiple Sclerosis

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Multiple sclerosis (MS) is a chronic autoimmune disorder associated with sensory and motor impairments. In Ireland, MS currently afflicts over 9,000 people, with approximately 290 people diagnosed with MS in Ireland each year. A range of disease-modifying therapies (DMTs) are available for symptom management in MS, nevertheless, most DMTs present with side-effects. There is a need for new therapeutic strategies in MS. Although MS is a complex disease, there is evidence indicating the role of neuroinflammation in MS pathogenesis. The NLRP3 inflammasome has a well-established function in innate immunity, and current evidence suggests that targeting the inflammasome is a key therapeutic target in the disease. Cannabidiol (CBD) and tetrahydrocannabinol (THC) are protective in murine models of MS, and sativex (a 1:1 combination of THC:CBD), is clinically available for symptom management in MS. The goals of this study are to define the role of the NLRP3 inflammasome in MS and develop inflammasome assays to assess the efficacy of novel therapeutics, (components of *Cannabis sativa* L.), as inflammasome inhibitors in immune cells with relevance to MS. This study assessed the expression profile of inflammasome markers (IL-1β/IL-18) in plasma samples from healthy control cases and people with MS. In addition, this has developed inflammasome assays to assess the efficacy of THC/CBD as inflammasome inhibitors in immune cells with relevance to MS. Our findings indicate the components of the hemp plant *Cannabis sativa* L. have the propensity to target inflammasome signalling in immune cells with relevance to MS.

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Interferon-gamma induces tolerogenic phenotype and activity in monocyte-derived dendritic cells from patients with multiple sclerosis and healthy donors

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Purpose: Multiple Sclerosis (MS) is an autoimmune disease of the central nervous system. Our previous results have shown that low doses of interferon-gamma (IFN-γ) induce differentiation of murine bone marrow-derived dendritic cells (BMDCs) with tolerogenic phenotype and function and with therapeutic activity in experimental autoimmune encephalomyelitis (EAE), a pre-clinical model of MS. In this study, we determined the impact of IFN-γ on the differentiation and function of monocyte-derived dendritic cells (moDC) from patients with multiple sclerosis and healthy donors (HD).
Methods: Monocytes were isolated from peripheral blood mononuclear cells from untreated MS patients (n=11) or HD (n=9) by CD14-specific immunobeads. moDC were differentiated using GM-CSF and IL-4 (1000 U/ml) in the absence (UN-moDC) or presence of increasing concentrations (1 to 1000 ng/ml) of IFN-γ (IFN-γ-moDC). Lipopolysacharide (LPS, 1 mg/ml) was added during the last 24 h to obtain mature moDC (m-moDC) and to evaluate functional stability of IFN-γ-moDC (LPS-IFN-γ-moDC). Cell viability, DC yield and tolerogenic phenotype were determined by flow cytometry. The tolerogenic function was evaluated in a mixed lymphocyte reaction (MLR) assay by co-culturing IFN-γ-moDC or LPS-IFN-γ-moDC with Cell Trace Violet (CTV) labelled allogenic peripheral blood mononuclear cells from HD. $CD4^+$ and $CD8^+$ T **cell proliferation and activation were determined by flow cytometry.**
Results: IFN-γ-moDC from MS patients and HD exhibited a tolerogenic phenotype characterized by significantly lower levels of CD80 than m-moDC and significantly higher levels of PD-L1 than UN-moDC. Additionally, IFN-γ-moDC from HD had significantly lower levels of CD86 and CD83 than m-moDC. Remarkably, IFN-γ-moDC and LPS-IFN-γ-moDC from MS patients significantly inhibited $CD4^+$ T cell proliferation and activation. IFN-γ-moDC from HD significantly inhibited $CD4^+$ T cell proliferation and activation. IFN-γ-moDC from MS patients and HD significantly inhibited $CD8^+$ T **cell proliferation and activation.**
Conclusion: Our results demonstrate that IFN-γ induces differentiation of moDC from MS patients and HD with tolerogenic phenotype and function.

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Therapeutic role of interferon-gamma in experimental autoimmune encephalomyelitis is mediated through a tolerogenic subset of splenic CD11b+ myeloid cells

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Cumulative evidence has established that interferon-gamma (IFN-γ) has both pathogenic and protective roles in multiple sclerosis (MS) and the animal model, experimental autoimmune encephalomyelitis (EAE). However, the mechanisms whereby IFN-γ may promote neuroprotection in EAE are not well understood. In this study, we addressed the impact of IFN-γ on peripheral and CNS infiltrating $CD4^+$ T cell subpopulations in EAE. The frequency of regulatory T (Treg) cells in spinal cords from chronic EAE mice treated with IFN-γ was significantly increased with no effect on Th1 and Th17 cells. Consistently, depletion of FOXP3-expressing cells blocked the protective effects of IFN-γ, indicating that the therapeutic effect of IFN-γ depends on the presence of Treg cells. However, IFN-γ did not trigger direct *in vitro* differentiation of Treg cells. *In vivo* administration of blocking antibodies against either interleukin (IL)-10, transforming growth factor (TGF)-β or program death (PD)-1, revealed that the protective effects of IFN-γ in EAE were also dependent on TGF-β and PD-1, but not on IL-10, suggesting that IFN-γ might have an indirect role on Treg cells acting through antigen-presenting cells. Indeed, IFN-γ treatment increased the frequency of a subset of splenic $CD11b^+$ myeloid cells expressing TGF-β-Latency Associated Peptide (LAP) and program death ligand 1 (PD-L1) in a signal transducer and activator of transcription (STAT)-1-dependent manner. Furthermore, splenic $CD11b^+$ cells from EAE mice preconditioned *in vitro* with IFN-γ and myelin oligodendrocyte glycoprotein (MOG) peptide exhibited a tolerogenic phenotype with the capability to induce conversion of naïve $CD4^+$ T cells into TGF-β-secreting Treg cells. Remarkably, adoptive transfer of splenic $CD11b^+$ cells from IFN-γ-treated EAE mice into untreated recipient mice ameliorated clinical symptoms of EAE and limited central nervous system infiltration of mononuclear cells and effector helper T cells. Therefore, IFN-γ promotes beneficial effects in EAE by endowing splenic $CD11b^+$ myeloid cells with tolerogenic and therapeutic activities.

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1739 – P3.01.40

Interferon-gamma induces tolerogenic dendritic cells with suppressive activity in experimental autoimmune encephalomyelitis

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Purpose: Multiple Sclerosis (MS) is an autoimmune disease of the central nervous system. Our previous results have shown that interferon-gamma (IFN-γ) suppresses experimental autoimmune encephalomyelitis (EAE), a pre-clinical model of MS, by inducing splenic $CD11b^+$ myeloid cells with tolerogenic and therapeutic activities. Here, we determined the effect of IFN-γ on the differentiation, tolerogenic phenotype, and therapeutic activity of murine bone marrow-derived dendritic cells (BMDCs).
Methods: BMDCs precursors from mice were differentiated into dendritic cells (DC) with GM-CSF (20 ng/ml) for 7 days in the absence (iDC) or presence of IFN-γ (IFN-γ-DC). Different concentrations of IFN-γ (5 to 50 ng/ml) were added starting from day 0, 2 or 4 of differentiation. Lipopolysacharide (LPS, 100 ng/ml) was added during the last 24 h to obtain mature DC (mDC) and to evaluate phenotypic and functional stability of IFN-γ-DC (LPS-IFN-γ-DC). Cell viability, DC yield, phenotypic profile, and expression of indoleamine 2,3-dioxygenase 1 (IDO-1) and Aryl hydrocarbon receptor (AhR) were determined by flow cytometry. The tolerogenic function was evaluated in a mixed lymphocyte reaction (MLR) assay. IFN-γ-DC or LPS-IFN-γ-DC pulsed with ovalbumin (OVA) peptide were co-cultured with Cell Trace Violet (CTV) labelled $CD4^+$ T cells isolated from the spleens of OT-II mice at different DC:T cell ratios and $CD4^+$ T cell proliferation and activation were determined by flow cytometry. EAE mice were i.v. transferred with IFN-γ-DC or LPS-IFN-γ-DC pulsed with myelin oligodendrocyte glycoprotein (MOG) peptide at the peak of disease and clinical progression was monitored daily.
Results: DC differentiated in the presence of IFN-γ exhibited a tolerogenic phenotype characterized by significantly lower levels of CD80, CD86, and MHC-II, and significantly higher levels of PD-L1 than mDC. LPS-IFN-γ-DC showed a stable phenotype. Induction of tolerogenic IFN-γ-DC was associated with increased expression of IDO-1 and AhR. Tolerogenic IFN-γ-DC and LPS-IFN-γ-DC inhibited OVA-specific $CD4^+$ T cell proliferation and activation. Remarkably, adoptive transfer of either IFN-γ-DC or LPS-IFN-γ-DC ameliorated clinical symptoms of EAE.
Conclusion: Low doses of IFN-γ induce differentiation of murine tolerogenic DC with therapeutic activity in EAE. Therefore, our results reveal a previously unknown protective mechanism of IFN-γ in a neuroinflammatory context.

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Neuroimmune interactions shape vascular inflammation: evidences from mouse and human studies

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Purpose: Atherosclerosis is a chronic inflammatory disease of medium and large-size arteries that affects millions of people. Atherosclerotic plaques emerge in the inner intimal layer of arteries and plaque instability triggers clinically significant cardiovascular disease including heart attack and stroke. As plaques lack innervation, the impact of neuronal control on atherosclerosis remains unknown. However, the immune system responds to plaques by forming leukocyte infiltrates in the outer connective tissue coat of arteries, i.e. the adventitia. Because the peripheral nervous system uses the adventitia as its principal conduit to reach distant targets, we hypothesized that the peripheral nervous system may directly interact with diseased arteries via adventitia immune cells to sense and affect atherosclerosis.
Methods: We used detailed aorta imaging, gene expression analyses, tissue clearing approaches, retrograde virus tracing, in-vivo ultrasound plaque imaging, extracellular nerve recording, systemic and local sympathetic denervation, normo- and hyper-lidemic mouse models, human cardiovascular tissues including coronary arteries from ischemic/dilated cardiomyopathy patients, and abdominal aortas from abdominal aortic aneurysms with or without atherosclerosis and arteries/aortas from healthy donors.
Results: In hyperlipidemic mice and human atherosclerotic tissues, we identified wide-spread neuroimmune interactions between nerves, immune cells and arteries as a key component that drives atherosclerosis: diseased adventitia segments showed expanded axon networks including growth cones at axon endings near immune cells and media smooth muscle cells. Murine vascular neuroimmune interfaces established a structural artery-brain circuit: abdominal adventitia nociceptive afferents entered the central nervous system through spinal cord lower thoracic dorsal root ganglia, and were traced to higher brain regions including parabrachial and central amygdala neurons; and sympathetic efferents projected from medullary and hypothalamic neurons to the adventitia through spinal intermediolateral neurons and both celiac and sympathetic chain ganglia. Moreover, central and peripheral components of the artery-brain circuit were activated in parallel to disease progression. When this crosstalk is disrupted by systemic or local sympathetic denervation, plaque-associated immune cell aggregates in the adventitia are destabilized, plaques shrink and become more stable.
Conclusion: Our data demonstrated that the tripartite neuroimmuen cardiovascular interactions shape vascular inflammation such as atherosclerosis, and suggested that these interactions could be targeted to treat atherosclerosis.

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1812 – P3.01.42

Astaxanthin-loaded polyarginine nanocapsules inhibit astrocyte reactivity

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Astaxanthin (AST) is a carotenoid obtained from natural sources that has extremely strong antioxidant, anti-inflammatory, and neuroprotective activity. However, the therapeutic application of AST is hindered by its low aqueous solubility and poor stability. In order to improve AST stability and bioavailability as well as its potential application in neuroinflammation, we have developed polyarginine (PARG) oil-in-water nanocapsules (NCs) loaded with AST. PARG is a cationic polymer that has been reported to enhance interaction and transport through cell membranes. The NCs were synthesized using a modified solvent displacement method. PARG was tested at different concentrations (0.06 - 0.25 mg/mL). Stable PARG-NCs were obtained in the range of 157-204 nm, with PDI values of between 0.1-0.2, and positive zeta potential between 33.8 and 57.5 mV, confirming the adherence of polyarginine on the oily droplet´s surface. To determine cytotoxicity of PARG-NCs, rat astrocytes (DI TNC1) were incubated with NCs synthetized with different concentrations of PARG (0.06, 0.125, and 0.25 mg/ml) and at volumes from 5-25% of cell culture medium volume. After 48 hours, cell viability was assessed by flow cytometry. The results showed that cell viability was higher than 90% in the presence of PARG-NCs at concentrations of 0.06 mg/mL and 0.125 mg/mL PARG and at volumes lower than 15%. To evaluate the *in vitro* anti-inflammatory activity of AST-PARG-NCs, rat astrocytes activated with interferon-gamma (100 ng/ml) and lipopolysaccharide (100 ng/ml) were treated with PARG-NCs loaded with 5, 10 or 20 µM AST for 24, 48, 72 and 96 hours. The expression of the glial fibrillary acidic protein (GFAP) was determined by flow cytometry. The results showed that activated astrocytes treated with PARG-NCs containing 5 and 10 µM AST for 72 h or with PARG-NCs containing 5, 10 and 20 µM AST for 96 h exhibited a significantly lower expression of GFAP than control cells. Blank (empty) PARG-NCs or free AST had no significant effect. Therefore, these results indicate that PARG-NCs are safe and might be useful as a nanosystem for AST delivery with potential anti-inflammatory application in neuroinflammatory diseases.

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1830 – P3.01.43

DICAM+ infiltrating monocytes: a mode to control neuroinflammation?

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Background and purpose: Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS). CNS-infiltrating monocytes are found in large numbers within the injured areas of the brain where they play a central role in driving disease progression through demyelination and tissue destruction. However, infiltrating monocytes can also poses a protective function. With this study we investigate a potential protective role of infiltrating monocytes expressing DICAM (dual Ig domain containing cell adhesion molecule) in MS.
Methods: In this study, freshly drawn cerebrospinal fluid (CSF) from 21 treatment-naïve patients with relapsing-remitting MS (RRMS), 14 symptomatic controls and 12 patients treated with natalizumab was analysed by flow cytometry to measure frequency and absolute numbers of DICAM expressing monocytes. Soluble DICAM in CSF was measured by ELISA, and *in vitro* LPS-stimulation studies was performed on freshly isolated blood monocytes.
Results: Analysing CSF of treatment-naïve patients with RRMS showed a reduced frequency of infiltrating-monocytes compared to controls, and furthermore, a decrease in the proportion of infiltrating monocytes that expressed DICAM. When patients were treated with natalizumab, an antibody blocking migration of blood leukocytes to the CNS, we observed that $DICAM^+$ monocytes were still recruited to the CSF and that the level of soluble DICAM in CSF was significantly increased compared to untreated patients. Stimulating monocytes *in vitro* with LPS induced production of proinflammatory cytokines; a production that was effectively reduced by a signal through surface bound DICAM.
Conclusion and discussion: The high frequency of infiltrating $DICAM^+$ monocytes in controls and natalizumab-treated patients in which disease is effectively controlled suggests an immunomodulatory role of these cells. Also, the ability of surface expressed DICAM to regulate the outcome of cell-stimulation in an anti-inflammatory direction, indicates a direct role of DICAM in controlling neuroinflammation. Studies from other groups have shown that DICAM can interact with DICAM on other cells and likewise control their inflammatory profile. Our observation of an increased level of soluble DICAM in natalizumab-treated patients therefore may contribute to control of disease activity through a paracrine action. Altogether, this makes DICAM a possible therapeutical molecule to control inflammation.

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1842 – P3.01.44

Significance of second-generation psychotic drugs in regulation mast cell response: an in vitro study

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Purpose: One of the most significant health issues facing the globe today is mental illness and neurodegenerative diseases, which presents a public health dilemma. Epidemiological indicators point to a rise in the prevalence of cognitive conditions like schizophrenia, bipolar, manic, or addiction-related disorders. Pharmacological treatment with neuroleptics is the most common therapy used in these states. Neuroleptics are psychotic drugs divided into two classes: “classic” first-generation antipsychotics and second-generation or "atypical" antipsychotics. The concept that inflammation contributes to the development of cognitive disorders, proposed over a century ago, has gained substantial support considering recent research findings. Immune cells, notably mastocytes, play a pivotal role in the development and functioning of the central nervous system. Mast cells profoundly affect their environment and neighbouring cells, including the behaviour or activation of cells involved in neurogenesis and neurodegeneration, such as astrocytes, microglia, and neurons. Considering the hypothesis that mast cells play a role in neuroinflammation, this study aims to assess the *in vitro* response of these cells to second-generation antipsychotics: aripiprazole, olanzapine, risperidone, and quetiapine.
Methods: The study used mast cells isolated from the peritoneal cavity of Wistar rats. Flow cytometry was used to examine the impact of selected drugs on mast cell viability and drug-induced changes in cell surface receptor expression. Histamine release from mast cell granules was measured using a spectrofluorimetric method to assess degranulation levels. The Boyden chamber method was used to determine mast cell migration to neuroleptics.
Results: Risperidone has the highest mast cell toxicity. All tested drugs, regardless of the concentration, cause strong degranulation and histamine release from mast cells, with olanzapine being the most potent activator of degranulation. Strong mast cell chemotactic activity toward olanzapine and quetiapine has been demonstrated. The expression level of the TLR4 receptor decreases under the influence of all tested drugs, regardless of the concentration.
Conclusion: New methods for the pharmacological treatment of neuropsychiatric disorders may be developed based on information on the causative roles played by mast cells and neuroinflammation in the onset of these conditions.

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1910 – P3.01.46

Selective alteration to CD4 subset differentiation by heterozygous IRF4 L116R protects against neuroinflammation

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The decision of naïve T cells to differentiate into a specific Th cell subset after antigen encounter is a critical pivot point of the immune response and, when out of balance, can lead to autoimmunity, allergy or immunodeficiency. Th subset differentiation is determined by expression of transcription factors including IRF4. We here describe a point mutation (L116R) in the DNA-binding domain of IRF4 leading to a dysregulation of CD4-T cell subsets and their functions. This point mutation does not alter overall protein expression. In sharp contrast to IRF4 null mice neither the CD4/CD8 ratio nor T cell activation and memory is altered in naïve mice carrying the point mutation. However, $IRF4^{L116R}$ T cells are reduced in their capability to differentiate into Th1, Th17 and Treg cells in a dose dependent manner contrasting with the findings in $IRF4^{KO}$ T cells. Particularly striking is the loss of Th1-differentiation in T cells from homozygous I$rf4^{L116R/L116R}$ mice while T cells completely lacking IRF4 show no reduction in Th1 differentiation. Furthermore, despite maintained ability to generate Th17 cells, expression of the $IRF4^{L116R}$ variant protects mice against disease development in the Th17 cell dependent EAE mouse model of neuroinflammation. This contrasts with IRF4 knock-out mice, where expression of one wild-type allele of IRF4 is sufficient for full disease development. Together our results show that the L116R point mutation in IRF4 can selectively alter the differentiation and function of some CD4 T cell subsets and suggest that the L116R mutation is not a classical loss- or gain of function variant.

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1911 – P3.01.47

Serum Neurofilament light as a biomarker of cognitive status in patients with polymyalgia rheumatica

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Purpose: In Polymyalgia Rheumatica (PMR), cognitive impairment is prevalent, affecting up to 80% of those with the condition.[1] The identification of a serum biomarker for the early detection of cognitive decline, and prediction of cognitive impairment severity is an unmet need. Therefore, the aim of this study is to test the hypothesis that serum neurofilament light (NfL) is a biomarker of cognitive impairment in patients with polymyalgia rheumatica.
Methods: Participants were consecutively recruited from a dedicated PMR clinic. Each participant underwent cognitive testing using the Montreal Cognitive assessment (MOCA) test, with a score of <25 identifying those with cognitive impairment. 10 healthy controls were also included. The serum NfL levels of each participant were assayed.
Results: In total, the study enrolled 19 patients with PMR and cognitive impairment, and 20 PMR patients with normal cognitive status. Using analysis of variance (ANOVA), a significant difference in serum NfL levels was observed between cognitively impaired PMR patients and age matched healthy controls (p=0.015). Moreover, a significant difference in serum NfL levels were observed between the combined (normal and impaired cognition) PMR patient results and those of the healthy controls (p=0.02). However, there was no significant difference in serum NfL levels between cognitively impaired PMR patients and PMR patients with normal cognition. There was also no statistically significant difference in the acute phase reactants, c-reactive protein and erythrocyte sedimentation rate, between groups at the time of testing.
Conclusion: Whilst the purpose of this pilot study was to assess the role of serum NfL as a biomarker for cognitive impairment in PMR, the significantly higher serum NfL in the combined PMR group versus healthy controls was an unexpected finding. Whilst the reasons for this are potentially multifold, our findings provide impetus for further research with larger patient cohorts to assess the association of serum NfL with cognitive trajectories in PMR patients, in addition to elucidating the precise disease specific relationship in PMR.

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1918 – P3.01.048

Flow cytometry live cell-based assay display higher sensitivity to detect autoantibodies against myelin oligodendrocyte glycoprotein (MOG-IgG)

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Purpose: This study aims to comprehensively compare the diagnostic performance of two distinct live cell-based assays, indirect immunofluorescence assay (IFA) and flow cytometry (FACS), for the detection of autoantibodies against myelin oligodendrocyte glycoprotein (MOG-IgG) that are diagnostic of MOG antibody-associated disease (MOGAD).
Method: The presence of MOG-IgG was assessed in 244 serum samples and 27 cerebrospinal fluid (CSF) samples by both IFA and FACS assays. Samples were obtained from 248 patients (23 paired serum/CSF samples). HEK293 cells were transfected with a MOG GFP-tagged cDNA clone and incubated with sera (1/160 dilution) or CSF (1/2 dilution). Finally, MOG-IgG from patients were detected using AF-594-conjugated goat F(ab)2 anti-human IgG (IFA) or AF-647-conjugated goat Fc anti-human IgG (FACS) secondary antibodies. For FACS, the IgG binding index of each sample was calculated as the ratio of the cell population´s average MFI: MFI GFP-Positive/MFI GFP-Negative. The mean IgG index value for negative samples tested by both techniques was 1.5, so we established a conservative cutoff ratio of 2.
Results: Out of the 244 sera examined, 197 yielded negative results, and 35 displayed positivity by both assays. Similarly, 25 out of 27 CSF samples were found to be negative, with one positive result for FACS and IFA. Notably, FACS identified 12 positive sera and 1 positive CSF sample that remained undetected by IFA. Conversely, no samples were positive only by IFA. Among the 12 discrepant sera, 6 patients had suggestive features of MOGAD, 3 samples were from patients with previous positive results and diagnosed with MOGAD, 1 patient had a diagnosis of multiple sclerosis, and 2 patients had no available clinical data. Furthermore, the discordant CSF sample came from a patient with MOGAD and positive MOG-IgG in serum. Overall, FACS-based determination of MOG-IgG demonstrated an almost perfect agreement with IFA (Cohen's kappa = 0.81; 95% CI [0.70-0.91]).
Conclusion: The findings of this study underscore a remarkable level of concordance between IFA and FACS in the identification of MOG-IgG. Moreover, the determination based on FACS showed higher sensitivity than that based on IFA, but it remains to determine whether its specificity is also superior.

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1928 – P3.01.49

Liposomal packaging enhances treatment efficacy in neuroinflammation

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Purpose: Liposomes are drug vehicles which may be applied for treatment targeting in multiple sclerosis (MS). These nanoparticles typically accumulate at sites of disrupted vasculature, as seen in the inflamed central nervous system (CNS), and are also commonly internalised by phagocytic innate immune cells, offering an opportunity to modulate both spatial- and cellular-level drug targeting.
Methods: To address these aims, mice were induced with MS neuroinflammation model experimental autoimmune encephalomyelitis (EAE) and dosed with fluorescence-labelled liposomes or liposome-packaged chemotherapeutic mitoxantrone (MX). The cell specific uptake and longevity, as well as the treatment impact of liposome packed MX, was characterised using multicolour flow cytometry and imaging.
Results: On a spatial level, liposomes were found to traffic to the CNS in an inflammation-dependent manner. Further, liposomes were found to preferentially interact with a specific innate immune cell population also depleted by treatment with liposome-packaged MX. Alteration of delivery modality was also found to improve treatment efficacy, where weekly intravenous delivery of 0.5mg/kg liposomal MX was sufficient to prevent clinical signs of EAE in contrast to freely delivered MX, which offered minimal protection.
Conclusion: These results highlight the applicability of liposome drug vehicles in treatment of neuroinflammation and indicate potential key mechanisms enhancing treatment efficacy.

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1947 – P3.01.50

The peripheral immune landscape in a humanized mouse model of Alzheimer's disease

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Purpose: Increasing epidemiological, clinical, and experimental evidence suggests that Alzheimer's disease (AD) pathogenesis is closely associated with systemic abnormalities, including dynamic processes in the peripheral immune compartment and inflammation. Recent reports in transgenic AD animal models propose a role of dysregulated peripheral immune system in memory impairment. In this regard, the contribution of peripheral immune response in driving different pathological stages in AD is less understood. Therefore, utilizing a novel App knock-in mouse model ($App^{NL\text{-}G\text{-}F}$) exhibiting robust Aβ pathology, neuroinflammation and memory impairment, we have explored the relation of Aβ pathology development in the brain with alterations in the peripheral immune compartment.
Methods: Using flowcytometry, we characterized the $App^{NL\text{-}G\text{-}F}$ mice at different stages of Aβ pathology (2-month-old: early, pre-plaques; 7-month-old: plaques + early memory impairment; 12-month-old: late stages) and investigated the distribution and activation status of various splenic immune cells.
Results: Among various immune cells from innate immunity, significant increases in macrophage number in $App^{NL\text{-}G\text{-}F}$ mice were observed. While the percent distribution of B cells from adaptive immunity was reduced in the 12 month-old mice, no significant changes in total T cell distribution in any age group compared to wild-type controls were observed. B cells showed reduced expression of activation molecule CD69 and PD-L1 in 7 and 12-months old $App^{NL\text{-}G\text{-}F}$ mice. Among T cell subsets, the CD4, CD8 and regulatory T (Treg) cell distributions in $App^{NL\text{-}G\text{-}F}$ mice were comparable to control mice in all age groups. However, a rise in follicular T helper (Tfh) cells was observed at 2-month of age only, with altered level of activation molecule ICOS expression on Tfh cells in $App^{NL\text{-}G\text{-}F}$ mice.
Conclusions: These findings in AD mice model show a reduction in B cell and their activation status during aggressive Aβ plaque deposition in brain, suggesting a possible contribution of B-cells in Aβ pathology reinforcing further investigation. Moreover, our data also indicate a possible role of Tfh cells in the early stage of Aβ pathology.

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1951 – P3.01.51

Flow cytometry live cell-based assay for detection anti-aquaporin 4 antibodies (AQP4-IgG)

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Purpose: This study aims to comprehensively compare the diagnostic performance of two distinct live cell-based assays, indirect immunofluorescence assay (IFA) and flow cytometry (FACS), for the detection of anti-aquaporin 4 autoantibodies (AQP4-IgG) associated with neuromyelitis optica spectrum disorder (NMOSD)
Method: The presence of AQP4-IgG was assessed in 135 serum samples and 17 cerebrospinal fluid (CSF) samples by both IFA and FACS assays. Samples were obtained from 137 patients (15 paired serum/CSF samples). HEK293 cells were transfected with GFP-tagged cDNA clone with AQP4 (M23 isoform) and incubated with sera (1/20 dilution) or CSF (1/2 dilution). Finally, AQP4-IgG from patients were detected using AF-594-conjugated goat F(ab)2 anti-human IgG (IFA) or AF-647-conjugated goat Fc anti-human IgG (FACS) secondary antibodies. For FACS, the IgG binding index of each sample was calculated as the ratio of the cell population's average MFI: MFI GFP-Positive/MFI GFP-Negative. The mean IgG index value for negative samples tested by both techniques was 2, so we established a conservative cutoff ratio of 2.5.
Results: Out of the 135 sera examined, 123 yielded negative results, while 11 displayed positivity in both assays. Similarly, 17 out of 17 CSF samples were found to be negative. Notably, FACS identified 1 positive serum that remained undetected by IFA. Conversely, no samples were positive only by IFA. Overall, FACS-based determination of AQP4-IgG demonstrated a perfect agreement with IFA (Cohen's kappa = 0.95; 95% CI [0.9-1]).
Conclusion: The findings of this study underscore a remarkable level of concordance between IFA and FACS in the identification of AP4-IgG. Although FACS-based determination seems to be more sensitive than IFA, it remains to be determined whether it is also more specific.

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1953 – P3.01.52

Sex-dependent effects of short-term aerobic exercise on age-related neuroinflammation in the mouse

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Modern society is ageing rapidly, posing increased risk of the development of physiological impairments due to age-related inflammation, which plays a role in the development of non-communicable diseases including neurodegeneration. This is occurring in parallel with an increase in sedentary behaviour, which is considered to be one of the leading causes of death worldwide. Extensive evidence has shown the multiple effects of regular exercise to prevent or reverse the detrimental effects of a sedentary lifestyle and to potentially alleviate age-associated low-grade chronic inflammation.
In this study, we investigated the impact of short-term, low-to-moderate intensity treadmill running on age-related neuroinflammation. Male and female C57BL/6J mice, aged between 3 and 18 months old, underwent 7 consecutive days of endurance aerobic exercise, followed by assessment of spatial memory using the novel object location test. Subsequently, gene and protein expression were assessed using an array of different assays to look at potential age-, sex- and activity-related effects. The bioenergetic function of primary microglial cells from both exercising and non-exercising mice was also assessed to investigate their metabolic signature and see whether our exercise regimen had any immunomodulatory impact on these cells.
Findings suggest that some markers of age-related neuroinflammation or cognitive impairment are not impacted by exercise, but that responses to both age and exercise varied widely within experimental groups. Neither sex, age nor prior exercise had an impact on the ability of mice to perform the spatial memory task. Immunohistochemical analysis of microglial markers of inflammation revealed an age-associated effect in both sexes, but with a less exacerbated effect in the aged and exercised male mice when compared with females. Moreover, cytokine expression was not affected by exercise, independently of the mice's sex.
Taken together, these results suggest that male and female mice respond in a physiologically different manner to both ageing and exercise, reinforcing the need to further investigate the underlying molecular mechanisms associated with the observed effects and the potential translational impact of these data. Notably, the broad variation in individual age-related responses to exercise we observed here suggests that a more personalised exercise regimen aimed at attenuating age-associated inflammation.

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2078 – P3.01.53

Unlocking the gut-brain axis: microbiota's impact on neuroinflammation

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Multiple sclerosis (MS) is one of the most common chronic inflammatory autoimmune diseases of the central nervous system (CNS), typically emerging in young individuals in the most productive period of their lives. Its multifactorial pathogenesis includes genetic and environmental factors such as communication of the gut microbiota with peripheral and central immune cells. Bacterial extracellular vesicles (BEVs) released from the gut microbiota are attracting increased attention for their potential role as biological shuttle system facilitating not only inter-kingdom but also inter-organ communication. Uncovering novel routes of microbiota-brain crosstalk and the effects of gut microbial components on disease-relevant cell types unveil new targets for therapeutic approaches, which are particularly needed for progressive forms of MS.
Bulk RNA sequencing has identified differential gene expression in human induced pluripotent stem cell (hiPSC)-derived microglia upon BEV challenge. Furthermore, *in vitro* stimulation of primary murine glial cells with BEVs induces altered cytokine profiles in response to vesicles derived from various bacterial species. *In vivo* studies in mice injected with BEVs were conducted to examine their effects on the blood brain barrier integrity, immune cell infiltration into the brain, and their impact on CNS-resident cell types, validated through immunohistochemistry and flow cytometry analysis. Additionally, isolation of BEVs from serum samples of MS and inflammatory bowel disease patients also indicates compromised barrier integrity in both inflammatory conditions.
These findings suggest that within an inflammatory environment, gut microbiota-derived extracellular vesicles shuttle along the gut-brain axis to enter the brain, where they interact with CNS-resident glial cells participating in the onset and progression of autoimmune CNS inflammation in MS. Concluding, the study underscores the potential of the role of BEVs in MS pathogenesis, offering avenues for future research and therapeutic interventions.

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2123 – P3.01.54

Intestinal inflammation as a precursor for Parkinson's disease

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Ulcerative Colitis (UC) is a chronic inflammatory disease affecting the large intestine by dysregulated immune response, which can modify gut physiology and impact distant organs. Gut-brain axis represents an emerging field that pinpoints the communication between these organs, where the enteric nervous system (ENS) plays an important role. Recently it has been suggested that inflammation may influence the function of the ENS. However, the mechanisms linking UC-associated mucosal to ENS dysfunction and its potential implications for Parkinson's Disease (PD) progression remain poorly elucidated. In this project, our objective is to delineate the relationship between mucosal inflammation and ENS function in the context of UC and connecting mechanisms to PD. Employing *in silico* and immunohistochemistry analyses, we aim to elucidate the involvement of the ENS in inflammatory processes. While the precise triggers driving this inflammation remain elusive, inflammatory cytokines pivotal in UC, such as TNF-α, Type I Interferons, and Th17-related cytokines, emerge as compelling candidates. Furthermore, we have differentiated induced pluripotent stem cells (iPSCs) into enteric neuronal-like cells (ENL) to model human ENL inflammation. Altogether, our work so far investigated initial inflammatory-related triggers that could lead to PD-associated changes in the ENS of UC patients. These results could unravel new mechanisms that connect gut inflammation to the development of PD, offering potential avenues for therapeutic intervention. Funding Support: KFO5024-50553912 (A01, A04 and B01).

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2160 – P3.01.55

Investigating the impact of long-term endurance exercise on brain inflammaging in male and female C57BL/6J mice

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Chronic noncommunicable diseases (NCDs), such as diabetes, cancer, cardiovascular disorders, or neurodegenerative conditions are the main cause of morbidity and mortality worldwide. Research suggests that systemic, low-grade inflammation may be a prevailing contributor to the development of NCDs, owing to immunological imbalance which leads to systemic dyshomeostasis. This is further aggravated by heightened inflammation associated with age, often termed 'inflammaging'. Several lines of evidence indicate that modifiable behavioural risk factors, such as physical activity levels, could impact the extent of inflammaging and NCD progression. In this work, we set out to examine the effects of long-term (12+ months), low-intensity endurance exercise on immunological health, and, in particular, on neuroinflammation, which has been implicated in the development of age-related neurodegenerative conditions. C57/BL6J mice of both sexes have been subjected to regular, low-intensity treadmill running beginning in young adulthood. Furthermore, to investigate the potential immunomodulatory effect of specific exercise-related moieties, such as lactate, exercise is combined with pharmacological treatment to prevent lactate uptake into cells. To track potential anti-inflammatory and pro-cognitive effects longitudinally, hippocampus-dependent spatial memory, which is susceptible to inflammation-associated deterioration has been assessed bimonthly using the object displacement paradigm. Results indicate that throughout youth and middle age, the impact of exercise is not significant in either sex. On completion, further behavioural testing will be conducted to assess hippocampus-dependent memory and learning. Subsequently, studies will be conducted to assess peripheral and central inflammatory and activation markers using immunohistochemistry, qPCR, Western blot, and multi-array chemiluminescent immunoassay (MSD) to investigate potential sex-, age-, and activity-dependent effects. Metabolic flexibility and immunometabolic signature of primary microglia, the principal innate immune cell of the central nervous system will also be assessed using Seahorse assay.
Disease burden from NCDs is on the rise globally, and therefore the elucidation of physiological mechanisms and identification of risk reducing strategies are of great importance. Our study will help investigate the potential age-countering impact of long-term, regular, low-intensity endurance exercise on immunological health and the role exercise-associated lactate may play in these effects.

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2172 – P3.01.56

Comparative analysis of brain-derived neurotrophic factor (BDNF) and amphiregulin (Areg) secretion in multiple sclerosis patients: implications for regenerative therapy

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Background: Multiple sclerosis (MS) is an auto-immune disease characterized by inflammation and demyelination. Regulatory T cells (Tregs) and their secreted factors, such as brain-derived neurotrophic factor (BDNF) and amphiregulin (Areg), offer potential for regenerative therapy, besides immunomodulatory potential. BDNF promotes remyelination, axonal plasticity and neuronal cell survival, while Amphiregulin (Areg) aids in tissue repair. This study compares BDNF secretion by Tregs as well as BDNF and Areg secretion in serum between MS patients and healthy controls (HCs).
Material and methods: Peripheral blood mononuclear cells were isolated via density gradient centrifugation, and serum was obtained from serum separation tubes and stored at -80°C. $CD45RA^+$ populations were purified using magnetic activated cell sorting and fluorescence activated cell sorting, followed by 24-hour cell culture and stimulation. Supernatant was collected and stored at -20°C. BDNF and Areg secretion in serum and supernatant were measured using electrochemiluminescence immunoassay.
Results: Sixteen MS patients and 12 HCs were recruited with a mean age of 50 years (IQR: 40 - 58 years) in MS patients and of 53 years (IQR: 31 – 56 years) in HCs. BDNF secretion in serum did not significantly differ between patients (6661 pg/mL; IQR: 5337 – 8299 pg/mL) and HCs (5367 pg/mL; IQR: 3069 – 8266 pg/mL). Among MS patients, those with higher Expanded Disability Status Scale (EDSS) scores showed no significant difference in BDNF levels compared to those with lower scores. Areg levels were uniformly low and showed no significant difference between patients (0.059 pg/mL;, IQR: 0,035 – 0,082 pg/mL) and HCs (0.053 pg/mL; IQR : 0,030- 0,067 pg/mL). Additionally, MS patients exhibited significantly lower amount of $CD45RA^+$ Tregs (56.071, IQR: 42.644 – 133.594 cells/ 100 mL of blood) than their healthy counterparts (190.556, IQR: 76.667 – 232.105 cells/ 100 mL blood).
Conclusion: MS patients demonstrated a significantly lower number of CD45RA+ Tregs compared to HCs. However, BDNF and Areg levels in serum did not significantly differ between MS patients and HCs. Further research is needed to elucidate the regenerative potential of Tregs, BDNF, and Areg in MS.

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2200 – P3.01.57

Cerebrospinal fluid biomarkers soluble CD27 and B-cell maturation antigen in multiple sclerosis and neuroinfection

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Purpose: The symptomatic overlaps between multiple sclerosis (MS) and neuroborreliosis (NB) can make them difficult to differentiate clinically. Cerebrospinal fluid (CSF) soluble CD27 (sCD27) is typically considered a marker of T-cell inflammation, but it may also reflect B-cell activation in MS and NB. CSF soluble B-cell maturation antigen (sBCMA) is a biomarker of mature B-cells and plasma cells. Assessment of T- and B-cell activation may help differentiate between these diseases. This study aimed to investigate the potential of CSF sCD27 and sBCMA to differentiate between untreated relapsing remitting MS (RRMS), NB, enteroviral meningitis (EM) and symptomatic controls (SC).
Methods: sCD27 and sBCMA were measured in CSF from age-matched RRMS (n=43), NB (n=67), EM (n=28) and SC (n=38), using electro-chemiluminescence immunoassay. Kruskal-Wallis with Dunn's test post-hoc was used for multiple comparisons, Mann-Whitney for comparing two groups, and Spearman's rank correlation analysis to assess correlations.
Results: The median concentration of sCD27 was 11.92µg/L (Q1=8.26, Q3=24.48) for RRMS, 40.66µg/L (Q1=15.56, Q3=88.04) for NB, 5.20µg/L (Q1=3.24, Q3=10.46) for EM and 2.49µg/L (Q1=1.94, Q3=2.90) for SC. The median concentration of sBCMA was 2.06µg/L (Q1=1.23, Q3=3.32) for RRMS, 3.29µg/L (Q1=1.65, Q3=9.63) for NB, 1.09µg/L (Q1=0.69, Q3=1.50) for EM and 0.9µg/L (Q1=0.76, Q3=1.63) for SC. Using multiple comparison, sCD27 effectively discriminated between all groups ($p \leq 0.01$-0.04). sBCMA was unable to discriminate between the groups of SC and EM, nor RRMS and NB. Comparing individual groups to SC, sCD27 was significantly increased in all groups (all $p<0.01$), while sBCMA was increased in RRMS ($p<0.01$) and NB ($p<0.01$), but not EM ($p=0.18$). Compared to RRMS, sCD27 and sBCMA were higher in NB ($p \leq 0.01$) and lower in EM ($p \leq 0.01$). The levels of sCD27 and BCMA correlated strongly in RRMS ($r_s=0.96$, $p<0.01$) and NB ($r_s=0.93$, $p<0.01$), less in EM ($r_s=0.79$, $p<0.01$) and SC ($r_s=0.44$, $p<0.01$).
Conclusion: The findings highlight sCD27 as a highly sensitive and dynamic inflammatory biomarker in CSF, with discriminatory ability in neuroinflammatory and neuroinfectious conditions of the central nervous system. The strong correlation between CSF sCD27 and sBCMA in RRMS and NB, indicates that sCD27 predominantly reflects B-cell activation in these conditions.

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2255 – P3.01.58

Positivity for anti-NMDAR antibodies exclusively detected by cell-based assay is not associated with clinical presentation and diagnosis of NMDAR encephalitis

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Purpose: The evaluation of anti-NMDAR antibodies (aNMDAR) is commonly performed using solely a commercial indirect immunofluorescence cell-based assay (CBA) in clinical laboratories, while tissue-based assays for screening and/or confirmation are conducted in a few centers. The clinical relevance of aNMDAR exclusively detected by CBA remains uncertain and may lead to misdiagnosis of autoimmune encephalitis.
Methods: We retrospectively examined 157 patients with a positive result for aNMDAR by CBA (Autoimmune Encephalitis Mosaic 6 kit, Euroimmun, Lübeck, Germany) referred to the Immunology Department at Hospital Clinic from March 2018 to March 2024. All samples were also tested by rat brain immunohistochemistry (IHQ). Here, we aimed to evaluate the clinical significance of aNMDAR detected only by CBA (with negative results by IHQ).
Results: Of the total cohort, 137 patients (87.3%) had positive results for aNMDAR by both CBA and IHQ. However, 20 patients (14.6%) presented positive results for aNMDAR only by CBA, with negative results by IHQ. Among these cases, 35 samples were analyzed, including cerebrospinal fluid (CSF)-serum paired samples from 15 patients, serum samples from 4 patients, and a CSF sample from 1 patient. The median age was 43 years (interquartile range: 27-58), and twelve patients were men (60%). Clinical manifestations leading to neurological consultation and antibody evaluation included: CNS inflammatory disorders (6), epilepsy with a non-inflammatory cause (3), primary psychiatric disorders (2), CNS infections (2), encephalopathy with an unknown cause (1), CNS neoplasms (1) and other nonspecific syndromes (4). In patients exhibiting clinical features mimicking autoimmune encephalitis, aNMDAR presence was also not confirmed by indirect immunofluorescence on cultured live hippocampal neurons.
Conclusion: Positive aNMDAR results exclusively identified by CBA are not associated with a well-characterized clinical presentation and diagnosis of NMDAR encephalitis. Positive results for aNMDAR detected by CBA must be confirmed by a tissue-based assay and interpreted within the appropriate clinical context to be considered clinically relevant.

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2266 – P3.01.59

Fas/FasL contributes to HSV-1 brain neuroinflammation and neurodegeneration

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The Fas/FasL pathway plays a key role in immune homeostasis and immune surveillance. In the central nervous system (CNS) Fas/FasL is involved in axonal outgrowth and adult neurogenesis. However, little is known about the role of the Fas/FasL pathway in herpes-induced encephalitis, neurodegeneration and memory impairment.
In this study, we used a neuropathogenic clinical strain of herpes simplex virus type 1 (HSV-1) in Fas- and FasL-deficient mice (lpr and gld) to study primary and latent (over 120 days) infection of the brain.
HSV-1 CNS infection induced the infiltration of Fas- FasL-bearing monocytes and T cells in the brain and also to an up-regulation of Fas and FasL expression on resident astrocytes and microglia within infected sites. Upon infection, Fas- and FasL-deficient mice (lpr and gld) were partially protected from encephalitis with a decreased morbidity and mortality compared to WT mice. Fas/FasL deficiency promoted cell-mediated immunity within the CNS, partially due to decreased tightness of the blood-brain barrier. Fas receptor stimulation abrogated HSV-1 induced activation and inflammatory reactions in microglia from WT mice, while lack of Fas or FasL led to a more pronounced activation of monocytes and microglia and also to an enhanced differentiation of these cells into a pro-inflammatory M1 phenotype. Latently infected Fas- and FasL-deficient mice showed increased deaths, with similar viral titers and proinflammatory response as lantently infected WT mice. However, Fas- and FasL-deficient mice showed less neurodegeneration markers (beta-amyloid and tau proteins) and better results in learning (NOR and labirynth tests).
Our data indicate that the Fas/FasL pathway leads to excessive neuroinflammation during HSV-1 infection, which is associated with a diminished anti-viral response and an excessive neuroinflammation further leading to neurodegeneration and memory impairments.

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2267 – P3.01.60

Functional characterization of conditioned media derived from mesenchymal stem cells transfected with anti-mir-29 family using antagomirs on cell lines of neurodegenerative diseases

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Purpose: The main objective of this work is to analyze the effect of conditioned medium (CM) from human mesenchymal stem cells transfected (tMSC) with antagomir of different components of the miR-29 family in relevant cellular models for studying neurodegenerative diseases generated from cell lines such as SH-SY5Y.
Methods: The study methodology involved characterizing the CM from mesenchymal stem cell (MSC) clones based on their immunomodulatory effect in various assays, including cell proliferation assessment, lymphocyte degranulation, Treg cells promotion, and microglial activation. Subsequently, the effect of tMSC CM with miR-29 in neurodegenerative disease cellular models was analyzed. Proliferation, cellular apoptosis, cell degranulation, neuronal ultrastructure, microglial activation, and cytokine concentration were assessed.
Results: The functional diversity of different mesenchymal stem cell (MSC) clones has been characterized, highlighting their immunomodulatory capacity through inhibition of lymphocyte proliferation, cytotoxic cell degranulation, promotion of Treg cells, along with suppression of microglial activation. Results from the analysis of miRNA levels in MSC clones revealed distinct expression patterns among miR-29 family members before transfection. MSC1 and MSC3 exhibited elevated levels of miR-29a, while miR-29b was downregulated. MSC2 showed a slight increase in miR-29c expression. Evaluating the effect of MSC conditioned media (CM) on apoptosis in SH-SY5Y cell cultures, both under normal conditions and neurodegeneration models induced by H2O2 and 6-hydroxydopamine, revealed a protective effect against apoptosis, particularly evident with untransfected CM. Related to miR-29b-1-5', similar but less pronounced effects were observed with miR-29a-5' in SH-SY5Y cell cultures. Transfecting MSC with antagomir for miR-29a-5' resulted in a modest yet significant reduction in apoptosis. These results highlight the significant roles of miR-29b-1-5' and miR-29a-5' in protecting against apoptosis in neurodegeneration models. These preliminary findings regarding the protective effects of miR-29 family members are undergoing further investigation through ongoing studies to validate and expand upon these observations.
Conclusion: In summary, this study reveals that mesenchymal stem cell (MSC) clones exhibit diverse immunomodulatory capabilities and that MSC conditioned media, especially without transfection (CMt-), have a significant protective effect against apoptosis in neurodegeneration models, suggesting therapeutic potential in neurodegenerative diseases.
Funding: This research was supported by grant CIGE/2021/162 funded by Generalitat Valenciana.

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2284 – P3.01.61

Autophagy regulation in peripheral blood mononuclear cells of patients with myasthenia gravis

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Myasthenia gravis (MG) is chronic autoimmune disease where autoantibodies mediate destruction of the neuro-muscular junction leading to weakness in voluntary muscles. The etiology is still unknown. Autophagy is a major mechanism of cellular homeostasis and it involves degradation of large intracellular cargo, such as damaged organelles or protein aggregates. It is a house-keeping process in the immune cells which regulates their energy balance and survival. Autophagy is influenced by immunological signals and it affects the responses of the immune cells. We analyzed the mRNA expression of autophagy mediators in peripheral blood mononuclear cells of MG patients. The study included 23 MG and 23 age/sex-matched healthy control subjects (HC). Autophagy related 5 (Atg5), Atg12, Microtubule-associated protein 1 light chain 3B (LC3B) and p62 mRNA levels were determined by RT-qPCR. Our results demonstrate upregulation of Atg12, LC3 and p62 in MG. Atg5 mRNA expression was unaltered in MG patients compared to healthy participants. These findings warrant further clarification of the role of autophagy-related mediators in MG pathogenesis.

The study was supported by an unrestricted grant from argenx BV (Ghent, Belgium), Ministry of Education, Science and Technological Development of the Republic of Serbia (Contract No. 451-03-9/2021-14/200110), and Serbian Society for the Peripheral Nervous System.

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P3.02 NOVEL APPROACHES TO VACCINOLOGY

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145 – P3.02.01

Modulation of HIV-1 Env antibody responses in mice with rationally designed calcium phosphate nanoparticles.

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Antigen-functionalized nanoparticles have become a major focus in experimental HIV-1 vaccine research in recent years. Biodegradable calcium phosphate (CaP) nanoparticles offer inherent advantages compared to biological and polymer-based nanoparticles. We evaluated CaP nanoparticles randomly loaded with soluble recombinant Env trimers (rCaP) in different immunization approaches. rCaP themselves induced poor anti-Env antibody responses in mice. However, functionalization of rCaP nanoparticles with a universal T helper epitope of Tetanus Toxoid (TT) enhanced the magnitude of the anti-Env antibody response, indicating an initially suboptimal MHC-II restricted T-cell help. If mice were previously immunized against TT, we were able to recruite pre-existing TT-specific T-helper cells and could further enhance anti-Env humoral immune responses via intrastructural help (ISH). We also substituted the TT peptides in the rCaP nanoparticles with an adjuvant (CpG). The magnitude of anti-Env IgG antibody levels was comparable to the ISH approach, although the anti-Env IgG subtype distribution varied. However, in contrast to CpG-adjuvanted rCaP, the ISH approach did not elicit unfavourable Env-specific IFN-γ secreting CD4 T cell responses. Thus, CaP nanoparticles represent a flexible HIV-1 Env antigen delivery platform that can be adjusted for efficient induction and modulation of humoral immune responses. As a next step, we developed and verified a highly selective, orthogonal bio-conjugation of Env trimers to the surface of CaP nanoparticles (oCaP) via post-translationally introduced aldehyde groups. The resulting oCaP were superior over rCaP in the activation of Env-specific B cells (*in vitro*) and induction of antibody responses (*in vivo*). This method maintains the native-like protein conformation and has a broad potential application in functionalization of nanoparticle platforms with stabilized candidate antigens for both vaccination and diagnostic approaches.

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323 – P3.02.02

A two-component vaccination strategy based on polyfunctional calcium phosphate nanoparticles

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Biodegradable calcium phosphate nanoparticles (CaP) offer a customizable vaccination platform. Proteins attached to the surface of CaPs act as an antigen display for B-cell recognition or guide the nanoparticles to target cells. Additionally, small molecules and/or adjuvants can be incorporated into the CaP core to be released under acidic conditions in the lysosomes of target cells.
Since the requirements for recognition of viral antigens differ fundamentally between T- and B-cell responses, we suggest a novel two-component vaccination strategy, in which the T-CaP component is optimized for the induction of T-helper cell responses through the targeting and activation of dendritic cells (DCs). On the other hand, the B-CaP component presents viral glycoproteins in the correct conformation to B-cells. By separating the induction of T-helper cell responses and B-cell responses, we also aim to improve the understanding of the requirements for the induction of long-lived effector antibody responses against the trimeric structures of the HIV-1 envelope protein (Env).
We have developed a highly selective orthogonal bio-conjugation of stabilized native-like Env trimers to the surface of calcium phosphate nanoparticles. This method maintains the trimeric conformation and has broad potential application for other stabilized candidate antigens. Moreover, we demonstrated that B-CaPs displaying orthogonally arranged Env trimers on their surface are superior in activating Env-specific B-cells (in vitro) and inducing Env-specific antibody responses (in vivo) compared to CaPs with randomly oriented Env trimers coupled.
To induce CD4+ T-helper cells, we generated T-CaPs displaying both a CD11c antibody for the targeting of DCs and an isotype non-targeting antibody. Preliminary data demonstrate an increased binding of CD11c-functionalized CaPs to murine DCs in vitro.
The optimized T- and B-CaPs are intended to be explored as a two-component nanoparticle vaccine in a humanized mouse system with regard to their immunogenicity.

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325 – P3.02.03

Design considerations towards a RIG-I adjuvanted CMV mRNA vaccine

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Cytomegalovirus (CMV) is a prevalent herpes virus that typically causes mild symptoms in healthy individuals but poses significant risks to individuals with weakened immune systems and pregnant women, often resulting in congenital infections with severe birth defects. Consequently, there is an urgent need for CMV vaccines to protect vulnerable populations and newborns from severe complications.
For this purpose, we are developing a novel concept for an mRNA vaccine targeting major CMV antigens, namely the immediate early protein IE1 and the gH/gL/gO trimeric surface complex. In order to maximize the mRNA's translational efficacy and stability, we aim at minimizing the innate immunogenicity of the mRNA itself while adding a RIG-I-based controlled adjuvant activity. By incrementally activating RIG-I, we aim to increase MHC expression and costimulatory cytokines. This will improve vaccine-induced T- and B-cell responses, leading to protective memory.
The effect of RIG-I activation on mRNA translation, stability, and cytokine release is assessed in various cell lines. Subsequently, mice are vaccinated with different mRNA constructs to confirm the beneficial effect of selective RIG-I stimulation on the induction of neutralizing antibodies and on antiviral CD8 T cell responses. By exploring our targets individually or in combination, we hope to determine the best approach for achieving optimal effectiveness against MCMV and advance to HCMV vaccine development.
In summary, our adjuvanted mRNA vaccine provides the ability to control the crucial balance between efficient antigen expression and dosed RIG-I-based innate immune stimulation to boost the adaptive immune response, ensuring effective immunity against CMV. This novel strategy holds great potential for successful vaccination against other infectious diseases and cancer.

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403 – P3.02.04

Inorganic fullerene-like tungsten disulfide nanoparticles strongly modulate the immune response in vitro

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Purpose: Tungsten disulfide (WS_2) nanoparticles have been extensively investigated in the biomedical field as theranostics due to their specific properties, including good biocompatibility. However, almost nothing is known about their effect on the immune system. This study aimed to investigate the effects of inorganic fullerene-like WS_2 (IF- WS_2) nanostructures on the immune response in vitro.
Methods: IF-WS2 nanoparticles, were used in three in vitro culture models: peripheral blood mononuclear cells (PBMCs) stimulated with phytohemagglutinin (PHA); monocyte-derived dendritic cells (MoDCs) co-cultivated with purified T cells; monocyte-derived macrophages (MoMs) and U937 cell line cultivated under M1 or M2 polarizing conditions. Several parameters were analyzed: cytotoxicity; internalization of nanoparticles by immune cells (morphological and flow cytometric analysis); T-cell proliferation of CellTrace Far Red prestained cells; production of cytokines and chemokines in culture supernatants and intracellularly; analysis of cell markers in/on MoDCs, MoMs, and T cells.
Results: IF-WS_2 were non-cytotoxic up to the concentration of 200 µg/mL. However, the concentrations of 25 µg/mL and higher inhibited PHA-stimulated proliferation of PBMC, T cells in co-culture with IF-WS2-treated MoDCs, but not purified T cells stimulated with CD3/CD28 beads. Morphological and flow cytometric data showed a dose- and time-dependent internalization of IF-WS_2 by MoMs and MoDCs but not lymphocytes. IF-WS2 decreased the production of pro-inflammatory cytokines/chemokines (IL-1β, TNF-α, IL-8, MCP-1, and GRO-α) by PHA-stimulated PBMC. The Th1 (IFN-γ), Th17 (IL-17A, IL-17F, and IL-22), and Th21 (IL-21) cytokines were down-regulated, whereas Th2 (IL-4, IL-5, and IL-3), Th9 (IL-9) cytokines and a T regulatory cytokine (IL-10) were up-regulated. These effects were also seen in IF-WS2-MoDC/T-cell cocultures and were correlated with inhibited differentiation, and maturation of MoDCs as well as the induction of their tolerogenic properties as judged by increased expression of tolerogenic markers (ILT4 and IDO-1), decreased production of IL-12 and the increased potency to induce differentiation of Tregs. In addition, IF-WS2 nanoparticles were capable of decreasing M1 and increasing M2 polarizing properties of both MoMs and U937 cells.
Conclusion: IF-WS2 nanoparticles exert a very potent modulation of the immune response in vitro through their action on antigen-presenting cells.

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435 – P3.02.05

Evaluation of the dose sparing effects of mmCT, dmLT and alum on two different vaccines in a neonatal mouse model

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Purpose: Childhood vaccinations give long term protection against many infectious diseases, but multiple vaccinations are usually required. In many cases, vaccine demand exceeds production capacity. Adjuvants can enhance magnitude and duration of immune responses and may reduce vaccine dose needed to induce protective immunity, allowing dose sparing and cost savings. Here we assessed dose sparing effects of the adjuvants dmLT, mmCT and alum on neonatal antibody (Ab) response to a pneumococcal conjugate vaccine Pn1-CRM_{197}, and of mmCT and alum to an influenza hemagglutinin (HA) vaccine.
Methods: Neonatal mice were immunized subcutaneously with fractional doses of Pn1-CRM_{197} or HA (2, 1, 0.75, 0.5 and 0.1 µg) with mmCT, dmLT or alum and compared with a full dose of the vaccine (4 µg) without adjuvant. Pn1- or HA-specific IgG antibodies were measured by ELISA in serum collected biweekly up to 8 weeks after immunization, and Pn1- or HA-specific IgG+ antibody secreting cells (ASCs) in bone marrow (BM) were enumerated by ELISpot 8 weeks after immunization.
Results: Ab levels of neonatal mice immunized with a full dose of Pn1-CRM_{197} or HA were low. Inclusion of mmCT or dmLT enhanced Pn1-specific IgG elicited by fractional doses of Pn1-CRM_{197} providing 8- and 5.3-fold dose sparing of the vaccine, respectively, and inducing protective levels against bacteremia (91-75%) and pneumonia (45-50%) in neonatal mice. mmCT also enhanced HA-specific IgG elicited by fractional doses of HA compared with full dose of HA without adjuvant, where inclusion of mmCT provided 8-fold dose sparing of the vaccine. Additionally, mmCT significantly enhanced Pn1- and HA-specific IgG ASCs in the BM 8 weeks after immunization compared with full dose vaccine only. On the contrary, mice immunized with fractional doses of Pn1-CRM_{197} or HA and alum, elicited Pn1- or HA-specific IgG in serum and IgG ASCs in BM comparable with full dose vaccine alone.
Conclusion: The adjuvants dmLT and mmCT are promising candidates for dose sparing and could be used at times when vaccine demand exceeds vaccine production capacity.

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854 – P3.02.06

Spatial analysis of rhesus macaque lung tissue shows a key role for vaccination or previous SARS-CoV-2 challenge in immune pulmonary status

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Purpose: Much has been learned about how SARS-CoV-2 drives lung immunopathology. However, studies of post-mortem human lung tissue have confounding factors including different viral loads, infection timepoints and treatments. Animal studies allow us to standardise these factors, examine a wider range of pathological states and study the impact of vaccination on protection against or enhancement of lung pathology. We have applied spatial biology techniques to study lung tissue responses to SARS-CoV-2 and propose these methods as novel tools to assess preclinical vaccine efficacy and to determine protective and pathologic mechanisms.
Methods: Using spatial transcriptomics (Nanostring GeoMx) and multiplex immunofluorescence (Leica Cell DIVE), we characterise the immune mechanisms driving pathology and protection in the lung across vaccine and challenge scenarios in rhesus macaques challenged with SARS-CoV-2. Lung tissue from vaccinated (DNA, mRNA, viral vector and formalin-inactivated vaccines), unvaccinated, and re-challenged macaques were analysed. Spatial transcriptomic profiling was carried out on both lesioned and non-lesioned areas of lung tissue from 2 macaques from each group – one with the highest in-group pathology score and one with a low pathology score. Multiplexed imaging was carried out on all of the macaques across the cohorts to examine the cellular profiles and carry out spatial analysis.
Results: Peripheral vaccinations resulted in different vaccine platform-dependent transcriptional profiles and immune infiltrates in the lung following challenge, which is a novel finding. Whole-virus exposure (whether by formalin-inactivated vaccine or previous challenge) strongly induced innate immune pathways and greater antigen presentation in the lung. Viral vector vaccination induced a highly diverse variety of immune pathways.
Conclusion: Adaptive immune cell counts in the lung increase following peripheral SARS-CoV-2 vaccination and challenge compared to challenge alone, suggesting potential recruitment of adaptive immune cells from the periphery. The profile of the resulting immune response differs according to vaccine platform and overall better-protected lung tissue was associated with immune memory, NK cell cytotoxicity, and T cell activation. Pathological responses were associated with greater presence of macrophage markers, while pathways associated with regulation of macrophage responses were upregulated in well-protected tissues. Future vaccines should consider these data to maximise protective efficacy and minimise tissue pathology.

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932 – P3.02.07

RIG-I ligand adjuvanted mRNA vaccines

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Purpose: mRNA-based vaccines have been proven to be a breakthrough technology with potential for advancing immunotherapies against cancer and infectious diseases. To ensure the vaccination success, several critical factors must be addressed including the optimization of translation of the encoded antigen as well as minimizing the mRNA immunogenicity. However, it has been shown that the adaptive immune responses to *non-mRNA-based vaccines* can be improved by immune adjuvants, originally termed 'dirty little secret of immunologists' to enhance their efficacy. Balancing the immunostimulatory effects of the mRNA vaccine seems to be crucial: while a defined amount of adjuvant can promote controlled innate immune activation beneficial for the resulting adaptive immune response, excessive activation of the innate immune system might suppress mRNA translation and might lead to the degradation of the mRNA. Here, we show a novel concept to introduce controlled adjuvant activity to a non-immunogenic mRNA.
Methods: We focus on the controlled activation of RIG-I, one of the key innate pattern-recognition receptors that sense viral RNA. Our group has identified the ligand of RIG-I to be a short, blunt-ended, double-stranded RNA with a 5'-triphosphate (3p-dsRNA). In this concept, we hybridize specifically designed and chemically modified 3p-dsRNAs to the 3' untranslated region of the mRNA.
Results: We could show *in vitro* that the RIG-I ligand adjuvanted mRNA vaccine induces an innate antiviral type I IFN response while still translating the encoded protein. Moreover, CD11b+ CD11c+ myeloid bone marrow-derived dendritic cells upregulate the expression of activation marker CD69 and CD86, and enhance presentation on MHC I and MHC II compared to a non-adjuvanted mRNA. By controlling the number of 3p-dsRNAs, we can precisely modulate the immunostimulatory properties of the mRNA, achieving an optimal balance between antigen translation and immune activation.
Conclusion: We designed a novel 3p-dsRNA-based adjuvant for an mRNA vaccine. This balanced innate immune activation allowed efficient translation, while enhancing antigen presentation and upregulation of cell surface markers, as well as the expression of co-stimulatory cytokines. Together, this combination should lead to an optimal induction of the adaptive immune response.

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1301 – P3.02.08

Recapitulating memory B cell responses in a lymphoid organ-chip to evaluate mRNA vaccine boosting strategies

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Purpose: Predicting the immunogenicity of candidate vaccines and biologicals in humans based on animal model data remains a challenge. To address this issue, we developed a lymphoid organ-chip (LO chip) model based on a microfluidic chip seeded with human PBMC at high density within a 3D matrix.
Methods: The lower channel of an $S1^R$ chip (Emulate) was seeded with human PBMC at $6x10^8$/mL within a collagen-based extracellular matrix, to mimic the high cellular densities characteristic of lymphoid tissue. The upper chip channel, used as a vascular-like compartment, was continuously perfused with nutrients and antigens for 6 to 14 days.
Results: Perfusion of the SARS-CoV-2 Spike protein mimicked a vaccine boost by inducing a massive amplification of Spike-specific memory B cells, plasmablast differentiation, and Spike-specific antibody secretion. The magnitude of Spike-specific B cell amplification in the LO chip, with a median 32.7x increase at d6, was higher than in 2D and 3D static cultures, highlighting the added value of a dynamically perfused culture. Features typical of lymphoid tissue, including the formation of activated CD4+ T cell/B cell clusters, the induction of ICOS in CD4+ T cells within the clusters, CXCL13 chemokine secretion, and the emigration of matured plasmablasts, were recapitulated in the LO chip. Importantly, myeloid cells were competent at capturing and expressing mRNA vectored by lipid nanoparticles, enabling the assessment of responses to mRNA vaccines perfused in the LO chip. Comparison of responses to Wuhan monovalent and Wuhan/Omicron bivalent mRNA vaccine boosts showed equivalent induction of Omicron neutralizing antibodies, suggestive of immune imprinting, as reported *in vivo*.
Conclusion: We developed a versatile lymph node-on-chip system suitable for the rapid evaluation of B cell recall responses. The model is responsive to protein and mRNA-encoded antigens, highlighting its potential in the preclinical evaluation of vaccine boosting strategies.

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1309 – P3.02.09

Improving paediatric vaccine responses: a neonatal mouse model of pneumococcal vaccination

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Worldwide, millions of children die every year due to infectious diseases. While vaccinations have saved millions of lives, there remains a significant need to enhance childhood vaccine efficacy. Infants receive vaccinations against dangerous infections but achieve full protection only after several booster vaccinations. This is because their immune systems are not fully mature and do not function in the same way as an adult's immune system, leaving a "window of vulnerability" in a child's life before booster vaccinations can take effect.
To maximise the effectiveness of vaccines, adjuvants can be added to boost the immune response. Most vaccines and adjuvants are developed and tested in adults; thus, effective adjuvants for the paediatric population are often overlooked. Improving current vaccine efficacy could lead to a decrease in the number of booster injections required for full protection. This, along with offering increased protection earlier in life, could impact significantly on children's health globally.
While many adjuvants currently in use are less effective in neonates and young children than in adults, we have identified cytosolic Poly(I:C) (cPIC) as an innovative adjuvant for paediatric vaccines using human cord blood, blood from healthy children, neonatal murine splenocytes and neonatal murine bone marrow derived macrophages. We have subsequently carried out proof-of-concept studies in a neonatal mouse model of pneumococcal vaccination, illustrating that addition of paediatric-relevant adjuvants to current vaccines can improve vaccine responses in early life.
When administered in the neonatal phase of life, inclusion of a RIG-I agonist with PCV13, an alum-containing paediatric vaccine, can improve pneumococcal-specific antibody responses compared to PCV13 alone. cPIC can also significantly increase B cell maturation to plasma cells and increase antigen-specific T cell responses when compared to vaccination with PCV13 alone. The increase in this range of vaccine-induced responses will likely confer increased protection to pneumococcal infection earlier in life. Moreover, these results provide proof-of-principle that inclusion of cPIC into current, alum-containing paediatric vaccines can improve paediatric responses to vaccination.

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1384 – P3.02.10

Investigating SARS-Cov2 antigen cross-reactivity of secreted (monoclonal) antibodies using droplet microfluidic linked deep sequencing

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Introduction: In recent years, the detection and characterization of antibodies from individual cells has made steady progress, allowing for the integration of read-outs, such as quantity, specificity and affinity. In this context, droplet microfluidics are powerful as they confine a cell and its microenvironment permitting a direct link between the cell and its secreted antibody inside individual pico-reactors. The aim of these technologies is not only to characterise the antibody repertoire at single-cell resolution but also to isolate a specific cell of interest for B-cell antibody sequencing. This harnessing of the natural repertoire can then lay the groundwork for developing antibody therapeutics with unique specificity and function. During the SARS-Cov2 pandemic the need for such antibodies, in particular of neutralizing function or cross-reactivity become evident. However, so far developed antibodies were losing effectiveness and were out-performed by the fast evolution of the RNA virus. To address this, powerful modelling techniques from antigen libraries coupled to next-generation sequencing were developed to scan escape mutations and antibody epitopes. While this approach permits for more analysis depths, it is aimed at characterizing antibody-virus binding with known antibodies or polyclonal serum, lacking single-cell resolution and the ability for antibody discovery. Therefore, new technologies are needed which allow to characterize and isolate secreted antibodies with additional information about their potential cross-reactivity.
Methods & Results: Using droplet microfluidics, we report a workflow to encapsulate single-cells into water-in-oil droplets, where secreted antibodies are captured using antibody-capture nanobeads. By further incorporating barcoded T7-phage libraries expressing SARS-Cov2 receptor binding domain (RBD) protein variants at high purity into each droplet, secreted antibodies can be further analysed for antigen specificity. Finally, linking to conventional fluorescent activated (droplet) sorting followed by deep sequencing results in antigen analysis from a single secreted antibody. This workflow allows for mutational profiling of the antigen (RBD), which was first demonstrated in a murine vaccination model, showing both antibodies against common and novel RBD variants. In a next step combined sequencing of antigen variant and antibody-producing cells will allow for the isolation of antibodies binding unique virus variants and discovery of potentially cross-reactive antibodies.

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1572 – P3.02.11

Comprehensive profiling of vaccine-induced tissue response using ex vivo natural human skin modules

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Purpose: In the rapidly evolving field of vaccine research, there is an urgent need for methodologies that accurately replicate human skin responses prior to clinical trials. Here, we introduce a new approach employing bio-stabilized, injectable *ex vivo* human skin modules (HypoSkin®) to investigate the early onset of tissue response induced by the injection of mRNA vaccines within the skin ecosystem.
Methods: Here we combine multiplex cytokine assays, bulk RNA sequencing, and proteomics analysis, to explore the global skin response following subcutaneous administration of the mRNA-1273 COVID-19 vaccine.
Results: Using our analytical pipeline, we reveal that vaccine injection elicits a distinct release pattern of cytokines and chemokines crucial for T cell and monocyte recruitment, strongly suggesting that immune and/or structural cells naturally present in human skin retain the ability to release immunomodulatory substances in response to vaccine administration within the modules. Additionally, by examining subtle modifications in gene expression, we can discern the precise sequential events occurring between the exogenous substance and the skin's biological processes. These events encompass the recognition and response to a foreign agent, the adjustment of skin homeostasis, the activation of stress responses, and the initiation of immune reactions, providing deeper insights into global modulation of the human skin ecosystem following vaccination.
Conclusion: Our investigation demonstrates that, by combining tissue-level analytic tools with injected HypoSkin®, we can capture the global dynamics of the natural human skin ecosystem response after injection of mRNA vaccine. This comprehensive approach not only enhances our understanding of vaccine efficacy but also provides a versatile platform for evaluating novel vaccination strategies and predicting outcomes in preclinical settings.

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1892 – P3.02.12

An investigation of novel vaccination strategies for inducing gastric mucosal immunity

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Helicobacter pylori (*H. pylori*) is the most prevalent chronic bacterial infection worldwide, colonising the gastric mucosa of approximately half of the world's population. Indeed, *H. pylori* is the leading cause of gastric cancer, and effective treatment of this infection is hindered by the ongoing rise in antimicrobial resistance. Thus, there is an urgent unmet medical need for novel strategies to prevent *H. pylori* infection.
Previous preclinical and clinical studies indicate that protection against *H. pylori* infection requires local gastric secretory IgA antibodies and mucosal T cell responses, including tissue-resident memory T cells. The development of a safe and efficacious mucosal subunit vaccine against *H. pylori* capable of inducing these responses, represents an easy, affordable solution to confer population-wide protection, but is hampered by the lack of well-tolerated, effective mucosal vaccine adjuvants. Further, incomplete knowledge of the gastric mucosal immune system renders it challenging to specifically tailor vaccination strategies for targeting this mucosal surface. Therefore, currently, the optimal vaccination strategies for inducing gastric cellular and humoral responses are unknown. However, crucial parameters include the route of vaccine administration and the mucosal adjuvants used. Here, we explore these parameters in different systemic prime-mucosal pull and mucosal-prime boost vaccination approaches, to elucidate the most effective vaccination strategies for inducing robust gastric mucosal immunity. Of note, intranasal immunisation of mice with a combination adjuvant formulation consisting of the invariant NK T cell ligand, α-galactosylceramide, and a novel chitin-derived polymer; 'C100', emerged as a promising approach for inducing gastric antigen-specific mucosal immunity.

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2085 – P3.02.13

Determining the immune response to vaccines in healthy individuals and cancer patients using a systems immunology approach

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Cancer patients have been shown to have a dysregulated immune system, leaving them more susceptible to infections and altering their response to vaccines. So far, the molecular and cellular mechanisms underlying these altered responses have not been well understood. To study this, we established a cohort of healthy persons and cancer patients, diagnosed with either multiple myeloma or non-small-cell lung cancer, who received the seasonal influenza vaccine and had blood drawn at different timepoints before and after. We hypothesise that either baseline differences or an altered immune response following vaccination lead to the divergent outcomes observed between the groups. To investigate this, we will assess data generated using high-dimensional cytometry, single-cell epigenomic profiling, cytokine profiling, and transcriptomics. Firstly, we will determine baseline differences between healthy donors and cancer patients using blood samples taken on day 0, before the administration of the vaccine. Secondly, using this data, we will attempt to identify a signature that can predict vaccine success measured by antibody levels and functionality. To do so, correlation and machine learning algorithms will be used. Lastly, samples taken on day 1, 7, and 30 will be used to track the initial immune response to the vaccine. This data will be integrated using MEFISTO and multifactorial response networks (MMRN) and changes over time between groups will be identified. In the long term, the knowledge gained through this study can contribute to the development of immune modulators to improve the vaccine response in cancer patients. Furthermore, vaccines can act as a surrogate for an infection and insights gained from this study can help to stratify individuals based on their risk for severe infection. This, in turn, can help to use limited resources in an optimal way by protecting the most vulnerable persons.

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2177 – P3.02.14

High affinity “non-cognate” ligand proteins as a new strategy for hepatitis C virus vaccine

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Purpose: Hepatitis C virus (HCV) is an enveloped, positive-sense, single-stranded RNA virus responsible for hepatitis C infection representing severe global health problem. Most of HCV-infected develop chronic infection. Among them, almost 1/4 may develop liver cirrhosis and 1/4 have chance of developing hepatocellular carcinomas. Number of therapeutic strategies with high efficacy is currently used, including direct-acting antivirals. Nowadays, no efficient HCV vaccine is available, which is caused by many reasons including genetic diversity of virus or shortage of animal models. However, number of promising vaccine candidates, such as mRNA, recombinant viral vectors, peptides, virus-like particles or DNA vaccines, undergo preclinical or clinical testing. Our novel strategy is based on the identification of proteins binding to a paratope of selected broadly neutralizing antibodies (bNAb), that could mimic cognate HCV envelope epitope and thus serve as an immunogen for eliciting neutralizing antibodies.
Methods: We tested this “non-cognate ligand” concept using highly complex combinatorial library derived from human myomesin-1 protein domain. By employing phage display, we identified proteins (binders) binding one of the selected anti-HCV bNAb and thus mimicking nature viral antigen. We immunized experimental mice with selected binders and analyzed immune response by ELISA and neutralization assays using set of HCV pseudotyped viruses of various genotypes (GT1-6) and levels of sensitivity for neutralizing antibodies.
Results: After immunization of experimental mice several binders elicited serum antibodies targeting HCV E2 envelope antigen analyzed by ELISA and neutralizing 5-6 of 15 HCV pseudoviruses, mostly of the genotype 1, 2 and 3 with the highest or medium sensitivity for neutralizing antibodies.
Conclusion: We confirmed that “non-cognate ligand” concept is effective to elicit antibodies neutralizing HCV pseudoviruses. This strategy seems to be a new promising approach in development of HCV and other infection preventing vaccine.

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2205 – P3.02.15

Design and development of a multi-epitope vaccine prototype against SARS-CoV-2

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Purpose: SARS-CoV-2 caused COVID-19 pandemic overwhelms global health care. COVID-19 vaccination is crucial for preventing infection and controlling the pandemic. Multiple safe and effective vaccines are available worldwide.
Nanotechnology provides the opportunity for construction of modern transport devices such as nanoparticles for a variety of applications in the field of medicine. A novel experimental protocol for the formation of saponin-cholesterol-phospholipid nanoparticles of vesicular structure has been developed and applied to prepare stable nanoparticles using escin or glycyrrhizin as saponin.
Here, we describe the design and development of a next-generation multi-epitope vaccine for SARS-CoV-2, consisting of epitopes recognizable by T-cells.
Methods: Structure-based and sequence-based immunoinformatics methods were used to derive models for selection of MHC binders specific for the mouse strain used in the study among a set of human SARS-CoV-2 T-cell epitopes identified by convalescent patients with COVID-19. The binders were synthesized and included in a multi-epitope vaccine prototype.
The methods for nanoparticle construction include a sonication, thus forming stable unilamellar vesicles. Tests and assays for cell viability, erythrocyte hemolysis, flow cytometry, and fluorescent microscopy analyses have been performed.
Results: The immunogenicity of the vaccine prototype designed in the present study was tested on humanized-ACE2 transgenic B6.Cg-Tg(K18-ACE2)2Prlmn/J mice by *in vitro*, *in vivo*, and *ex vivo* immunoassays. The animals were immunized with a mix of predicted MHC-I, or MHC-II, or MHC-I/MHC-II peptide epitopes in CFA, and boosted with peptides in IFA. Immunization with SARS-Cov-2 epitopes remodels lymphocyte profile. A weak humoral response and significant production of IL-4 and IFN-γ from T cells were found after vaccination of the animals.
By selecting appropriate component ratios, stable and safe particles were formulated with respect to the tested bio-cells. The versatility of these nanoparticles allows for the encapsulation of various molecules, either within the vesicle interior for water-soluble components or within the vesicle walls for hydrophobic components. These particles are successful included into isolated mouse macrophages.

Conclusion: The multi-epitope vaccine prototype presented in this study demonstrates immunogenicity in mice and shows potential for human vaccine construction.

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P3.03 PATTERN RECOGNITION RECEPTORS

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596 – P3.03.01

Development and functional analysis of inducible RNA agonists for spatiotemporal control of RIG-I activation

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RIG-I (Retinoic Acid Inducible Gene I) is an intracellular innate immune receptor that detects 5'triphosphorylated blunt end double-stranded viral RNA. Upon activation, RIG-I initiates a type I interferon response, leading to an antiviral state of the cells. Based on the activity to induce an immunogenic cell death in tumor cells, RIG-I agonists are currently developed for cancer immunotherapy. Tumor-targeted delivery of RIG-I agonists is not yet available. Therefore, we aim at inducible targeted activation of systemically administered RIG-I agonists in tumor tissue. Another objective is to use targeted activation of RIG-I to achieve progress in antiviral prevention and therapy.
To investigate the spatiotemporal control of RIG-I activation, we developed synthetic inducible RNA agonists, which were tested in various experimental setups, including primary human immune cells, THP-1 cells, and several cancer cell lines. Additionally, we generated a reporter cell line expressing EGFP under the control of the interferon-beta promoter, enabling non-invasive tracking of RIG-I stimulation at the single-cell level.
We found that activation of our inducible RIG-I agonists within the cells stimulated the production of type I interferon, supporting our concept of inducible RIG-I activation. In the absence of activation, inducible agonists were inactive.
The newly developed inducible ligands allow the targeted activation of RIG-I. Spatiotemporal control of RIG-I activation *in vivo* is expected to improve the therapeutic efficacy of RIG-I agonists while minimizing systemic side effects. Our findings open new avenues for RIG-I-mediated cancer immunotherapy and pave the way for future clinical applications of inducible oligonucleotide agonists of innate immune pathways.

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931 – P3.03.02

RIG-I like receptor activation by mRNA incorporating modified nucleosides

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The remarkable success of nucleoside-modified, *in vitro*-transcribed (IVT) mRNA vaccines against SARS-CoV-2 has brought mRNA technology to the medical mainstream, with more than 12 billion vaccine doses administered worldwide to date. Effective IVT mRNA translation requires the inhibition of innate immune RNA receptors. The co-transcriptional incorporation of modified nucleosides such as N1-methylpseudouridine (m1Ψ) and 5-methylcytosine (5mC) has been utilized to prevent activation of endosomal Toll-like receptors by transfected mRNA. While inhibitory effects on cytosolic RIG-I like receptor (RLR) activation through the reduction of double-stranded IVT byproducts have been suggested, a systematic investigation and direct comparison of nucleoside-modified mRNA sensing by the RLRs RIG-I and MDA5 has not been performed.
Here, we investigate the effects of commonly used modified nucleosides on the activation of MDA5 and RIG-I by IVT mRNA preparations and structurally defined RLR-agonistic RNAs. We quantify mRNA translation as well as activation of the type I interferon response in primary immune cells and gene-edited monocytic cell lines and analyze the differential contribution of MDA5 and RIG-I to the innate immune response against IVT mRNA.
Using this approach, we find that most nucleoside modifications have a less pronounced effect on RLR activation than previously reported, in particular on MDA5 activation. In addition, the effect of modified nucleosides on innate immune activation varies with both length and secondary RNA structure. Our results highlight the importance of rigorous assessment of innate immune stimulation for individual mRNA sequences and nucleoside modifications, with implications for the clinical effectivity of mRNA formulations and their potential inflammatory side effects.

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945 – P3.03.03

Temperature regulates toll-like receptor signalling in macrophages

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The innate immune system is the primary response to infection and injury. Innate immune cells express pattern recognition receptors (PRRs), which recognise pathogen- and danger-associated molecular patterns. PRR signalling, such as that of Toll-Like Receptors (TLRs) may induce both local inflammation and systemic fever. Febrile temperatures directly inhibit pathogenic replication and regulate immune function – including lymphocyte recruitment and activation of $CD8^+$ cytotoxic T-cells. Peripheral sites exhibit reduced local temperatures, such as the nasopharynx at ~33°C and trachea at ~35°C, which is reflected in the optimal culture and infection temperature of upper respiratory viruses. However, the role of temperature itself on PRR signalling is poorly understood.
We incubated macrophages for 16 hours at physiologically-relevant temperatures – 34°C (peripheral), 37°C (core) and 39°C (febrile) with TLR-agonists LPS (TLR4), Pam3CSK4 (TLR1/2), R837 (TLR7) or Poly(A:U) (TLR3).
In murine immortalised bone-marrow derived macrophages (iBMDM), TLR-dependent TNF secretion was upregulated at 34°C, and downregulated at 39.5°C. This behaviour was inverted for CCL5, and IL-6 showed minimal variation. Observations were consistent at transcript level (RT-qPCR), and cell viability was unchanged. Of interest at 39.5°C, the inflammasome sensor NLRP3 was also downregulated at protein level. Similar responses were observed in primary human monocyte-derived macrophages (hMDMs).
We also evaluated TLR-induced interferon regulatory factor (IRF) induction using a THP-1 reporter cell line. The IRF response to TLR activation was strongly enhanced at 34°C and suppressed at 39.5°C. Despite this, western blotting demonstrated both free and conjugated ISG15 was downregulated at 34°C and upregulated at 39.5°C, illustrating the complexity of temperature regulation.
Our data demonstrate that subtle, physiological-range changes in temperature have significant effects on TLR signalling. This has substantial implications for our understanding of dysregulated innate immune signalling in autoimmunity. With substantial climate-driven changes in environmental temperature on the horizon, it is critical we understand the interactions between temperature and innate immunity.

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1141 – P3.03.04

Characterization of STING agonist-induced monocyte cell death reveals combination of apoptosis, pyroptosis and caspase 8 activation

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Purpose: The cyclic-GMP-AMP synthase – stimulator of interferon genes (cGAS-STING) pathway senses double-stranded DNA in cytoplasm, a signal of invading pathogens or cell damage. The activation of the cGAS-STING pathway induces secretion of proinflammatory cytokines by immune cells that in turn modulate antiviral and antitumoral responses. Therefore, synthetic activators of the cGAS-STING pathway, such as STING agonists, are of therapeutic interest.
We have recently demonstrated that STING agonists not only trigger cytokine production in peripheral blood mononuclear cells (PBMCs) but also induce cell death of monocytes. Here we investigated the mechanisms of monocyte death in terms of apoptosis, pyroptosis and necroptosis.
Methods: STING agonist-induced monocyte cell death was assayed in PBMCs using multiparametric flow cytometry-based immunophenotyping combined with FAM-FLICA staining of active caspases, or phospho-flow for kinase activation. Additionally, we demonstrated the direct effect in enriched monocytes using reporter-based assays and western blot. We investigated the activation of caspases 3, 7, 1 and 8, along with kinases RIP1, RIP3 and pseudokinase MLKL. The cytokine secretion was analyzed with multiplex assay.
Results: PBMCs secreted a broad cytokine portfolio in response to STING agonist treatment. STING agonists triggered activation of apoptotic caspases 3 and 7 and pyroptotic caspase 1 in monocytes already upon 4-hour treatment. However, phosphorylation of RIP kinases or MLKL pseudokinase was not detected. STING agonists also induced caspase 8 activation and cleavage of RIP1 kinase.
Conclusion: Activation of the cGAS-STING pathway induces proinflammatory cytokine secretion in PBMCs, yet it is linked with a rapid monocyte cell death. STING agonist-induced monocyte cell death combines both apoptotic and pyroptotic processes, while necroptosis is not involved. We propose that necroptosis is blocked by active caspase 8, as RIP1 kinase cleavage fragment was detected upon STING agonist treatment. We suggest that the immunogenic monocyte cell death could be an important immunoregulatory mechanism for inhibition of proinflammatory cytokine secretion, and subsequently activation of secondary innate and adaptive immune processes.

The work was supported by the project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) – Funded by the European Union – Next Generation EU.

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1979 – P3.03.05

The RIG-I/MAVS pathway promotes Wnt signaling-dependent osteogenic differentiation and calcification of human aortic smooth muscle cells

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Purpose: It has been already well established that erroneous detection of endogenous nucleic acids by the innate immune system drives pathogenic processes of autoimmunity. Along with that, gain-of function mutations in the innate immune receptor retinoic acid-inducible gene-I (RIG-I) are associated with Singleton-Merten Syndrome (SMS). This rare genetic disorder is characterized by dental and skeletal abnormalities, as well as early and severe aortic calcification. Based on this etiological connection, we aimed to understand the underlying molecular mechanisms connecting RIG-I activation and signaling to aortic calcification.
Methods: To examine the effect of RIG-I activation on interferon (IFN) response and osteoblastic differentiation processes, we stimulated primary human aortic smooth muscle cells (HAoSMC) with a specific RIG-I ligand and performed a qRT-PCR to quantify the relative gene expression. To induce calcification *in vitro*, we cultivated the cells in a pro-calcifying medium (PCM) and visualized the calcium depositions by staining with Alizarin Red.
Results: We found that RIG-I activation in HAoSMC did not only trigger a type I IFN response, but also increased mRNA levels of the osteogenic factors bone morphogenic protein 2 (BMP-2), bone sialoprotein 2 (BSP2) and osteopontin (OPN). Moreover, HAoSMC cultivated in PCM showed production of calcium depositions. Additional RIG-I stimulation in PCM-cultivated cells showed an earlier and stronger process of calcification. Lack of RIG-I or its downstream signaling molecule mitochondrial antiviral-signaling adaptor protein (MAVS) decreased the expression of interferon-stimulated genes and osteogenic factors. Calcification was also reduced in both RIG-I- and MAVS-deficient cells. In addition, we could show that Frizzled-5 (FZD5) and Ras GTPase-activating protein-binding protein 1 (G3BP1), two factors that are connected to the Wnt-signaling pathway, play a major role in these calcification processes, as loss of these molecules resulted in a loss of calcification.
Conclusion: Our results demonstrate that RIG-I and the downstream signaling molecule MAVS are involved in the inflammatory process that drives aortic pathogenesis. In addition, we could show that this pathway is connected to the Wnt-signaling pathway, which seems to have a crucial role in aortic calcification. Further studies are required to identify the underlying molecular mechanisms in greater detail.

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P3.04 POLYMORPHISMS AND MUTATIONS IN IMMUNOGENETICS

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55 – P3.04.01

Model for the study of drug hypersensitivity based on Bucilamine and the HLA-DRB1*08:02 allele in Colombian Amerindian populations.

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Purpose: Allergic diseases and hypersensitivity reactions are common disorders that consist of an extensive genetic component that includes the Major Histocompatibility Complex molecules, which have certain alleles associated with the development of hypersensitivity to medications, among which are the HLA-DRB1*08:02 allele as a predisposing factor for hypersensitivity to Bucillamine; This drug being the starting point for the study of the relationship between hypersensitivity reactions to medications and the expression of certain MHC alleles. Objective: To find the existing relationship between drug hypersensitivity and the expression of the specific allele in Amerindian populations of the Sierra Nevada de Santa Marta and to suggest the application of the proposed methodological model in studies that seek to relate drug allergies with previously identified alleles.
Methods: A systematic search for information was carried out in certain databases such as Sience, ScienceDirect, Elsevier and Pubmed, the frequencies obtained were tabulated and organized according to their expression to later be analyzed using the MEGA7 software.
Results: A significant frequency of the HLA-DRB1*08:02 allele in the Ijka (61.7%), Arhuaco (41.5%), Kogi (17.9%) and Arsario (15%) populations.
Conclusion: Careful management of Bucilamine and drugs molecularly similar to it is recommended in susceptible Amerindian populations. At the same time, the use of the proposed model is suggested for the study of other drugs that can trigger allergic reactions based on the expression of HLA alleles.

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140 – P3.04.02

Assessing the impact of SERPING1 gene exons 5 and 6 duplications on mRNA expression in type 1- Hereditary Angioedema.

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Objective: This study aimed to evaluate the effect of duplications within exons 5 and 6 of the SERPING1 gene on mRNA expression in a patient diagnosed with Type 1 Hereditary Angioedema (HAE-1).
Introduction: HAE-1 is a rare genetic disorder characterized by recurrent and unpredictable swelling episodes due to aberrations in the C1 inhibitor (C1-INH) protein. The molecular landscape of the disorder encompasses various genetic variants, including missense and nonsense mutations, deletions, duplications, and splice defects in the SERPING1 gene. Approximately 8% of these variants are large deletions or duplications, yet their impact on mRNA expression remains largely unexplored.
Methods: We present the case of a 44-year-old female patient diagnosed with HAE-1. Genomic DNA underwent Sanger sequencing for SERPING1 gene mutations, revealing no pathogenic variants. Subsequent MLPA analysis identified a duplication spanning exons 5 and 6. To delineate the variant boundaries, Reverse-Transcription PCR (RT-PCR) was performed. PBMCs were isolated and stimulated with interferon-gamma to induce C1-INH gene expression. RNA extraction and cDNA synthesis were performed, followed by RT-PCR using SERPING1-specific primers. Quantitative PCR (qPCR) analysis was employed to measure the relative expression of C1-INH mRNA transcripts, normalized to reference genes, enabling comparative analysis between patient and control samples.
Results: Contrary to expectations, RT-PCR analysis only amplified the wild-type allele, indicating potential aberrant mRNA degradation of the duplicated allele. Quantitative analysis revealed that mRNA expression of the patient was significantly reduced (57%) compared to healthy controls (100%), indicating a significant decrease in C1-INH mRNA production and that only mRNA from the wild-type allele was being amplified.
Conclusions: Our findings suggest that genetic duplications in SERPING1 gene may disrupt mRNA integrity, leading to the activation of nonsense-mediated decay (NMD), a mRNA degradation mechanism activated upon detection of premature termination codons (PTC) in transcripts. Therefore, the complete absence of transcripts from the duplicated allele could be attributed to the activation of this degradation mechanism. This hypothesis is based on the observation that genetic duplications often disrupt mRNA integrity, potentially leading to PTC generation and subsequent degradation by NMD, as previously suggested for other genetic variants of the SERPING1 gene (Colobran et al. 2014).

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715 – P3.04.03

High risk PNPLA3 rs738409 single nucleotide polymorphism is associated with higher concentrations of CCL2 in alcoholic end-stage liver disease

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Purpose: PNPLA3 rs738409 single nucleotide polymorphism (G allele) has been pointed out as the most robust genetic predictor of severe metabolic dysfunction-associated steatotic liver disease (MASLD), associated with an increased risk for liver-related events such as decompensation or hepatocellular carcinoma (HCC). In the present research, we have analysed the association between the PNPLA3 genotype and the concentration of 13 proinflammatory cytokines in patients with alcoholic end-stage liver disease (ESLD).
Methods: Upon Ethical approval, DNA was isolated from the whole blood, and patients (106 transplant candidates with alcoholic ESLD, without HCC as determined by explant histology, and sex and age matched control patients) were genotyped for PNPLA3 rs738409 by PCR using the TaqMan assays. Concentrations of 13 selected cytokines (IL-1β, IFN-α2, IFN-γ, TNF-α, CCL2, IL-6, IL-8, IL-10, IL-12p70, IL-17A, IL-18, IL-23, IL-33) were determined in plasma by flow cytometry using the commercially available assay (LEGENDplex™ Human Inflammation Panel 1).
Results: Comparison of patients with ESLD and healthy controls revealed that there is a statistically significant ($p<0.001$, Mann Whitney test) difference in concentration of 11 cytokines (IL-1β, IFN-α2, IFN-γ, TNF-α, CCL2, IL-6, IL-8, IL-10, IL-12p70, IL-23, IL-33). There was no statistically significant difference in the concentration of IL-17 and IL-18. Forty patients with ESLD had CC genotype, 40 were heterozygotes and 26 had GG genotype, there was no difference in sex and age between the genotypes. Patients with the GG genotype had a significantly higher concentration of CCL2 (213 (136 - 265) pg/mL, median with interquartile range) than patients with GC (150 (93 - 238)) and CC genotype (139 (117 - 186)), $p<0.001$ (Kruskal Wallis test followed by Mann Whitney). No significant association was found for the remaining 12 measured cytokines ($p>0.05$ for all analyses).
Conclusion: Higher concentrations of CCL2 are associated with the PNPLA3 GG genotype in patients with alcoholic ESLD. The possible contribution of CCL2 to the development of liver-related events in alcoholic liver disease will be further evaluated. The work was supported by Croatian Science Foundation (IP-2022-10-2285, UIP-2017-05-1965, IP-2020-02-2431, DOK-2021-02-6365).

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1486 – P3.04.04

Follow-up with digital PCR of a patient with VEXAS syndrome treated with azacitidine

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VEXAS (vacuoles, E1 enzyme, X-linked, autoinflammatory, somatic) syndrome is a recently identified, adult-onset, autoinflammatory recessive X-linked disorder. It is caused by somatic mutations in the UBA1 (ubiquitin-like modifier activating enzyme 1) gene in monocytes. This enzyme plays a crucial role in the activation of ubiquitin, a protein involved in intracellular protein degradation and cellular homeostasis. VEXAS syndrome is characterized by rheumatological manifestations such as recurrent fever, skin and lung inflammation, auricular and nasal chondritis, vasculitis, deep vein thrombosis and arthralgia.
Most pathogenic mutations associated with VEXAS syndrome involve substitutions of methionine-41, with c.122T>C (p.Met41Thr) being around half of them. One treatment approach involves targeting and eliminating the UBA1-mutated monocyte population. However, the optimal standard of care for this condition is still undefined. Treatment with azacitidine, a medication that has been reported to maintain a hematological response and provide optimal symptom control, has shown to be promising in this regard.
The purpose of this work was to find a way to measure the levels of UBA1 mutations in patients with VEXAS and to establish whether the treatment with azacitidine reduces that amount. A protocol to use digital PCR (dPCR) has been developed to follow-up the levels of the main UBA1 mutation. Two probes have been designed: one probe targeting the wild-type gene (targeted with VIC probe) and another probe targeting c.122T>C (targeted with FAM probe). With the aim of validate this technique, we elaborated a calibration line using blood cells samples: one from a patient with the mutation and another from a patient with the wild type gene (both genes analyzed with next generation sequencing (NGS), obtaining R^2=0.99 and detection limit of 0.1%).The protocol was used to follow-up a patient who started the treatment with azacitidine, finding out a diminution of the mutated cells level (from 77% to 65% of mutated cells after the first azacitidine dose).
In conclusion, dPCR has been proved to be effective in detecting and monitoring the percentage of UBA1 mutated cells and is thus a way of quantifying the reduction of those mutated cells in patients with VEXAS receiving treatment with azacitidine.

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1534 – P3.04.05

New scenario: interleukin-2 receptor gamma gene mutation as a variant of uncertain significance

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Introduction: The interleukin-2 receptor gamma (IL2RG) gene is involved in lymphocyte growth and differentiation. IL2RG mutations are associated with moderate or severe X-linked combined immunodeficiency, characterized by the absence of T and NK lymphocytes. Variants in this gene can be reported as uncertain significance when no validated association with the patient's phenotype is found.
Methods: Genetic study was performed using a Primary Immunodeficiency panel by next-generation sequencing.
Results: We report the case of a 50-year-old patient with a history of recurrent sinusitis. He presented Hodgkin's lymphoma with nodular sclerosis at the age of 28 and diffuse large B cell non-Hodgkin lymphoma associated with Epstein Barr virus at the age of 46, treated with chemotherapy and local radiotherapy, achieving complete remission in both cases. Subsequently, he developed a relapse of diffuse large B-cell lymphoma at the age of 49, treated with chemotherapy and autologous transplantation. In the study of the first lymphoma, the patient presented sustained IgG, IgA and IgM hypogammaglobulinemia, decreased immunoglobulin production in vitro, absence of response to vaccines and decreased memory B lymphocytes. In view of these data, common variable immunodeficiency was suspected and substitutive treatment with intravenous immunoglobulins was started. In addition, CD4/CD8 ratio was inverted with $CD4^+$ T cell lymphocytopenia, requiring prophylactic antibiotics. Maintaining normal values of NK cells. CD132 (common gamma chain) expression in NK, B and CD4-CD8 T lymphocytes showed no alterations compared to a healthy control. The genetic study detected a heterozygous variant in TNFRSF13B gene (exon 4, c.542C>A), with pathogenicity prediction, associated with common variable immunodeficiency type 2, and a hemizygosis variant in IL2RG gene (exon 7, c.918C>A) of uncertain significance.
Conclusion: It is important to report new variants, which require future functional validation studies, to discover new scenarios with less severe forms of the disease. As well as if its association with other mutations could modify the expected phenotype of an immunodeficiency.

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1571 – P3.04.06

HLA polymorphism in *Leishmania infantum* infection

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Leishmania infantum infection is a zoonotic disease with a high incidence worldwide. The Mediterranean coast of Spain, located in the south of the country, is an endemic area. The infection produces a wide clinical spectrum, ranging from asymptomatic to patients who develop visceral disease or different varieties of cutaneous disease. The life cycle of the parasite is vectored by the female mosquito of the genus Phlebotomus. After the bite, *L. infantum* infects the monocytic-macrophagous series found in the tissues and replicates in them producing the disease. At the immunogenetic level, no genetic association with risk or protection against L. infantum infection has been described. Therefore, we propose a study that recruits four different cohorts to find human leukocyte antigen (HLA) alleles or haplotypes that may be associated with *L. infantum* infection. The cohorts collected were as follows: i) patients with visceral or cutaneous disease (SP), ii) patients with asymptomatic infection (AP), iii) cohabitants of patients with visceral or cutaneous disease (cohabitants), and iv) control group not suffering from the disease (control). After recruitment, we performed HLA typing of each group by high-resolution genotyping of HLA class I (A, B, and C) and II (DRB1 and DQB1). We performed the comparison at the allelic and haplotypic levels. In addition, we compared the frequencies of patients homozygous or heterozygous for the Bw4/Bw6 epitopes, as well as the frequency of the C1 and C2 groups of the HLA-C locus. The results showed that the HLA-B*38 allele (Pc=0.026) was increased in the cohabitant compared to the control group. On the other hand, haplotype 18.3 (HLA-A*02:01 ~B*18:01 ~C*05:01 ~ DRB1* 11:02 ~DQB1*03:01) was more frequent in patients with symptomatic disease than in the control group. The same was true for haplotype 60.3 (HLA-A*02:01 ~ B*40:01 ~C*03:04 ~DRB1* 13:02 ~DQB1*06:04). However, the latter data do not support Bonferroni correction and significance is lost. In conclusion, we show a possible protective role of the HLA-B*38 allele to infection, while haplotypes 18.3 and 60.3 may be related to the risk of developing symptomatic disease. This may help to better understand the disease and the possible development of vaccines.

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European Journal of Immunology Clinical-Basic-Translational

2114 – P3.04.07

Impaired reprogramming of the autophagy flux in maturing dendritic cells from Crohn's disease patients with core autophagy gene-related polymorphisms

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Crohn's disease (CD) is an inflammatory bowel disease whose pathogenesis involves inappropriate immune responses towards gut microbiota on genetically predisposed backgrounds. Notably, CD is associated with single-nucleotide polymorphisms affecting several genes involved in autophagy, the catabolic process that ensures the degradation and recycling of cytosolic components and microorganisms. In a clinical translation perspective, monitoring the autophagic activity of CD patients will require some knowledge on the intrinsic functional status of autophagy. Here, we focused on monocyte-derived dendritic cells (DCs) to characterize the intrinsic quantitative features of the autophagy flux. Starting with DCs from healthy donors, we documented a reprogramming of the steady state flux during the transition from the immature to mature status: both the autophagosome pool size and the flux were diminished at the mature stage while the autophagosome turnover remained stable. At the cohort level, DCs from CD patients were comparable to control in term of autophagy flux reprogramming capacity. However, the homozygous presence of *ATG16L1 rs2241880 A>G* (T300A) and *ULK1 rs12303764 (G/T)* polymorphisms abolished the capacity of CD patient DCs to reprogram their autophagy flux during maturation. This effect was not seen in the case of CD patients heterozygous for these polymorphisms, revealing a gene dose dependency effect. In contrast, the *NOD2 rs2066844 c.2104C>T* (R702W) polymorphism did not alter the flux reprogramming capacity of DCs. The data, opening new clinical translation perspectives, indicate that polymorphisms affecting autophagy-related genes can differentially influence the capacity of DCs to reprogram their steady state autophagy flux when exposed to proinflammatory challenges.

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2217 – P3.04.08

Investigation of the role of the toll-like receptor 3 (TLR3) Leu412Phe (TLR3 L412F) single nucleotide polymorphism in the pathogenesis of long COVID

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Purpose: the role of the *TLR3* L412F (rs3775291) single nucleotide polymorphism in the pathogenesis of long COVID has not been reported to date. TLR3 is central to the innate immune response against a number of viruses, including SARS-CoV-2. *TLR3* L412F has previously been shown to reduce cellular TLR3 activity and has been implicated in acute COVID-19 disease severity.
Methods: we carried out a case-control study to investigate the frequency of *TLR3* L412F in long COVID patients (n=183) and healthy controls (n=263). In addition, we investigated the effect of *TLR3* L412F on pulmonary function (% predicted FVC, DLCO, MIP and MEP), serum ACE and vitamin D levels, and olfactory function (SNIFF score) in long COVID patients.
Results: our case-control study observed no significant association between *TLR3* L412F and the development of long COVID. Furthermore, *TLR3* L412F had no significant effect on pulmonary function, serum ACE and vitamin D levels, or olfactory function in long COVID patients.
Conclusion: This is the first study to report the effect of *TLR3* L412F in long COVID patients. This study suggests that *TLR3* L412F does not significantly contribute to long COVID pathogenesis.

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P3.05 T LYMPHOCYTE REGULATION AND FUNCTION

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19 – P3.05.01

The role of NFκB inducing kinase in regulatory T cells and tissue regulatory T cell subtypes

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Purpose: Investigating the role of the non-canonical NFκB pathway, particularly NFκB-inducing kinase (NIK), in regulatory T cells (Tregs) and their subtypes across various tissues, with a focus on understanding its impact on Treg function and its involvement in inflammatory conditions.
Methods: Our study employed various approaches, including experimental models and single-cell data analysis. The investigation encompassed the role of NIK in thymically-derived and peripherally induced cells. We conducted assessments of NIK's effect in peripheral tissues and secondary lymphoid organs using experimental animal models. An alternative model of T cell transfer colitis was utilized to investigate NIK's role in inflammatory conditions. Furthermore, single-cell data were generated and analyzed through RNA sequencing and T-cell receptor sequencing.
Results: NIK deletion in all T cells led to a decrease in thymic Treg cell precursor numbers, whereas specific NIK deletion in Tregs didn't produce the same effect. Moreover, NIK was found to play a notable role in maintaining Helios$^+$ Treg subtypes and in modulating the suppressive capacity of Tregs in the periphery. In the colon, NIK signalling appeared to be important for sustaining colonic Helios$^+$ Tregs and facilitating their suppressive function. Additionally, NIK signalling was observed to be involved in the maintenance of ST2$^+$ Tregs in the visceral adipose tissue, which are critical for tissue repair and regeneration. Conversely, NIK overexpression resulted in an increase in specific Treg subtypes in the colon and induced an inflammatory phenotype under steady-state conditions.
Conclusion: Our study highlights the influence of NIK and the non-canonical NFκB pathway on Treg development and function across diverse tissues, emphasizing their crucial roles. Further investigations, including single-cell sequencing analyses and multiparameter flow cytometry, are needed to unravel the intricate mechanisms underlying NIK signalling in Tregs and its therapeutic implications. Integration of T cell transfer colitis model with single-cell data analysis aims to deepen our understanding of NIK's effects on Tregs in vivo. Ongoing investigations into tissue-specific Treg markers such as ST2 and KLRG1 seek to elucidate molecular pathways influenced by NIK signalling. This study contributes significantly to our comprehension of NIK signalling in Tregs and its potential therapeutic implications.

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114 – P3.05.02

Hybrid SARS-CoV-2 immunity induces durable cellular pan-coronavirus immunity also in the elderly

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Different vaccines and SARS-CoV-2 variants resulted in highly diverse hybrid immunity in the global population. Age, virus mutations, comorbidities, and other causes of compromised immunity influence the degree of protection in the individuals. Whilst antibody binding is often impaired by escape mutations, T cell responses are less affected and critical for long-term protection. Conserved pan-coronavirus-epitopes might further reduce the risk of T cell escape and provide immunity against potential future coronavirus spillovers. Consequently, monitoring cellular responses is critical to provide the right recommendations regarding vaccination schemes and virus containment measures especially in susceptible individuals. We utilized peptide pools of dominant SARS-CoV-2-specific and dominant pan-coronavirus epitopes with or without spike-specific epitopes to distinguish infection and vaccination status in T cell-based assays and to assess pan-coronavirus immunity. Pan-coronavirus-specific T cells are ubiquitous, boosted especially by combinations of infection and vaccination, and long-living. Their frequencies and TCR avidities are comparable throughout age but display reduced polyfunctionality in the elderly.

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118 – P3.05.03

Role of co-stimulatory molecules expressed by dendritic cells in formation of immunological synapse

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Dendritic cells (DCs) play a key role in the adaptive immune response, as they activate T cells by the formation of the immunological synapse and it's well known, that the DC has substantial influence on the executed immune response by varying the presented co-stimulatory receptors and secreted cytokines.
We know that that the arrest of scanning T-cells by DCs is not a "find and stop" scenario. They need multiple transient contacts with target cells prior to forming a stable synapse. The nature of these transient contacts and their duration plays a decisive role in the outcome of T-cell activation. However, to this day, the mechanism behind the arrest of scanning T-cells is still not fully understood.
Interestingly, maturation of DCs is important for the stability of these contacts. This suggests that co-stimulatory molecules, which are induced upon DC maturation, might regulate the formation of the immunological synapse. The fact that arrest and activation does not uniquely relies on adhesive interaction further suggests a potential role for the engagement of co-receptors in this process.
To investigate the influence of different co-receptors and cytokines presented by DCs on the arrest and subsequent synapse formation of scanning T-cells, we developed a quantitative live cell-imaging assay. It relies on the tracking of motile T-cells on a monolayer of CHO cells, which consists of a few cells that express a cognate peptide linked to MHC and co-stimulatory molecules, surrounded by many wild-type cells. This approach allows the quantification of the influence of given co-stimulatory molecules on T cell arrest and synapse formation.
Our data reveal diverse effects of TNFR family members on the propensity of T cells to establish stable contacts with APCs. While CD70 expression had no effect on CD4 or CD8 T cell arrest, CD40 increased the probability of experienced CD4 T cells to form immunological synapses. Conversely, presence of 4-1BB-L on pMHC-I expressing cells appeared to decrease the probability of naïve CD8 T cells to form stable contacts. Altogether, our data suggest that expression of specific TNFRs at the surface of cells presenting antigenic peptides regulates T cell arrest and synapse formation.

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123 – P3.05.04

Ligand-independent signalling of T cell inhibitory receptors.

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Purpose: Cytotoxic CD8+ T cells play a pivotal role in eradicating intracellular pathogens and malignant cells, providing long-term protective immunity. The activation and regulation of these cells are tightly controlled by a delicate balance between activating and inhibitory receptor signalling. Tumor cells exploit inhibitory pathways by overexpressing ligands for inhibitory receptors, evading immune surveillance. A recent study has also unveiled the existence of tonic signalling (also known as ligand-independent signalling) by PD-1. In this study, we extend our understanding of inhibitory receptor signalling by examining tonic signalling in other immunotyrosine based inhibitory motif-containing inhibitory receptors, specifically KIR2D1 and LILRB1 and their effect on early T cell activation. Furthermore, we also compare the potency of ligand-induced inhibitory signalling among all three inhibitory receptors.
Methods: We have used a system that allows us to precisely manipulate the cell surface ligand densities for both activatory and inhibitory receptors on antigen-presenting cells. This enables us to investigate inhibitory receptor signalling in the absence of ligands (tonic signalling) and the level of inhibitory ligands necessary to inhibit different strengths of activation (inhibitory potency).
Results: Our results confirm that PD-1 exhibits ligand-independent tonic signalling, and that this activity is mediated by the cytoplasmic tail. However, we find no evidence of tonic signalling in KIR2D1 and LILRB1, indicating that ligand-independent inhibitory signalling may be specific for PD-1. Furthermore, distinct differences in the ligand-dependent potency of inhibition were observed, with PD-1 demonstrating the highest inhibitory efficacy compared to LILRB1 and KIR2D1. We also employed endogenous PD-1 ligand (PD-L2) to validate receptor signalling while maintaining physiological receptor-ligand binding properties.
Conclusion: In conclusion, the study provides valuable insights into the intricate interplay between activating and inhibitory receptors on T cells which significantly influences the outcome of T cell responses. The knowledge gained can be applied to enhance the efficacy of future T cell-based immune therapies, particularly in the context of cancer treatment.

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129 – P3.05.05

Histone deacetylases 1 and 2-mediated control of intestinal CD4+ T cell immunity

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Some effector CD4+ T cell subsets display cytotoxic activity, thus breaking the functional dichotomy of CD4+ helper and CD8+ cytotoxic T lymphocytes. However, molecular mechanisms regulating CD4+ cytotoxic T lymphocyte (CD4+ CTL) differentiation are poorly understood. We previously showed that the combined activity of histone deacetylases 1 and 2 (HDAC1 and HDAC2) are crucial for maintaining CD4 lineage integrity and that HDAC1-HDAC2 levels are key determinants of CD4+ CTL differentiation in lymph nodes and spleen. However, it is not known whether HDAC1/HDAC2 are also required for the proper differentiation, regulation and function of intestinal CD4+ CTLs. In order to address this question, we are performing a comprehensive analysis, including flow cytometry, immunohistology and scRNA-seq approaches, of intestinal T cells from mice with a deletion of both Hdac1 and one Hdac2 alleles ($HDAC1^{cKO}$-$HDAC2^{HET}$). We are characterizing CD4 and CD8 subset composition as well as transcription factor and cytokine expression. Our preliminary results indicate alterations in intestinal $CD4^+$ and $CD8^+$ $TCR\alpha\beta^+$ T cells composition within the small intestine intraepithelial lymphocyte (SI-IEL) and lamina propria (SI-LPL) populations at steady-state. These data suggest that $HDAC1^{cKO}$-$HDAC2^{HET}$ mice have a distinct intestinal T cell composition in comparison to wild-type mice that might result in a compromised function of intestinal immunity in homeostasis and during infection with Citobacteria *rodentium*. Data from our ongoing study will be presented.

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183 – P3.05.06

Two Step Selection for Bias in β Chain V-J Pairing

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The β chain rearrangement in T cells is a two-step process where first D_β and J_β bind, and only then V_β is joined to the complex. We here show that the frequency of human and mouse V_β J_β combinations deviates from the one expected based on each gene usage frequency. This bias is observed mainly in functional (F) rearrangements, but also slightly in non-functional (NF) rearrangements. Preferred V_β J_β combinations in F clones are shared between donors and samples, suggesting a common structural mechanism for these biases in addition to any host-specific antigen-induced peripheral selection. The sharing holds even in clones with J_β1 that share the same D_β1 gene. V_β J_β usage is correlated with the Molecular Weight and Isoelectric Point in F clones. The pairing is also observed in the Double Positive cells in mice thymocytes, suggesting that the selection leading to such a pairing occurs before thymic selection. These results suggest an additional structural checkpoint in the beta chain development prior to thymic selection during the T cell receptor expression. Understanding this structural selection is important for the distinction between normal and aberrant T cell development, and crucial for the design of engineered TCRs.

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238 – P3.05.07

Blockade of PD-1 and costimulation of the CD27 pathway drive the Eomes-dependant proliferation of CD8 T cells with an exhausted phenotype

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Immune checkpoint blockade (ICB) therapies have strikingly advanced the oncological treatments over the past decade. This includes antagonistic and agonistic antibodies that block inhibitory or activate costimulatory receptors, thus modulating effector T cells function. However, it is observed that many cancer patients treated with ICB develop immune-related adverse events (IRAEs) that can force patients to arrest their treatment. A better understanding of the mechanisms underlying IRAEs could help counteracting them and avoiding ICB treatments disruption. Preclinical studies have shown promising results with synergistic blockade of PD-1 and activation of members of the TNF receptor superfamily such as CD27. Our project aims at carefully studying the roles of these two pathways in homeostatic conditions and at understanding the impact of in vivo mAb treatments that target them. We showed that C57BL/6 mice treated with blocking PD-1 mAb develop proliferating T cells expressing exhaustion markers in the liver, which is dependent on the transcription factor Eomes. A scRNA-seq/TCR-seq on PD-1^{KO} mice showed that exhausted T cells induced in the liver are oligoclonal and resident memory T cells (T_{RM})-derived. We also showed that prolonged stimulation of the CD27 signalling pathway reduces the T_{RM} subset differentiation while driving the expansion of an exhausted-like (Tex) population. Interestingly, $CD27^{KO}$ mice expressed more steady-state hepatic T_{RM}, which do not develop into Tex cells upon PD-1 blockade, thus suggesting an interplay between the two signalling pathways for Tex cells development. We also showed that, despite inducing similar exhausted-like populations, individual treatments induced distinct phenotypic profiles among T cells, suggesting that their effects are partly mediated by different factors. Accordingly, combination therapy (PD-1 blockade and agonist anti-CD27 mAbs) resulted in increased exhausted CD8 T cells expansion, compared to individual treatments. Overall, our results suggest a role for PD-1 and CD27 pathways in liver memory T cells homeostasis.

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301 – P3.05.08

Study of TSLP signaling through dendritic cells in driving skin T cell responses

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Purpose: Atopic dermatitis (AD) is a chronic inflammatory skin disease that affects up to 20% of children and 3-5% of adults worldwide. AD typically presents an aberrant type 2 immune response, shown by skin infiltration of T helper 2 (Th2) cells, eosinophils and basophils, accompanied Tfh cell response and an elevation of blood immunoglobulin E levels. The keratinocyte-derived cytokine TSLP has been recognized to play a key role in AD pathogenesis, but how TSLP signals through its receptor (TSLPR) expressed on dendritic cells (DCs) to drive T cell response remain to be investigated.
Methods: We used a TSLP overexpressing AD model originally established by the lab. We employed DC-selective knock-out mouse lines and gene reporter mouse tools, combined with flow cytometry and single cell transcriptomic analyses, and DC-T cell coculture to investigate TSLP-driven T cell responses.
Results: We demonstrated that TSLP-TSLPR signaling in DCs drives simultaneously Th2 and regulatory T cell (Treg) responses. We showed that TSLP-activated DCs upregulated their expression of the costimulatory molecule OX40L and took use of our newly generated OX40L knockout mice to characterize the implication of OX40L from DC in Th2/Treg responses, as well as OX40L-reporter mice to identify and characterize these DCs from surface marker expression to transcriptomic profiles.
Conclusions: Our results provide insights on how skin TSLP signals through DCs to drive different T cell responses.

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331 – P3.05.09

Identification of functional peptide-specific Nsp3, NC and M SARS-CoV-2 T-cell responses in hybrid immunity

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LBR & RPC contributed equally to this work.

Purpose: To avoid viral adaptation to vaccines, it is critical to identify and characterize SARS-CoV-2 (CoV-2)-specific T-cell responses outside the Spike (S) protein. Here, we identify CoV-2 T-cell responses targeting peptides in Nsp3, Membrane (M), and Nucleocapsid (NC) proteins and characterize the functional profile in individuals with hybrid immunity.
Methods: Comprehensive screening using a mega-matrix ELISpot assay identified a total of 27 targeted peptides distributed across the S, Nsp3 (2 peptides), M (3 peptides) and NC (1 peptide) CoV-2 proteins in individuals that received 3-dose mRNA vaccination after CoV-2 infection (n=10). For further characterization, T-cell responses to these targets were analyzed by flow cytometry. PBMCs were stained with T-cell lineage markers (CD4, CD8, CD27, CCR7), activation (CD69) and functional markers (CD107a, IFN-γ, IL-2, TNF-α). We assessed and compared phenotypes and polyfunctionality of responding T cells by SPICE v6.1.
Results: Six individuals presented responses against the S region, while three participants presented responses in Nsp3 and M and one in NC. Concerning S peptides, CoV-2-specific $CD4^+$ and $CD8^+$ T-cell responses produced CD107a, IFN-γ, IL-2, and TNF-α. We observed a trend towards an increase in the frequency of $CD107a^+$ $CD8^+$ (p=0.06) compared to $CD4^+$ T-cells. T-cell responses against Nsp3 and NC peptides expressed all cytokines tested. By contrast, CoV-2 T-cell responses against M peptides were mostly monofunctional, expressing IFN-γ^+. Regarding polyfunctional T-cell responses against S peptides, only a fraction of S-specific $CD4^+$ T-cells expressed IFN-γ^+-$IL2^+$. S-specific $CD8^+$ T-cells were IFN-γ^+CD69^+ and TNF-α^+CD69^+. Most S-specific T-cell responses were associated with CD69 expression without cytokine expression. Interestingly, Nsp3 and NC–specific $CD4^+$ and $CD8^+$ T-cell responses were mostly polyfunctional (IFN-γ^+-$IL\text{-}2^+$, IFN-γ^+-TNF-α^+, $IL\text{-}2^+$-TNF-α^+, IFN-γ^+-$IL\text{-}2^+$-TNF-α^+). The M-specific $CD4^+$ and $CD8^+$ T-cell responses had a monofunctional profile, and a small proportion of $CD4^+$ T-cells expressed IFN-$\gamma^+IL\text{-}2^+TNF\text{-}\alpha^+$.
Conclusion: This study reveals novel and polyfunctional $CD4^+$ and $CD8^+$ T-cell responses in individuals with hybrid immunity targeting peptides of Nsp3, NC, and M CoV-2 regions. These findings may inform future vaccine design.

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410 – P3.05.10

Next-level efficiency: StraightFrom Spleen Isolation Kits - standardizing isolation of untouched T cells with minimal hands-on time

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Background: Magnetic isolation of T cells from mouse spleen is a widely used technique in immunology and cell biology research. Studies using magnetically isolated T cells contribute to the understanding of the immune system, the development of new vaccines and the advancement of immunotherapy. Therefore, the selection of a reliable cell isolation method is critical for consistent experimental results.
Objectives: Development of gentle isolation strategies for highly purified T cells that preserve cell functionality. These strategies should be compatible with downstream applications and should standardise, simplify and accelerate cell isolation workflows and ensure experimental reproducibility.
Methods: Simultaneous spleen dissociation and labeling of non-target cells with an antibody cocktail was performed using the GentleMACS dissociator. Subsequent automated magnetic labelling of cells and isolation of untouched T cells (CD4, CD8 and Pan T) was performed using the autoMACS NEO Separator. Target cell purity, yield, cell activation status and functionality were assessed.
Results: Utilizing our streamlined StraightFrom Spleen workflow, we efficiently isolated untouched CD4, CD8, or Pan T cells with remarkable purity and yield. Unstimulated cells cultured overnight exhibited no expression of CD25 or CD69. However, upon overnight stimulation with the T cell Activation/Expansion Kit, a notable upregulation of the T cell activation markers was observed. Subsequently, the isolated T cells exhibited successful proliferation in culture, affirming their functional viability. These findings underscore the robustness and efficacy of T cell isolation through the StraightFrom Spleen Isolation Kits.
Conclusion: In summary, we present a novel and expedited cell isolation approach characterized by minimal manual intervention. Our accelerated isolation protocols enable the swift procurement of functional cells with superior purity and yield, offering a compelling alternative to the labor-intensive multi-step procedures commonly employed in both academic and industrial settings.

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429 – P3.05.11

Oxysterol-mediated inhibition of CD8 T cell metabolism and function

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Intermediates of both cholesterol synthesis and cholesterol metabolism can have diverse roles in the control of cellular processes that go beyond the control of cholesterol homeostasis. For example, oxidized forms of cholesterol, called oxysterols, have functions ranging from the control of gene expression, signal transduction and cell migration. This is of particular interest in the context of immunology and immunometabolism where we now know that metabolic processes are key towards shaping the nature of immune responses.
In this study we investigated the impact of two oxysterol species, 25-hydroxycholesterol (25HC) and 27-hydroxycholesterol (27HC), on the metabolism and function of CD8 T cells. 25HC is enzymatically generated by cholesterol 25 hydroxylase (Ch25h) expressed in inflammatory macrophages and tumour associated macrophages. 27HC is generated by Cyp27a1, which is highly expressed in some tumour types including Glioblastoma.
The data show that 25HC and 27HC both potently inhibit the activation of splenic CD8 T cells. In addition, when 25HC and 27HC were applied to fully differentiated cytotoxic T cells an inhibition in proliferation, metabolism and cytotoxicity was observed. This study has also investigated the mechanisms involved and revealed roles for 25HC/27HC-mediated inhibition of Srebp transcription factors and activation of LXR nuclear receptors. In addition, 25HC and 27HC were observed to directly affect the plasma membrane, altering the membrane order.
In conclusion, we show that 25HC and 27HC inhibit the metabolism and function of murine CD8 T cells through multiple mechanisms.

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The role of G-protein γ subunit 2 in the pathogenicity of CD4+ T cell in experimental autoimmune uveitis

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Autoimmune uveitis, an autoimmune non-infectious inflammatory eye condition, is studied using experimental autoimmune uveitis (EAU) *in vivo*. EAU is driven by T cells, demonstrated by the induction EAU in naïve mice via the transfer of retina antigen specific pathogenic $CD4^+$ T cells (PTCs). The purpose of this study is to understand the mechanism underlying PTCs pathogenicity in EAU. We performed RNA sequencing analysis on C57BL/6 mice comparing PTCs and endogenous $CD4^+$ T cells (ETCs) and found 135 differentially expressed genes (Padj<0.05). Of these, 106 genes were found to have human orthologues and from those genes, 22 gene candidates that were highly associated with human autoimmune diseases were identified. Three genes of interest are: CD226, linked to multiple sclerosis, type 1 diabetes, rheumatoid arthritis, and juvenile idiopathic arthritis; Klrk1/NKG2D, associated with Behçet's disease; and G-protein γ subunit 2 (Gng2), correlated with multiple sclerosis.
Published single-cell RNA sequencing studies of spontaneous uveitis in AIRE-/- knockout mice show that Gng2 expression peaks in Th1 cells, followed by Th17, T regulatory cells, and finally, Th2 $CD4^+$ T cell subtypes. Using quantitative real-time PCR, we found that in OTII transgenic mouse $CD4^+$ T cells and splenocytes, Gng2 mRNA expression increases after stimulation with anti-CD3/28 and is upregulated in retinas during early EAU. Similarly, in human T cell leukaemia cell lines (Jurkat and CCRF-CEM cells) GNG2 mRNA levels were enhanced after TCR stimulation. Using siGNG2 RNAi knockdown and the $G\beta_1\gamma_2$ small molecule inhibitor gallein, we observed that inhibiting Gng2 impedes chemotaxis toward CXCL12 in Jurkat and CCRF-CEM cells, while activation induced upregulation of Gng2 enhances chemotaxis toward CXCL12. Likewise, gallein also inhibited $CD4^+$ T cell chemotaxis towards CXCL12. Gallein also slows proliferation by inhibiting G2/M cell cycle entry in Jurkat and CCRF-CEM cells. In conclusion, targeting Gng2 in $CD4^+$ T cells could be a potential therapeutic strategy to treat autoimmune uveitis.

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452 – P3.05.13

Exploring Affinity-Dependent Metabolic Imprinting of Anti-Viral T Cell Responses

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T cell responses to viral infections are mediated by their recognition of specific viral peptide-MHC complexes by the T cell receptor (TCR). T cell functions are underpinned by changes in metabolism in response to TCR ligation, whereby the metabolic network is reprogrammed to meet the demands of the cell. To date, T cell metabolism studies have been largely based on bulk T cell populations, however each unique T cell can respond differently to a single viral antigen. Therefore, this project uses an antigen-specific approach to focus on how the affinity of a TCR determines T cell function, and how TCR affinity influences T cell metabolism to ultimately drive this response.
Specifically, this project explores antigen-specific T cells in the context of Epstein-Barr Virus (EBV). Primary EBV infection affects over 90% of the worldwide population and in some individuals, is associated with the long-term development of cancer and autoimmunity.
To investigate the immune and metabolic phenotypes of EBV antigen-specific T cells, primary cells were detected *ex vivo* using peptide-MHC tetramers. Tetramer staining was combined with metabolic probes to assess mitochondrial mass and membrane potential, as well as with SCENITH to explore the cells' dependence on glycolysis and oxidative phosphorylation.
Antigen-specific T cell clones were also generated *in vitro* by peptide-MHC tetramer staining and single-cell sorting. T cell clones were assessed for their variable TCR sequences by PCR and functional avidity by IFNɣ ELISA. A range of unique TCRs and distinct functional responses towards the same EBV epitope were identified. T cell clones with different TCR usage and functions will be compared for metabolic differences in response to antigen stimulation by various approaches, including extracellular flux analysis and SCENITH.
Overall, this project aims to explore the fundamental relationship between the TCR affinity, immune function and metabolic phenotypes of EBV antigen-specific T cells, before determining possible underlying mechanistic links. This understanding will enable investigations into differences between affinity-dependent T cell phenotypes in the periphery versus resident-memory populations at the site of EBV infection.

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488 – P3.05.14

SARM1, a TLR adapter with NADase activity, regulates the differentiation of distinct populations of CD4 T cells and their inflammatory activity

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During their activation, T cells undergo metabolic reprogramming and glycolysis becomes the main means of generating energy. NAD^+ is a crucial molecule during T cell activation, and high concentrations of this electron acceptor are required. Recently, a domain with the ability to degrade NAD^+ was discovered in the SARM1 protein. Knowing that this protein is expressed in $CD4^+$ T lymphocytes, we hypothesized that SARM1 could control T cell quiescence, activation, differentiation and memory formation by regulating NAD^+ bioavailability. We discovered that in vitro SARM1 can act in the differentiation of distinct populations of lymphocytes, and we also observed that in Th1 polarizing conditions there is a greater number of IFN^+ cells in the absence of SARM1, as well as an increase in the number of FOXP3 positive cells in cells under differentiation medium for Treg and Th17, the latter, in turn, has a reduction in IL-17 production. In vivo, we found that *Sarm1*$^{-/-}$ animals in homeostasis have more activated T lymphocytes, based on CD69 expression, but we observed no difference in the absolute number of T cell subpopulations. We also found that *Rag1*$^{-/-}$ animals transferred with $CD4^+$ T lymphocytes naive animals from *Sarm1*$^{-/-}$ animals develop more severe colitis when compared to those that received naive $CD4^+$ T cells, presenting greater weight loss, splenomegaly, greater shortening of the colon and greater compromise of the intestinal barrier, however we did not observe a difference in the number of T lymphocytes infiltrated the colon after 30 days of transfer. Our data suggest that SARM1 acts in the differentiation of Th1, Treg and Th17 cells, as well as a regulator of lymphocyte-mediated inflammation. Finally, we discovered that circulating $CD4^+$ T cells from patients with colitis have reduced SARM1 expression when compared to healthy donors, which corroborates our hypothesis that SARM1 is an immunoregulatory component of $CD4^+$ T lymphocyte biology.

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495 – P3.05.15

Polyphosphate regulates T-cell metabolism and differentiation

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Purpose: Inorganic polyphosphate (polyP) is a ubiquitous biopolymer, whose functions in mammalian cells have largely remained elusive. PolyP consists of up to several hundred phosphate moieties linked by energy rich phosphoanhydride bonds. As a negatively charged linear polymer, polyP is usually associated with divalent cations, suggesting its role in calcium signalling. Here, we explore the dynamic generation and intracellular function of polyP in murine CD4+ T-cell subsets.
Methods: We performed colorimetric phosphate detection assays, negative DAPI staining in UreaPAGE and used the fluorescently labelled PolyP-binding domain of polyphosphatase (PpxΔ12) to detect the intracellular biopolymer in T cells. To analyse differential PolyP levels in T-cell subsets, we applied in vitro T-cell differentiation assays and inhibitor treatments of isolated CD4+ T cells. To analyse the impact of decreasing polyP levels on CD4+ T cell functions, we performed protein transduction of recombinant exopolyphosphatase 1 from *S. cerevisiae* fused with a cell-permeable peptide (CPP-Ppx1). Efficient transduction of CPP-Ppx1 was assessed by immunoblotting and immunofluorescent microscopy.
Implications of the CPP-Ppx1-mediated degradation of polyP for metabolic rates and calcium mobilisation in T cells was analysed in a seahorse analyser and ratiometric calcium mobilisation assays, respectively.
Results: We found that polyP accumulates in T cells dependent on TCR stimulation strength and calcium mobilisation. Addition of IL-6 further increased, whereas TGF-β treatment decreased polyP levels in stimulated T cells. Consistently, Th17 cells displayed elevated levels of polyP compared to Treg cells. Moreover, the treatment of stimulated T cells with recombinant CPP-Ppx1 effectively transferred the enzyme into subcellular compartments and reduced the amount of polyP. Stimulated T cells treated with CPP-Ppx1 displayed altered ECR/OCR metabolism rates, intracellular calcium levels and T-cell activation. Validating these phenotypic alterations, we also found increased proliferation in T cells that express an inducible Ppx1 transgene, suggesting that polyP acts as a rheostat that restricts calcium signals in stimulated T cells.
Conclusion: Targeting polyP in CD4+ T cells may provide a novel approach to modulate calcium signalling, T-cell metabolism and differentiation.

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505 – P3.05.16

RORA proximal enhancers regulate its expression during early human Th17 cell differentiation

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Distal regulatory elements, e.g., enhancers influence cell identity by regulating transcriptional status of a given cell. Global methylation and acetylation profiling of histone-3 has been used as a marker to identify promoters and enhancers. Here, we utilized ChIP-sequencing to profile the enhancers of differentiating T helper (Th)-17 cells. Using ATAC-sequencing based profiling of differentially accessible Th17-specific chromatin loci, we identified potential enhancers responsible for fate specification of Th17 cells. Our analysis revealed autoimmune diseases associated single nucleotide polymorphisms (SNPs) were enriched near Th17-specific enhancers. We found 24 SNPs overlap with the binding sites of transcription factors active in Th17 cells. Furthermore, we identified an enhancer in the intron of RAR-related orphan receptor alpha (RORA) that was predicted to regulate RORA expression. Functional validation of this proximal RORa enhancer using CRISPR-Cas9-mediated deletion followed by luciferase reporter assay confirmed its role in regulation of RORA expression and thereby Th17 differentiation. These findings provide insights into the potential mechanism by which the RORA enhancer modulates Th17 differentiation. Additionally, they underscore the significance of regulatory SNPs within noncoding regions in conferring resistance or susceptibility to Th17 cell-mediated autoimmune pathologies.

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550 – P3.05.17

Identification of highly suppressive human regulatory T cells in old age

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Purpose: Regulatory T cells (Treg) are the main modulators of immune tolerance and homeostasis and their fitness is crucial to keeping the balance between health and disease. With age, the immune system gets dysregulated and older people become more susceptible to developing different ailments. T lymphocytes ($CD4^+$ and $CD8^+$ cells) are affected by aging processes showing a more pro-inflammatory phenotype and a less reactive status. On the other hand, well-functioning Treg cells express some markers related to aged T cells. It is not clear yet how Treg cells are modified in the course of aging. The aim of our study is to shed some light on the enigmatic field of aging Treg cells, trying to define differences between $CD4^+$ Treg cells from young and older donors.
Methods: The expression of different Treg markers was analysed using flow cytometry by staining freshly isolated human PBMCs. We also performed cell death assays to evaluate Treg susceptibility to the apoptotic stimulus Etoposide. In addition, the suppressive capacity of Treg cells was assessed by co-culturing sorted Treg on proliferating T cells.
Results: While the expression of Treg-related markers stays stable over age, we found that Treg cells from older donors express significant higher levels of the CD28 TCR co-receptor. We analysed some of the most prominent inhibitory molecules on Treg and found that $CD28^{high}$-Treg cells express more PD-1 than $CD28^{low}$-Tregs and this phenomenon increases with age. In addition, $CD28^{hi}$-Treg are mostly $CD45RO^+$ indicating an effector/memory phenotype. Moreover, $CD28^{high}$ expressing Treg suppress proliferating T cells more than their $CD28^{low}$ counterparts.
Conclusion: We have identified a subset of highly suppressive Treg cells that is more abundant in the older population compared to young. Our results propose high CD28 expression by Treg as a potential marker for aged Treg that would partially explain the restrained immune response in the elderly.

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554 – P3.05.18

Deciphering the impact of preeclampsia on γδ T cells in the decidua

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Preeclampsia, a severe pregnancy complication, is primarily diagnosed by hypertension and proteinuria, with serious risks including intrauterine growth restriction, placental abruption, and stillbirth. Human γδ T cells are enriched at the maternal-fetal interface, while little is known about their functional potential in preeclampsia.
Here, we characterize TCR repertoires, phenotypes and cytokine production of decidual γδ T cells among women with preeclampsia and those with a healthy pregnancy, and compare those to paired maternal and cord blood samples. Decidual γδ T cells show increased TNF-α and IFN-γ production in preeclampsia, suggesting a polarization toward a pro-inflammatory state. Furthermore, TCR repertoire analyses reveal that decidual γδ T cells consist of more adult-like TCR clones, and thus presumably derive from the maternal site.
In conclusion, an enrichment of inflammatory γδ T cells in the decidua during preeclampsia is evident, suggesting that γδ T cells are implicated in the pathogenesis of this pregnancy-related disease.

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557 – P3.05.19

Histone Deacetylase Class I, Especially HDAC2 Leads to Change of the CD4+ Lineage Genes and Switch the Differentiation to CD4+CD8α+ T Cells

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Purpose: Extrathymic $CD4^+CD8^+$ double-positive (DP) T cells in humans were discovered in 1986. In healthy humans, the frequencies of $CD4^+CD8^+$ T cells are very low. However, it has been shown that in certain virus or autoimmune diseases, the percentage of DP T cells in peripheral blood can increase. DP T cells are divided in two subsets: $CD4^+CD8\alpha\alpha^{low}$ and $CD8\alpha\beta^+CD4^{low}$ T cells. The development of DP T cells is still not fully understood, but there are hints of epigenetic factors that favor the expression of CD8 on $CD4^+$ T cells. Hence, we are interested in the expression of class I histone deacetylases (HDAC) in peripheral DP T cells.
Methods: Surface and intracellular expression of peripheral blood T cell markers and transcription factors were analyzed by flow cytometry. Expression of HDAC1, 2, and 3 was investigated in isolated peripheral $CD4^+$ T cells by western blot as well as RT-qPCR. Using an in-vitro assay, naïve $CD4^+$ T cells from peripheral blood of HD (n=5) were activated for 3d under Th0 polarizing conditions and HDAC class I were inhibited for 24h. Afterwards, $CD4^+$ T cells were investigated by flow cytometry.
Results: Our first results demonstrate an increased frequency of $CD4^+CD8\alpha\alpha^{low}$ T cells compared to $CD8\alpha\beta^+CD4^{low}$ T cells in peripheral blood of HD. Furthermore, $CD4^+CD8\alpha\alpha^{low}$ T cells show an increased expression of RunX3 and decreased ThPOK. The inhibition of HDAC class I increases the expression of cytotoxic markers. Coreceptor CD8α is upregulated on the surface of $CD4^+$ T cells. In contrast, ThPOK, CD27, and CD40L are downregulated, which are essential markers for the activation and lineage of $CD4^+$ T cells. The high level of HDAC2 is associated to an increased expression of CD8α and RunX3 and decreased MFI of CD27 on $CD4^+$ T cells. These first suggestions outline possible explanations for the $CD4^+CD8\alpha\alpha^{low}$ T cell lineage.
Conclusion: In summary, the regulation of class I HDAC functionality induces the cytotoxic pathway in peripheral $CD4^+$ T cells. We suggest that especially the role of HDAC2 is essential for the expression and stability of CD4 lineage genes and could be causative for $CD4^+$ T cell dysregulation.

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605 – P3.05.20

Memory CD8+ T-cell formation and pathogen origin: Investigating the connection

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Purpose: One of the remarkable features of the adaptive immune system is the creation of immunological memory. Upon recognition of its cognate antigen, CD8+ T cell undergoes phenotypical changes, transforming into short-lived effectors and long-lived memory subsets. Despite extensive exploration into the diversity of memory subpopulations, it is still not clear why and how these diverse memory subsets arise. In our research, we investigate whether the origin of pathogens (type of pathogen, mode of infection) influences the formation of memory subsets, thereby impacting the functional propertiea of the resulting immune memory.
Methods: To address our research question, we utilized established mouse infection models faturing transgenic OT-I mice (TCR recognizing ovalbumin peptide: OVA) and various pathogens expressing the OVA peptide. These pathogens included *Listeria monocytogenes* (Lm-OVA) for bacterial infection, Lymphocytic Choriomeningitis Virus (LCMV OVA) for systemic viral infection, and Influenza strain PR8 (PR8-OVA) for respiratory viral infection. At the peak of infection (day 8 pi.), blood analysis was conducted to observe the phenotype of OT-I effector cells across these diverse infections by flow cytometry. At the memory phase (day 60 pi.), we explored OT-I memory phenotypes in the spleen and lungs using single-cell RNA sequencing. To ensure relevance of our findings in a physiological matter, we further investigated also the endogenous response of polyclonal $CD8^+$ T cells.
Results: Bacterial infection predominantly formed central memory phenotype ($CD62L^+$, $CCR7^+$), systemic viral infection generated mainly effector memory phenotype ($CX3CR1^+$, $KLRG1^+$) and respiratory viral infection produced primarly tissue-resident phenotype ($CD69^+$, $CXCR6^+$) in lungs and another yet undescribed memory phenotype (expressing IFITMs, LGALS3 and ITGA1) in the spleen.
Conclusion: Obtained data suggest that the origin of pathogen influences already the trajectory of the immune response during the infection, and further affects formation of $CD8^+$ T-cell memory subsets.

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663 – P3.05.21

Human CD8+HLA-DR+CD45RC- T cells as a potential regulatory T cell population

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Purpose: Regulatory T cells (Treg) play an essential role in immune homeostasis and medical conditions such as autoimmune diseases and cancer. While their phenotype and functions are extensively described for $CD4^+$ Treg, $CD8^+$ Treg are contradictorily discussed due to a lack of precise markers. In our laboratory, an HLA-DR-expressing $CD8^+$ T cell population with regulatory capacities was described. Other groups proposed $CD122^+$ or $CD45RC^{low/-}$ as $CD8^+$ Treg markers, but none of the currently described $CD8^+$ Treg phenotypes is widely accepted. The suppressive capacity of $CD8^+HLA\text{-}DR^+$ Treg amongst different donors is very variable. These results and the necessity to define *bona fide* $CD8^+$ Treg markers prompted us to characterise these cells in more detail.
Methods: We investigated the expression of inhibitory molecules and the production of various cytokines to identify potential markers for $CD8^+$ Treg candidates. To that purpose, freshly isolated human PBMCs were stimulated with anti-CD3/anti-CD28, and T cells were analysed using flow cytometry.
Results: $CD8^+HLA\text{-}DR^+CD45RC^-$ T cells showed significantly higher percentages of the inhibitory molecules CTLA-4 and PD-1 compared to $CD8^+HLA\text{-}DR^+CD45RC^+$ T cells and $CD8^+HLA\text{-}DR^-CD45RC^-$ T cells. Additionally, IL-10 levels were significantly higher in $CD8^+HLA\text{-}DR^+CD45RC^-$ T cells compared to $CD8^+HLA\text{-}DR^+CD45RC^+$ T cells and $CD8^+HLA\text{-}DR^-CD45RC^-$ T cells, while pro-inflammatory cytokine levels where significantly lower.
Conclusion: Our results propose $CD8^+HLA\text{-}DR^+CD45RC^-$ T cells as a potential Treg population based on their cytokine profile and their expression of inhibitory molecules. The inhibitory phenotype of $CD8^+HLA\text{-}DR^+CD45RC^-$ T cells will be studied in co-culture experiments with sorted cells to analyse the impact of $CD8^+HLA\text{-}DR^+CD45RC^-$ T cells on T cell proliferation and survival.

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712 – P3.05.22

Biology and immunobiology of a population of innate-like T cells in healthy conditions and cancer

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Purpose: T lymphocytes expressing a mature $TCR_{\alpha\beta}$ are composed by conventional $CD4^+$ and $CD8^+$ T cells and unconventional T cells ($UTC_{\alpha\beta}$), including invariant natural killer T (iNKT) cells, mucosal-associated invariant T (MAIT) cells and intraepithelial lymphocytes (IELs). Recently, our group identified a population of $CD4^-$ $CD8^-$ $UTC_{\alpha\beta}$ that was not recognized by antigen-loaded CD1d and MR1 tetramers, indicating that the cells were not iNKT cells or MAIT cells. Due to the lack of CD4 and CD8 expression and positivity for $TCR_{\alpha\beta}$, we operationally called these cells double negative $TCR_{\alpha\beta}{}^+$ T cells ($DNT_{\alpha\beta}$). The biology and role of $DNT_{\alpha\beta}$ remain poorly understood.
Methods: We employed transcriptomic analyses and multiparametric flow cytometry to characterize $DNT_{\alpha\beta}$ in various tissues. *In vitro* experiments assessed their proliferation, cytokine production, and immune response polarization.
Results: $DNT_{\alpha\beta}$ were present in various tissues of healthy mice at different ages with spleen representing their main reservoir. $DNT_{\alpha\beta}$ were found in CD1d, MR1, β2m, and MHCII- deficient mice, suggesting potential restriction by alternative molecules. Transcriptomic analyses revealed that $DNT_{\alpha\beta}$ expressed a unique gene signature associated with T cell activation and a polyclonal $TCR_{\alpha\beta}$ repertoire. Upon cytokine stimulation, $DNT_{\alpha\beta}$ proliferated and produced effector molecules (IFN-γ, GRZB) at higher levels compared to conventional T cells, reflecting their ability to respond to environmental stimuli. TCR triggering further enhanced their proliferation and activation. We observed the presence of tumor-infiltrating $DNT_{\alpha\beta}$ in different cancer models. Interestingly, the conditioned medium of stimulated $DNT_{\alpha\beta}$ induced increased MHCII expression on macrophages, comparable to that induced by type 1 polarizing stimuli. In contrast, the expression of CD206, associated with type 2 polarization, was not increased, suggesting their capacity to polarize macrophages towards anti-tumor M1-like phenotype.
Finally, $DNT_{\alpha\beta}$ were found in peripheral blood and tissues of healthy donors and cancer patients, with phenotypic similarities to murine $DNT_{\alpha\beta}$.
Conclusion: We revealed the biology and functional properties of a previously poorly known population of $UTC_{\alpha\beta}$, paving the way for their use as therapeutic targets (cell therapy).

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734 – P3.05.23

High-throughput screening of small molecular compounds targeting FoxP3 identifies MAPK and STAT3 inhibitors as regulatory T cell inhibitors

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Purpose: Regulatory T cells (Tregs) have a central role in suppressing antitumor immunity and promoting tumor progression, and the Treg linage-defining transcription factor FoxP3 is key to maintaining the Treg suppressive functions. Thus, targeting FoxP3 expression in Tregs could be a promising anti-tumor approach.
Methods: We have developed a unique, phenotypic, cell-based, high-throughput flow cytometry assay for screening a library of 1522 approved drugs to identify drugs targeting FoxP3 expression.
Results: Here we found drug candidates that downregulate FoxP3 expression in Tregs, show low T cell toxicity, downregulate expression of suppressive Treg markers like LAG-3, PD-1 and ICOS, and inhibit Treg suppressive functions; these drugs converge at inhibiting MAPK and STAT3 signaling. To further characterize these drugs and determine their structure-activity relationship, we built new sub-libraries and searched for analog compound structures by *in silico* prediction. We found that several of these drugs also inhibit Treg suppressive functions in a manner similar to- or better than the original compound.
Conclusion: Although more studies are needed, these drug candidates could serve as probes for studying Treg functions, which could expand the repertoire of anti-tumor therapies. The high-throughput assay will also enable further discovery of drugs regulating Treg functions.

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959 – P3.05.26

Regulation of T cell homeostasis by extrinsic cell death pathways

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Purpose: The extrinsic cell death pathway is a critical regulator of activated T cells, responsible for AICD. Our knowledge of how extrinsic cell death pathways influence other stages of T cell development and function is less clear.
Methods: To investigate the role of extrinsic cell death in normal T cell development, we analysed mice with huCD2-driven T cell specific iCre mediated deletion of CASPASE8 from the earlier stages of T cell development, inducible Casp8ΔT^{iCD8} mice for peripheral deletion in $CD8^+$ T cells and mice expressing D138N kinase dead mutant of RIPK1 to explore the mechanism of death.
Results: We found that ablation of CASPASE8 had significant perturbations upon nonconventional thymic NKT cells development, while conventional thymocytes and nonconventional γδ T cells development was unaffected. In the periphery, CASPASE8 expression was crucial for conventional $CD8^+$ T cells and nonconventional γδ T cells survival, but appeared redundant for conventional $CD4^+$ T cells survival. The requirement for CASPASE8 by $CD8^+$ T cells appeared constitutive rather than simply developmental, since inducible deletion in peripheral $CD8^+$ T cells resulted in similar perturbations. We found that in the absence of CASPASE8, $CD4^+$ and $CD8^+$ naïve T cells were completely resistant to FasL-inducing apoptotic and necroptotic cell death, suggesting that naïve T cells are not susceptible to necroptotic cell death. We also observed that kinase dead RIPK1 partially rescued $CD8^+$ VM T cells, NKT cells and completely γδ T cells from cell death in the absence of CASPASE8 in vivo, implicating necroptosis as the mechanism of cell death in these populations.
Conclusion: CASPASE8 deletion from the earlier stages of development revealed that CASPASE8 has multiple roles in different stages of T cell subsets. CASPASE8 plays a crucial role for the survival of $CD8^+$ conventional T cells, although the mechanism of cell death is unclear. Nonconventional γδ T cells, DNNKT cells and $CD8^+$ VM T cells in the absence of CASPASE8 were susceptible to necroptotic cell death.

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1023 – P3.05.27

Pertussis booster vaccination in four age groups reveals different shaping of memory T cell responses related to primary-vaccine background

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Purpose: First and second generation whole-cell (wP) and acellular (aP) pertussis vaccines prevent severe disease and mortality in vulnerable infants. However, aP vaccines are considered less effective in reducing circulation of *Bordetella pertussis* (Bp), supposedly because they shape alternative immune memory. Here, we interrogated differential Bp-specific memory T cell responsiveness after aP booster vaccination in four age groups with wP or aP primary vaccination backgrounds.
Methods: PBMCs were collected before (D0), 28 days (D28) and one year (1yr) after aP booster vaccination from aP-primed children (7-10y, n=16), aP/or wP-primed adolescents (11-15y, n=14/14), wP-primed young adults (20-34y, n=22), and mostly wP- or non-primed older adults (60-70y, n=24) in a phase IV interventional study (Periscope-BERT). T cell responses were characterized by IFNγ/IL-13/IL-17A Triple FluoroSPOT (counting spot-forming cells (SFC)) and supernatant analysis (Th1/Th2/Th17 cytokines) upon stimulation with a peptide pool representing 132 known Bp CD4 T cell epitopes (Bp132), Filamentous Hemagglutinin (FHA), medium or aCD3 (all with aCD28).
Results: Overall, baseline (D0) levels of Bp-specific SFC and secreted cytokines were highest and especially skewed towards a Th2-dominated memory profile in younger aP-primed 7-10y and 11-14y-olds. D28 levels of specific IFNγ/IL-13/IL-17A SFC as well as Th1/Th2/Th17 cytokines were enhanced over D0 in all groups except in aP-primed 7-10y-olds. In this latter group a consistent ‘plunge’ in SFC and cytokine levels was observed on D28 compared to D0, across Th lineages. At 1yr post-booster, a trend towards (still) elevated levels of specific SFC and secreted cytokines over D0 could be seen, in all age (sub)groups.
Conclusions: aP-Primed age cohorts have an alternatively-shaped Bp-vaccine antigen-specific memory T cell compartment compared to wP-primed cohorts, with a Th2-skewed functionality and a differently regulated booster pattern. How this relates to less effective protection against Bp clearance and transmission is to be further elucidated. Generally elevated T cell responses one year post-booster indicate that enhancing immunological memory across differently primed age cohorts is feasible.

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1112 – P3.05.28

Splenic and circulating CD4+ T lymphocyte subsets in autoimmune and malignant hematologic disorders

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Purpose: T lymphocytes play a cardinal role in mediating the functions of other immune cells. Therefore, investigations of T lymphocyte counts and functions are critical in autoimmune disease and malignant disorders for elucidating the pathogenesis of such diseases as well as for elaborating management strategy and treatment options. Splenectomy represents the second line therapeutic intervention in many types of hematological disorders. Patients with immune thrombocytopenia (ITP) and hematologic malignancies (HM) undergoing splenectomy were included in this prospective study. We aim to explore the effector and suppressive CD4 T lymphocyte subsets (Th1, Th17 and Treg) in the spleen and blood, as well as to compare splenic and circulating (Th1/Treg, Th1/Th17 and Th17/Treg) cell ratios of patients and control group.
Methods: Mononuclear cells from peripheral blood and dissociated spleen tissue of patients with ITP, hematologic malignancies and controls group were purified and the expressions of the specific transcription factors (Tbet, RORγt and FoxP3) were quantified within the CD4+ compartment of T lymphocytes. Data were acquired on a FacsCalibur flow cytometer and analyzed using FlowJo® v10 software. Statistical analysis was performed using Prism Graph Pad and SPSS software. Mann-Whitney U-test was used to compare different study groups.
Results: Our data suggest no significant differences in the readouts of total CD4+ cells either in spleen or blood samples comparing ITP and HM patients. Frequencies of CD4+ T cell subpopulations: Th1, Th17 and Treg were comparable between the ITP patients and controls both in the spleen and blood samples. Importantly though, the percentages of splenic, but not circulating, FoxP3+CD4+ regulatory T cells were significantly increased in HM patients compared to control subjects and ITP patients. Our findings also showed significant difference in Th1/Treg balance in the spleen of ITP patients compared to patients with hematological malignancies.
Conclusion: Splenic T cell subsets can be considered as biomarkers to assess disease pathogenesis and, respectively, prospective therapeutic targets in patients with hematological disorders undergoing therapeutic splenectomy. The study also emphasize the importance of the biomarkers when they are used as ratios or correlative readouts.

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1113 – P3.05.29

Chronic stress fine-tunes the transcriptomics of ageing T cells in 'dirty' mice

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Purpose: Ageing has deleterious effects on the adaptive immune system, especially T cells. Since chronic stress accelerates ageing, we aim to investigate how stress modulates the transcriptomic profile and function of ageing T cells. Studying ageing T cells from tissues requires an immunologically superior mice model called 'dirty' mice that recapitulates adult 'dirty' human immune system.
Methods: We subjected aged (13-14mo) 'dirty' mice to chronic variable stress over 21 days. Immune cells, including various T cell subsets, were analysed from lymphoid and peripheral tissues by flow cytometry. T cells from different tissues were analysed by single-cell RNA-sequencing (scRNA-seq). Furthermore, T cell functionality under chronic stress was analysed by vaccinating aged mice with COVID-19 mRNA vaccine.
Results: T cell frequencies and absolute numbers in spleen remained quite robust under chronic stress. Further analysis of splenic T cells by scRNA-seq corroborated this finding wherein no significant differences were observed in the proportions of T cell subsets. Interestingly, there was an upregulation in the homeostatic genes under chronic stress. Since these genes are included in the proteostasis network, we vaccinated aged mice to investigate the impact of chronic stress on T cell responses. On stimulation, we observed increase in release of pro-inflammatory cytokines, IFN-γ and TNF-α, in stressed splenocytes but no significant changes in splenic T cell subsets. Additional analysis of immune cells in other lymphoid and peripheral tissues revealed an increase in T cells numbers under chronic stress. However, the proportions of T cell subsets in these tissues remain largely unchanged. Taken together, our data suggests the maintenance of proteostasis in splenic T cells after chronic stress in aged 'dirty' mice.
Conclusion: Loss of proteostasis is one of the hallmarks of T cell ageing. The upregulation of the homeostatic genes under chronic stress highlights a new direction in understanding the ageing T cell proteostasis that needs to be explored further. Comprehending the transcriptional dynamics of ageing T cells is clinically significant for developing effective disease management strategies in stressed individuals.

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1132 – P3.05.30

Breaking barriers: scrutinizing T cells that act at the forefront in people who develop multiple sclerosis

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Purpose: $CD4^+$ T-cells likely play a key role in breaching the blood-brain barrier (BBB) in multiple sclerosis (MS). Our previous work revealed that so-called Th17.1 ($CCR6^+CXCR3^+CCR4^{dim/-}$) cells predominate the cerebrospinal fluid (CSF) and accumulate in the blood after natalizumab (anti-VLA-4) treatment in people with MS. Especially Th17.1 cells expressing CCR5 and producing granzyme K (GZMK), may constitute a subset poised to cross the BBB as an early event in MS. Here, we assessed the skewing and effector program of GZMK-producing $CCR5^{high}$ Th17.1 cells as potential instigators of MS development.
Methods: We used a 37-color-based spectral cytometry panel that was designed to profile Th17.1 cells in paired blood and CSF from different MS cohorts. We combined spectral cytometry with bulk and single cell RNA sequencing to analyse Th17.1 cells from pre- and post-natalizumab blood. Th17.1 cells and other $CD4^+$ T-cell subsets were isolated from healthy blood to study *in vitro* induction of Th17.1-associated brain-homing/effector molecules using IL-2, IL-15, IL-12, IL-18 and/or IL-23.
Results: As a GZMK-defining marker, CCR5 co-expressed with CXCR6, IL-18R, CD69, CCR2, CD26 and KLRG1 on the Th17.1, which was hardly seen for Th1 ($CCR6^-CXCR3^+CCR4^-$) and Th17 ($CCR6^+CXCR3^-CCR4^+$). These co-expression profiles for Th17.1 cells were more prominent in MS CSF versus blood, and post- versus pre-natalizumab blood. Single cell RNA-seq analysis showed three clusters within Th17.1 cells with both GZMK (*TRYP2*) and granzyme A (*HFSP*), from which one was characterized by CXCR6 (*TYMSTR*). *Ex vivo* experiments confirmed CCR5 and CXCR6 co-expression in GZMK-producing Th17.1 cells. In the presence of IL-2 and IL-15, we found that Th17.1 cells responded to IL-12 to induce CCR5, CXCR6 and IL-18R surface expression. Only for Th17.1, IL-12 synergized with IL-18 to further upregulate CD69, a known early activation and tissue-residence marker.
Conclusion: This study reveals that GZMK-producing $CCR5^{high}$ Th17.1 cells are enriched for markers that represent pathogenic traits of BBB crossing in MS. These markers likely work together to enable a subset of Th17.1 cells to selectively activate, adhere to and break through the BBB, thereby facilitating the entry of other pathogenic lymphocytes into the MS brain.

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1153 – P3.05.31

NFAT single-deficient T cells as a therapeutic option for anti-leukemia and anti-lymphoma responses

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NFAT plays a pivotal role in transmitting signals during T-cell receptor engagement and activation of T cells. However, prolonged NFAT activation has been associated with abnormal T-cell function, particularly contributing to T-cell exhaustion and cytokine release syndrome (CRS). Besides tissue-specific transgenic mice, CRISPR/Cas9 technology allows the application of sophisticated manipulations of T cells to delete specific NFAT family members. Our protocols demonstrated efficient gene-editing of primary murine and human T cells without virus transduction. Nucleofection only transiently compromised the glycolytic reserve. In both murine and human T cells, cytokines were less expressed upon *Nfatc1* and/or *Nfatc2* knockout, while the cytotoxic activity was unchanged *in vitro*. NFATc1 was identified as crucial for the exhaustion of donor T cells in an allo-HCT model, as mice with *Nfatc1* $^{-/-}$ T cells exhibited significantly decreased exhaustion markers compared to wild type. These results provide valuable insights into the intricate role of NFAT in modulating T cell exhaustion, offering potential avenues for fine-tuning T-cell responses in cancer therapy while addressing challenges associated with prolonged NFAT activation.

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1227 – P3.05.32

Deformability cytometry of Jurkat cells for cell immunotherapy

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Purpose: CAR-T cell immunotherapy relies on the anticancer function of modified T lymphocytes, which is why a thorough understanding of their properties is essential for further optimization. Current research focuses primarily on molecular factors, but CAR-T cells also possess complex physical properties that can significantly influence the efficacy of the therapy, yet their impact remains poorly understood. Our research aims to deepen the understanding of the mechanical properties of T lymphocytes in various environments and thus contribute to the further development of CAR-T therapy.
Methods: We applied deformability cytometry and machine-learning-assisted image analysis to study the mechanical properties of the Jurkat cell line as a standard lymphocyte T model. We aim to elucidate how the deformability of Jurkat cells changes upon their activation, understand how the cytoskeleton governs their mechanics, and investigate the effects of exposure to pharmaceuticals, including lipid emulsions that are part of the intravenously administered nutrition for cancer patients. These emulsions, such as Omegaven (a fish oil emulsion) and SMOFlipid (a mixed oil emulsion), come into direct contact with T lymphocytes and have known immunomodulatory effects, yet their impact on leukocyte mechanics remains unexplored.
Results: Preliminary findings indicate that destabilization of the actin cytoskeleton with cytochalasin D has a milder effect on Jurkat cells compared to adherent epithelial cells, probably due to their small size and large nucleus that is the stiffest cellular component. Additionally, our results show that chemical activation of Jurkat cells by PMA (phorbol myristate acetate) and ionomycin does not affect cell deformability, whereas the more physiological activation with antibodies against CD3 and CD28 increases it. This finding highlights the potential importance of the T cell activation mode in therapeutic contexts. Finally, we demonstrate that the effect of lipid emulsions Omegaven and SMOFlipid on cell mechanics is small. However, Omegaven significantly reduces cell viability, which could be related to its adverse side-effects reported in clinical studies.
Conclusion: Our study represents the first systematic deformability cytometry of Jurkat cells and thus provides a crucial foundation for future research on primary lymphocytes and the optimization of CAR-T therapy.

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1253 – P3.05.33

Expression of cytotoxic T-lymphocyte associated protein 4 in interleukin-17A-producing T cells and regulatory T cells is critical for immune homeostasis: Implications for sarcoidosis pathogenesis

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Objective: Sarcoidosis is a granulomatous disease of unknown origin that primarily affects the lung. We previously found decreased expression of the co-inhibitory marker cytotoxic T-lymphocyte antigen 4 (CTLA-4) in Th17 cells and regulatory T cells (Tregs) in lung-draining mediastinal lymph nodes (medLN) of patients. However, the effects of low CTLA-4 expression in Th17 cells or Tregs are unknown.
Methods: Using the Cre-LoxP system, we generated mice in which the *Ctla4* gene was specifically targeted in IL-17A-producing cells on one (CTLA-4$^{IL17A\text{-}Hz}$) or both alleles (CTLA-4$^{IL17A\text{-}KO}$), as well as mice haplo-insufficient for CTLA-4 exclusively in Tregs (CTLA-4$^{FoxP3\text{-}Hz}$). The immune status of the mice was evaluated by multicolor flow cytometry at 8 and 30 weeks of age and during an immune response to collagen immunization.
Results: We did not find evidence for immune dysregulation in CTLA-4$^{IL17A\text{-}Hz}$ mice. In contrast, spleen and mesenteric LN of CTLA-4$^{IL17A\text{-}KO}$ mice showed a significant increase in the proportions of CCR6$^+$ T cells. Moreover, increased fractions of cells expressing the ICOS activation marker were found within total CD4$^+$ memory T (Tm) cells, CCR6$^+$ T cells and Tregs. Signs of substantial B cell activation were detected. Nevertheless, these immune alterations did not lead to any histopathological changes in tissues from CTLA-4$^{IL17A\text{-}KO}$ mice. Upon collagen immunization splenic CD4$^+$ T cells exhibited increased ICOS$^+$ cell proportions and cytokine production, but this was not associated with enhanced collagen-induced arthritis symptoms in CTLA-4$^{IL17A\text{-}KO}$ mice. In CTLA-4$^{FoxP3\text{-}Hz}$ mice, Tregs were expanded and, interestingly, the fractions of ICOS$^+$ cells were increased in the Tm, CCR6$^+$ T cell and Treg populations.
Conclusions: This study shows that expression levels of CTLA-4 in Tregs are critical for the maintenance of immune homeostasis. In IL-17A-producing T cells CTLA-4 expression levels appears less critical and only a complete deficiency induces immune dysregulation. We observed a reciprocal influence between CTLA-4 expression levels on Th17 cells and Tregs. This would be consistent with a model in which both Tregs and Th17-like cell subsets with reduced CTLA-4 expression in sarcoidosis patients may support increased T cell activation and inflammation.

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1305 – P3.05.34

Quantitative and functional heterogeneity in CD4+ T cell responses to primary COVID-19 mRNA vaccination in older adults

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Purpose: Upon vaccination, older adults generally mount lower and more heterogeneous specific T cell responses. Congruently, we previously observed a greater degree of variability in SARS-CoV-2 Spike-specific cellular responses induced by mRNA-1273 vaccination with older age, as measured by IFNγ ELISpot. More in depth understanding of the quantity, phenotype, and functional capacity of Spike-specific $CD4^+$ T cells in the aging and vulnerable population is crucial to improve (future) vaccination strategies.
Method: Hence, we interrogated the Spike-specific $CD4^+$ T cell responses of adult participants (25-78yrs of age) in the VITAL-Corona study before and 28 days after two-dose mRNA-1273 vaccination. Based on age and degree of Spike-specific cellular responsiveness by IFNγ ELISpot (cut-off 10 spot-forming cells/10^5 PBMCs), three distinct groups were defined; low versus high responding older adults (OA-LR versus OA-HR; 60+yr) and high responding younger adults (YA-HR; 25-45yr) as a control group. The quantity, phenotype and functional capacity of the specific $CD4^+$ T cells were further characterized upon 24h stimulation with a Spike-peptide pool using a combined flowcytometric Activation-Induced Marker (AIM) and Intracellular Cytokine Staining (ICS) assay.
Results: Notably, SARS-CoV-2-specific $CD4^+$ T cells were induced following primary COVID-19 vaccination in all three groups. However, the OA-LR group showed overall lower AIM^+ and/or cytokine-producing $CD4^+$ T cells compared to the OA-HR and YA-HR groups. Although a similar trend was found for any combination of 2 out of 4 tested AIM markers (i.e. CD69, CD134, CD137 and CD154), mainly the $CD69^+CD134^+$ $CD4^+$ T cell subset was impaired in the OA-LR group. Additionally, in the Spike-specific AIM^+ $CD4^+$ T cell compartment, reduced frequencies of IFNγ- and IL-10-producing, but similar levels of TNFα- and IL-2-producing $CD4^+$ T cells were observed in the OA-LR group.
Conclusions: Together, following primary COVID-19 mRNA vaccination, OA-LR mount reduced Spike-specific $CD4^+$ T cell responses based on (mostly $CD69^+CD134^+$) activation marker expression and with distinct functional capacities, as the response is skewed away from IFNγ production. These insights underpin the observed cellular responses measured by IFNγ ELISpot at the cohort level, and suggest that an AIM assay might be more suitable to evaluate T cell responses in older adults.

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1428 – P3.05.35

Contribution of circulating Th2-like Tfr and Th2-like Treg cell subsets to early detection in patients with lung adenocarcinoma

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Objective: This study aims to investigate alterations and clinical implications of circulating Th2-like Tfr and Treg subsets in early-stage lung adenocarcinoma (LUAD) patients.
Methods: Peripheral blood samples from 31 healthy individuals and 34 LUAD patients underwent flow cytometry analysis to identify CD4+ T cell subsets, specifically CCR6-CXCR3- Th2-like regulatory T (Treg) cells and CCR6-CXCR3- Th2-like follicular regulatory T (Tfr) cells. IL-4 secretion in Th2-like Treg/Tfr cells was quantified. Dynamics of Th2-like T cell subsets were monitored in 17 patients both pre- and post-cancer resection. Receiver Operating Characteristic (ROC) curves assessed the diagnostic potential of Th2-like T cell subsets for early-stage LUAD.
Results: LUAD patients exhibited a significant increase in both the percentage and absolute count of Th2-like Treg and Th2-like Tfr cells. Following tumor resection, there was a notable decrease in the absolute count of Th2-like Tfr/Treg cells. Additionally, LUAD patients showed a substantial decrease in IL-4 secretion by Th2-like Treg cells. ROC analysis revealed areas under the curves of 0.8155 and 0.7187 for percentages, and 0.7068 and 0.8321 for absolute numbers of Th2-like Tfr and Th2-like Treg cells, respectively, effectively differentiating early LUAD patients from healthy individuals.
Conclusion: The noticeable upregulation of circulating Th2-like Tfr and Th2-like Treg cell subsets in early-stage LUAD patients suggests their potential involvement in LUAD onset and progression, thereby contributing to its early diagnostic potential.

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1520 – P3.05.37

Development of a CRISPR/Cas9 system for functional studies of transmembrane adaptor protein knockout T cells

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Background: The bacterial CRISPR/Cas9 defense system targets foreign DNA for destruction via incorporation of copies of this foreign DNA into its CRISPR locus. During subsequent infections with the same pathogen, the pathogenic DNA is recognized and CRISPR-RNA (crRNA) transcribed from the CRISPR locus. Transactivating CRISPR-RNA (tracrRNA) then basepairs with the crRNA and forms a functional unit to act as a scaffold for the Cas nuclease, resulting in a guideRNA (gRNA). Hence, the CRISPR/Cas9 technology can be used for targeted deletion or the knock-in of genes of interest.
Objectives: In this project, we utilize CRISPR/Cas9 to delete **modifiers of TCR-mediated signaling** processes (e.g. the **T**-cell **R**eceptor **I**nteracting **M**olecule (TRIM) and the **S**HP2-**I**nteracting **T**ransmembrane adaptor protein (SIT)), **metabolic key enzymes** and **lineage transcription factors** in murine and human T cells and monocyte populations. Our aim is to dissect the precise immunological functions of these proteins in primary hematopoietic cells. In the long run we aim to use our findings to finely tune innate and adaptive immunity e.g. against tumors and, further, to improve chimeric antigen receptor (CAR) T cell therapy.
Methods: sgRNAs for targets are cloned into a retro-gRNA-eGFP vector, in which we exchanged the eGFP reporter with the Thy1.1 reporter. In addition, we crossed Rosa26-LSL-STOP$^{fl/fl}$-Cas9-EGFP mice with Cre deleter mouse strains (e.g. CD4-Cre), enabling cell type-specific Cas9 expression. By western blotting, FACS-analysis, qPCR- and calcium measurements as well as single cell RNA sequencing, we assess cell functionality, activity and metabolic fitness in wildtype cells and target knockout cells.
Results and conclusion: Our results demonstrate that our *in vitro* transduction system is functional and reproducible. Upon knockout of immune checkpoint proteins and SIT and TRIM target proteins, key cytokines and metabolic targets, activation status, proteins involved in antigen presentation and cytokine profiles are altered.

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Impact of Estrogen receptor signaling on Th17 cell polarization: possible contribution to Systemic lupus erythematosus development?

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Females tend to mount a more robust immune response, leading to an increased prevalence of many autoimmune conditions. Paradoxically, the female hormone 17-β-estradiol (E2) has been attributed an immunosuppressive role by inhibiting differentiation of healthy mouse naïve CD4 T cells (Tn) into Th17 cells, which promote inflammation in many autoimmune conditions, including Systemic lupus erythematosus (SLE). Therefore, we want to analyze the impact of E2 signaling via the estrogen receptors (ERs) in Th17 polarization in male and female human cells, and assess whether alterations in this pathway may contribute to SLE.
We measured the effect of E2, as well as agonists and antagonists of the two ERs (ERa and ERb), on the polarization of human CD4 Tn cells into Th17 cells *in vitro* by measuring the expression of the Th17-defining transcription factor RORγt and the functional marker CCR6. We observed that an ERα agonist inhibited Th17 polarization in healthy female donors, but not in males. Correspondingly, physiological levels of E2 led to a trend of reduced Th17 cell polarization only in females. Preliminary results suggest that E2/ERα signaling maintained their inhibitory effect on Th17 polarization in SLE. In contrast, signaling via ERβ inhibited CCR6 expression in both healthy female and male donors, even in the absence of E2 – indicating that ligand-independent ERβ signaling inhibits the pro-inflammatory potential of Th17 cells. Preliminary analyses indicate that this immunosuppressive effect by ERβ may be lost in SLE patients.
We have shown a sex-specific effect of ligand-induced ERa signaling on Th17 polarization, as well as an E2-independent effect of ERb on CCR6 expression in healthy females and males. While the role of E2 and ERa signaling appears to be maintained in SLE, preliminary results suggest that the estrogen-independent immunosuppressive effect of ERb may be lost in SLE. Understanding ER signaling in CD4 T cells in SLE will help shed light on the sex bias and pathology of the disease, as well as help inform estrogenic contraceptive choices for SLE patients.

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CD25-biased IL-2/anti-IL-2 mAb complexes potently expand antigen-primed CD8+ T cells and overcome Treg cell-mediated suppression of activated CD8+ T cells

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Purpose: IL-2 is a critical cytokine for the proliferation, survival, and expression of effector functions in activated T cells, but its therapeutic utility in cancer immunotherapy has been hindered by off-target effects leading to serious toxicity and concomitant expansion and increase in the suppressive activity of T regulatory (Treg) cells. Activated $CD8^+$ T cells are pivotal in cancer immunotherapy due to their cytotoxic activity leading to the elimination of tumor cells. The purpose of this study was to investigate the efficacy of CD25-biased IL-2/anti-IL-2 mAb complexes, formed by rmIL-2 and JES6-1A12 mAb (IL-2/JES6), in expanding antigen-primed $CD8^+$ T cells and inducing their effector functions. Since IL-2/JES6 also potently expands Treg cells, we investigated the possibility whether IL-2/JES6 is capable of overcoming Treg cell-mediated suppression of activated $CD8^+$ T cells.
Methods: We utilized a model of OT-I $CD8^+$ T cell adoptive transfer into congenic B6 mice with subsequent priming with ovalbumin and treatment with CD25-biased IL-2/JES6. Flow cytometry analysis evaluated the expansion of adoptively transferred $CD8^+$ T cells and the expression of activation and effector markers. *In vitro* Treg suppression assays examined the effect of IL-2/JES6 on Treg cell-mediated suppression of activated $CD8^+$ T cell proliferation.
Results: CD25-biased IL-2/JES6 potently expanded antigen-primed OT-I $CD8^+$ T cells as well as Treg cells. IL-2/JES6 promoted the upregulation of CD25, granzyme B, and perforin in expanded $CD8^+$ T cells. We demonstrated that IL-2/JES6 effectively abolished Treg cell-mediated suppression of activated $CD8^+$ T cell proliferation, indicating that IL-2 sequestering is a dominant mechanism in how Treg cells suppress activated $CD8^+$ T cells.
Conclusion: CD25-biased IL-2/JES6 potently and selectively expanded the antigen-primed $CD8^+$ T cells but also Treg cells. However, Treg cells were not able to suppress activated $CD8^+$ T cells in the presence of IL-2/JES6.

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1638 – P3.05.41

Histone Deacetylase 1 as a regulator of CD4+ T cell expansion in skin autoimmunity

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The skin constitutes the first line of defense against pathogens and toxins derived from the environment. It mounts host-protective responses while maintaining immune homeostasis. These functions are fulfilled by immune cells, including $CD4^+$ T cells. Histone deacetylases (HDACs) regulate the acetylation status of histones and non-histone proteins by removing acetylation marks on lysine residues. Hence, HDACs control several biological processes, such as differentiation and function of $CD4^+$ T cells. However, the exact role of HDAC1 in $CD4^+$ T cells, specifically in skin autoimmune diseases, is not well understood.
To assess the role of HDAC1 in the regulation of cutaneous $CD4^+$ T cells, we utilized a well-established mouse model of experimental skin autoimmune disease (K5/TGO) in which ovalbumin (Ova) is expressed by keratinocytes in a tetracycline-dependent manner. We adoptively transferred HDAC1-WT or HDAC1-deficient (HDAC1-cKO) naïve Ova-specific $CD4^+$ OTII T cells in K5/TGO/TCR$\alpha^{-/-}$ recipient mice. Transferred OTII cells responded to the neo-self-antigen Ova in the skin and elicited inflammation. Recipients of HDAC1-cKO T cells displayed increased skin inflammation. This was in line with a decreased fraction of peripherally induced $Foxp3^+$ Treg in HDAC1-cKO OTII recovered from K5/TGO recipients and with increased numbers of HDAC1-cKO T cells in the skin-draining lymph nodes (sdLNs).
Surprisingly, in a competitive setting of HDAC1-WT and HDAC1-cKO T cells, HDAC1-cKO T cells showed a disadvantage for expansion both in sdLNs and skin as well as reduced activation and skin-homing, which might indicate that HDAC1 impacts the potential of OTII to interact with antigen-presenting cells. To test this, we will study early events of T cell activation and proliferation and chemokine receptor regulation of HDAC1-cKO versus WT $CD4^+$ T cells. With the help of already obtained scRNA-sequencing data, we hope to further understand transcriptional changes and determine HDAC1-dependent regulators of T cell expansion and TCR signaling in skin autoimmunity.

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1651 – P3.05.42

Pro-inflammatory response of human memory CD8 T cells are kept in check by GZMB B regulatory B cells.

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Purpose: Memory CD8 Tcells are critical mediators of antiviral and antitumor immunity. However, an uncontrolled response can contribute to autoimmune diseases or allograft rejection. Elucidating the mechanisms by which memory CD8 T cells are regulated is important for understanding and treating various disease states. Emerging evidence highlights the role of B cells in the regulation of CD8 function. This study aims to evaluate the proliferation, cytotoxic and inflammatory function of CD8T memory cell subsets after coculture with GZMB regulatory B cells (GZMB Breg).
Methods: Purified CD8T cell subsets (naive CD45RA+CCR7+, effector memory [EM] CD45RA-CCR7- and EM expressing CD45RA [TEMRA] CD45RA+CCR7-) from healthy volunteers were stimulated with aCD3aCD28 beads + IL15 for 3 days with in vitro induced GZMB Breg or resting B cells. Proliferation, expression and production of cytokines and cytotoxic molecules (TNFa, IFNg, GZMB, PERF) were measured by spectral flow cytometry. A redirected cytotoxic assay was used to assess CD8T cell cytotoxicity. The activation of glomerular endothelial cells was assessed by flow cytometry after incubation with coculture supernatant.
Results: The proliferation of memory (TEMRA and EM) CD8T cells and their expression of cytotoxic molecules were inhibited by GZMB Breg compared to CD8T alone. In contrast, resting B cells promoted the inflammatory function of memory CD8T. The cytotoxicity of memory CD8T cells was greatly reduced after coculture with GZMB Breg, especially for TEMRA CD8 T cells. Activation of glomerular endothelial cells was reduced when the supernatant of memory CD8T cells was treated with GZMB Breg compared to CD8T with resting B cells or CD8T alone. Studies in kidney transplant recipients with rejection or stable grafts are ongoing.
Conclusion: We show that the diverse pro-inflammatory functions of human memory CD8T cells are strongly influenced by the B cell, with GZMB Breg inhibiting their function and resting (and potentially activated) B cells promoting their function. The mechanisms by which GZMB Breg cells control CD8 T cell function and the impact of allotransplantation on this regulation are under investigation.

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1705 – P3.05.43

Fast & efficient particle-free isolation of mouse CD304+ natural regulatory T cells

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CD4+ regulatory T cells (Tregs) (CD25+Foxp3+) are important in immune tolerance and homeostasis because of their role in suppressing effector immune responses. Isolation of Tregs is required for downstream molecular characterization and functional assays. Existing products that isolate mouse Tregs involve a time-consuming two-step protocol where enrichment for CD4+ T cells is followed by positive selection of CD25+ Tregs. These cells are often particle-bound and may impede IL-2 signaling due to antibody blocking of IL-2Rα (CD25), which can impact downstream applications. We have developed a novel, fast, and particle-free Treg isolation strategy that targets the mouse natural Treg marker, CD304 (neuropilin-1), and does not block IL-2Rα signaling. Natural Tregs were isolated from mouse spleen and lymph nodes using the Mouse Release CD304+ Regulatory T Cell Isolation Kit and assessed for purity by flow cytometry. Treg suppression and in vitro IL2-mediated expansion assays were performed to validate the function of isolated Tregs. Using this isolation strategy, Tregs were obtained in under 45 minutes with CD4+CD304+ Treg purities of 90.7 ± 3.0% and 92.1 ± 0.7% for spleen and lymph node, respectively (mean ± SD); the majority (~80%) of isolated cells were CD25hiFoxp3+. CD4+CD304- responder T cells were also obtained in parallel isolations with a purity of 94.1 ± 2.9% (mean ± SD). Further, CD4+CD304+ isolated Tregs exhibited consistent dose-dependent suppressive ability when co-cultured with responder T cells. CD4+CD304+ Tregs also displayed robust in vitro expansion when cultured with IL-2 and soluble Mouse T Cell Activators. In conclusion, this novel isolation strategy enables researchers to efficiently obtain pure and functional mouse natural Tregs.

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1711 – P3.05.44

The Role of Follicular Cytotoxic T Cells Subsets and Their Function in Chronic Lymphocytic Leukemia

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Objectives: The follicular cytotoxic T (T_{FC}) cells are new subset of $CD8^+$T cells, express CXCR5, and eliminate infected or malignant B cells. Recent studies suggested that some T_{FC} subsets may be involved in the regulation of antibody responses by interacting with B cells. The functional differences of different T_{FC} subsets and their roles in B cell differentiation, as well as their frequency and roles in Chronic Lymphocytic Leukemia (CLL), a malignant B cell leukemia was investigated in this study.
Method: Peripheral blood mononuclear cells (PBMCs) were isolated from the whole blood of donors and were stimulated by PMA/ionomycin for 5 hours. After culturing, intracellular levels of IL-4, IL-17, IL-21, IFN-γ, perforin, and granzyme-B in T_{FC} subsets were measured on flow cytometry. $CD40L^+T_{FC}$, $ICOS^+T_{FC}$, and $CD19^+$B cells were sorted. After B-T_{FC} co-culture, plasma cell differentiation was performed by flow cytometry, and IgG responses in supernatants were measured by ELISA.
Results: In healthy subjects, IL-4, IL-17, IL-21, and IFN-γ expressions were higher in $ICOS^+T_{FC}$ than $CD40L^+T_{FC}$ and $ICOS^-CD40L^-$ (DN)T_{FC}. $ICOS^+T_{FC}$ had high levels of perforin and granzyme B, but granzyme B expression in $CD40L^+T_{FC}$ was decreased. $CD40L^+T_{FC}$ induced plasmablast differentiation and IgG production in B lymphocytes more than other subsets.
Total, $ICOS^+$, and $CD40L^+T_{FC}$ of CLL patients were shown to be increased in comparison with healthy controls. IL-4, IL-17, IL-21, and IFN-γ expressions in $ICOS^+$ and $CD40L^+T_{FC}$ of CLL patients were also higher than healthy subjects. In CLL patients, $ICOS^+T_{FC}$ exhibited higher perforin and granzyme B expression compared to other T_{FC} subsets. Additionally, there was a positive correlation between $CD5^+CD19^+$ malign B cells and total T_{FC}.
Conclusions: Our results indicate that $ICOS^+T_{FC}$ exhibits a cytotoxic profile, while $CD40L^+T_{FC}$ is more involved in B cell differentiation. Considering the cytotoxic effect of T_{FC}, an increase in T_{FC} is observed in CLL patients along with an increased tumor burden. Given the involvement of $CD40L^+T_{FC}$ in plasma cell differentiation, it is conceivable that the tumor microenvironment in CLL patients might have a role the differentiation of T_{FC} towards CD40L-expressing cells.

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1718 – P3.05.45

Helios and KLRG1 refine the definition of functionally altered CD8+ T cell subsets that evolve by aging and CMV infection

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Throughout life, the $CD8^+$ T-cell population changes due to differentiation, resulting in subsets that exhibit altered responsiveness at old age. Partially driven by chronic infections with viruses such as cytomegalovirus (CMV), this responsiveness shifts from antigen-specific, T-cell receptor (TCR)-dependent signals to innate-like or bystander stimuli, such as inflammatory cytokines. CD45RA re-expression and loss of CD27 and CD28 help define subsets with altered responsiveness, but does not fully explain the heterogeneity observed within currently known subsets. Recent studies revealed novel aging-related markers, such as CD56, KIR, KLRG1, TIGIT, and Helios. We questioned if addition of these molecules could more accurately delineate $CD8^+$ T-cell subsets that show skewing from TCR-dependent to bystander responsiveness driven by aging and CMV infection.
We combined all above-described markers in one flow cytometric panel to phenotypically define new $CD8^+$ T-cell subsets using the clustering algorithm FlowSom. We stimulated human peripheral blood mononuclear cells from CMV^+ and CMV^- blood bank donors aged 20 to 40 years or 50 to 70 years either via TCR (by anti-CD3) or with bystander stimuli (interleukin-2 or TLR7/8-agonist R848) and measured induction of activation markers among the redefined $CD8^+$ T cell subsets.
Expression patterns of CD45RA, KLRG1, and Helios appeared to mark $CD8^+$ T-cells differentially responding to TCR or bystander stimuli more accurately than the combination of CD45RA, CD27, and CD28. Thereby, CD45RA expression marks increased TCR responsiveness, whereas co-expression of KLRG1 expression marks increased bystander responsiveness, and additional expression of Helios marks decreased TCR responsiveness. Moreover, we observed that with age and CMV infection, frequencies of subsets that preferably respond as bystander cells increase, and that the bystander responsiveness of these subsets becomes more pronounced.
Thus, inclusion of novel markers Helios and KLRG1 to the classical combination of CD45RA, CD27 and CD28 refines definition of $CD8^+$ T-cell subsets with different responsiveness that develop by aging or CMV infection. This refinement offers a more comprehensive understanding of the shift from TCR-mediated responsiveness to a less specific bystander responsiveness of T cells, which could improve our comprehension of immunosenescence in older adults.

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1721 – P3.05.46

TCF1 enhances regulatory T cells and is upregulated in SLE via the Wnt-β-catenin pathway.

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Purpose: This study aims to elucidate the features of T-cell factor 1 (TCF-1)-associated regulatory T (Treg) cells and their relevance in individuals with systemic lupus erythematosus (SLE).
Methods: We investigated the expression of the Tcf7 gene using multiple sequencing datasets from the NCBI-GEO database. Flow cytometry was utilized to detect TCF1 in peripheral blood T cell subsets. In TCF1-associated Treg subsets, we examined inhibitory molecules and assessed IL-10 secretion through in vitro culture. The involvement of the Wnt-β-catenin pathway was explored using the pathway inhibitor XAV939. Additionally, correlation and ROC curve analyses were conducted to assess the clinical significance of TCF1-associated Treg cells in SLE.
Results: High expression levels of the Tcf7 gene and TCF1 protein were observed in CD4+ T cells and Treg cells. Compared to TCF1-negative Tregs, circulating TCF1-positive Tregs demonstrated elevated expressions of CTLA4 and LAG3, along with increased IL-10 secretion. TCF1-positive Tregs in SLE patients exhibited heightened levels of inhibitory markers. Activation of the Wnt-β-catenin pathway was noted in TCF1-positive Treg cells of SLE patients. Impairment in the expression of inhibitory molecules and IL-10 secretion was observed upon addition of XAV939. Furthermore, TCF1-positive Treg cells showed negative correlations with CRP, ESR, and IL-2 levels. ROC curves illustrated that the abundance of TCF1-positive Treg cells could effectively distinguish SLE patients from healthy controls, as well as individuals with primary Sjogren syndrome and rheumatoid arthritis.
Conclusion: The increased presence of TCF1-positive Tregs in SLE patients suggests a heightened suppressive function regulated by the activated Wnt-β-catenin pathway. The TCF1-positive Treg subset holds promise in screening and aiding in the diagnosis of SLE.

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Investigation of Canine T Cell Receptor Beta Chain Constant Gene Variants: Implications for Immunotherapy of T cell malignancies

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Purpose: The pursuit of effective immunotherapies for human T cell malignancies has led to a focus on the mutually exclusive expression of T cell receptor beta chain constant gene variants 1 and 2 (TRBC1 and 2). In this context, dogs, with their comparable clinical and molecular characteristics of T cell malignancies, offer an appealing translational research model. This study aims to analyze the sequence diversity of TRBC1 and 2 in normal leukocytes of various dog breeds, as well as in a collection of canine lymphoma/leukemia cell lines, to identify potential targets for immune intervention.
Methods: cDNA sequences were isolated from six canine hematopoietic cell lines of T, B, and NK cell origin, along with 21 peripheral blood leukocyte samples representing 14 different dog breeds. *TRBC1* and *2* sequence differences were estimated using multiple sequence alignment tools. Additionally, the expression of *TRBC* in canine non-T cell lines was confirmed on the single-cell clones.
Results: Analysis reveals amino acid deletion/substitution in the transmembrane domain of canine *TRBC1* and *2* loci, and no amino acid differences in the extracellular domain. In particular, the canine *TRBC1* variant lacks a conserved transmembrane lysine residue. Similar to humans, canine T cell lines predominantly express *TRBC2* transcripts, however often both transcripts are present. Unexpectedly, *TRBC* expression is also detected in certain B and NK cell lines.
Conclusions: The absolute conservation of amino acids within the extracellular domain of canine TRBC constant chains 1 and 2 complicates the design of differentiating antibodies. The deletion of two amino acids from the transmembrane region of TRBC1, involving a conserved lysine interacting with CD3 chains, predicts functional differences. The presence of *TRBC* transcripts in non-T cell lines of hematopoietic origin suggests a potential mixed B/T or NK/T developmental origin, previously unappreciated. The simultaneous expression of *TRBC1* and *2* transcripts in the CLB70, B cell line, presents an unprecedented opportunity to identify transcriptional elements involved in constant chain usage, pending confirmation at the single-cell level. These findings have implications for both immunotherapy development, canine TCRβ function, and understanding the developmental origins of canine T cell malignancies.

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1743 – P3.05.48

Therapeutic targeting of the cyclic-AMP/prostaglandin E2 pathway in ovarian cancer

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Prostaglandin E_2 (PGE_2), the most abundant prostanoid in the human body, plays a crucial role in sustaining immune homeostasis. In ovarian cancer, however, PGE_2 signaling promotes an immunosuppressive microenvironment through its cognate receptors EP2 and EP4, primarily via the cyclic AMP (cAMP) signaling pathway. We, therefore, aimed at abrogating the PGE_2/cAMP-mediated suppression of metastatic ovarian cancer effector T cells and restoring immunity in these cells by inhibiting the PGE_2-EP2/EP4 receptors.
Here, we demonstrate that PGE_2 is highly upregulated in the plasma and ascites of metastatic ovarian cancer patients compared to plasma from age- and gender-matched healthy donors. Compared to healthy donors, we could also show that activated T cell subsets from ascites and peripheral T cells from these cancer patients produce low levels of IFNγ, IL-2, and TNFα but express high levels of the T cell exhaustion markers PD-1, TIM-3, HLA-DR, and CD39; all of which could be associated to the observed increased PGE_2 levels.
TPST-1495 is a dual EP2-EP4 antagonist currently undergoing a phase 1a/1b clinical trial for several cancer types (NCT04344795). Antagonism of the EP2/EP4 receptors by TPST-1495 countered PGE_2's immunosuppressive effects on activated T cells. TPST-1495 not only induced cytokine (IFNγ, IL-2 and TNFα) production and decreased the expression of both PD-1 and TIM-3, but also, countered the PGE_2 effect on these exhausted immune cells by enhancing their proliferation. Moreover, previous work in our lab demonstrated that protein kinase A and some of its substrates, including GSK3α, VASP, vimentin, and cAMP response-element–binding protein 1, which are downstream of the adenylyl acylase/cAMP pathway, could all be phosphorylated upon T cell stimulation with PGE_2. Combining fluorescent cell barcoding with phosphoflow cytometry, we could show that pre-stimulating T cells with TPST-1495 before exposure to PGE_2 significantly reduced the phosphorylation of these proteins.
This study underscores the potential of blocking the EP2/EP4 receptors to enhance anti-tumor responses, especially in cancers that show an incomplete response to immune checkpoint inhibitors.

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1751 – P3.05.49

Uncovering molecular mechanisms of rapid protection mediated by memory CD8+ T cells

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Cytotoxic $CD8^+$ T cells are cells of adaptive immunity protecting against pathogens or tumors. One of their hallmarks is the creation of long-lived memory T cells after a pathogen encounter or vaccination. Antigen-specific memory $CD8^+$ T cells provide rapid protection of the host upon re-infection with their cognate pathogen. It is known that memory T cells are better in the protection against *Listeria monocytogenes* than naive T cells on a per-cell basis, but the molecular mechanisms remain to be explained. Production of effector molecules such as granzyme B and IFNγ, or efficient trafficking into the site of infection via surface Core 2 O-glycosylation mediated by GCNT1 may be involved.
We addressed the importance of these molecules for the rapid protection against *Listeria monocytogenes* expressing model antigen ovalbumin (Lm-OVA) mediated by memory $CD8^+$ T cells. First, we generated OVA-specific memory $CD8^+$ T cells by adoptively transferring T cells from monoclonal OT-I Rag2KO mice or OT-I Rag2KO mice deficient in *Ifng*, *Gzmb*, or *Gcnt1* into a congenic host followed by the infection with Lm-OVA.
After 30-60 days, we adoptively transferred these OVA-specific memory $CD8^+$ T cells again into naive hosts, which were subsequently challenged with a high dose of Lm-OVA, and the bacterial burden was determined in the spleen and liver after four days.
Our results showed that disruption of *Ifng*, *Gzmb*, or *Gcnt1* does not interfere with memory formation following the primary Lm-OVA infection. Moreover, *Gcnt1* is dispensable for rapid Lm-OVA clearance by memory T cells. The experiments with memory T cells deficient in *Infg* and *Gzmb* are ongoing at the moment but will be presented at the conference.

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1804 – P3.05.50

A role for a novel factor in T cell biology

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Chaperones form complexes with cytoplasmic proteins, such as transcription factors, required for cellular functions. We identified one such factor that appears to be required as part of a signalling pathway downstream of the T cell receptor (TCR), since thymic development in conditional knockout (cKO) mice is compromised from the moment TCR signalling is required. Unpublished data from our lab demonstrates that this factor's conditional deletion results in reduced T but not B cell numbers, with marked naïve T cell lymphopenia. Hence, we aim to dissect the molecular pathways that cause the observed phenotype.

To accomplish this, we performed flow cytometry to characterize the immune populations and signalling cascades, such as calcium flux. Furthermore, mass spectrometry assays suggest that our protein of interest binds to cytoplasmic and mitochondrial proteins, thus we performed quantitative polymerase chain reaction (qPCR) and western blotting to assess the presence of key factors at the messenger ribonucleic acid (mRNA) and protein level and confirmed molecule interactions via reciprocal immune precipitations. Finally, we performed the mito stress test to profile mitochondrial function and, importantly, complemented key experiments with electron and immunofluorescence confocal microscopy.

Despite the relevance of calcium influx downstream of TCR signaling we observed that peripheral T cells from cKO mice display significantly lower amounts of calcium in intracellular stores and influx upon store depletion in comparison to control mice. Critically, we observed that this phenotype is progressive during developmental stages of thymocytes.

Furthermore, we observed that, upon *in vitro* and *in vivo* T cell activation, naïve but not memory T lymphocytes from KO mice display decreased activation and proliferation, which translates into a distinct cellular morphology. Naïve T lymphocytes deficient or our factor of interest also exhibited distinct mitochondrial metabolic profiles. The consequence of an altered immune response in cKO mice could be shown using an *influenza* virus infection, which resulted in increased weight loss.

Altogether, we identified a novel factor involved in T lymphocyte development and activation and hypothesize it acts as a molecular switch in downstream pathways from the TCR, impacting mitochondrial dynamics and calcium homeostasis.

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1819 – P3.05.51

Antigen-specificity determines effector function or regulatory capacity of T cells stimulated by B cells

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Purpose: It has been found that naive B cells promotes $CD4^+CD25^-$ T cells differentiation into regulatory T (Treg-of-B) cells. Previous studies mostly used wild-type B cells to prepare Treg-of-B cells. Treg-of-B cells express ICOS, LAG3, PD1, and CTLA4, produce higher amount of IL-10, and exhibit inhibitory ability in suppression assay as well as in murine models. We would like to explore whether antigen-specific B induced T cells with regulatory function or effector capacity.
Methods: In this study, spleens from C57BL/6 and OB1 (witch have ovalbumin-specific BCR) mice were employed for the isolation of B cells. Spleens from OTII (witch have ovalbumin-specific TCR) mice were utilized for $CD4^+CD25^-$ T cell isolation. Subsequently, Treg-of-B and T-of-OB1 were cultured for experimental analysis. Treg-of-B and T-of-OB1 cells were characterized by protein expression using flow cytometry, cytokine profiling using ELISA, inhibition using suppression assay and murine model of delayed-type hypersensitivity (DTH).
Results: After OVA stimulation, OB1 splenic B cells increased costimulatory molecules, including CD80, CD86, MHCII, and CD69. In contrast to regulatory Treg-of-B, we found that OVA-activated OB1 B cells induced T-of-OB1 cells witheffector function in *in vitro* suppression assay. In cytokine profiles, higher levels of IL-17, IFN-γ, IL-2, and IL-10 during the generation of T-of-OB1 cells compared with Treg-of-B cells. And activated T-of-OB1 cells secreted higher amounts of IFN-γ than Treg-of-B cells. Furthermore, T-of-OB1 could not inhibit IFN-γ-producing Th1 cells *in vitro* and the swelling in DTH model.
Conclusion: In summary, this study showed that antigen-specific B cells increased costimulatory capacity and induced T cells with effector function but not suppressive functions. These findings provided valuable insights into the mechanisms underlying the interaction between antigen-specific B cells and T cells and pave the way for further researches.

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1852 – P3.05.52

Increased number of Th1 cells and monocytes is associated with infected non-union in patients with long bone fracture

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Bone fracture non-union is classified as a failure of bone healing at least 6 months after fracture fixation. Confirming infection as the underlying cause is challenging in case of subclinical infection. Preoperative blood testing would be valuable in diagnosing infectious causes and facilitate early initiation of appropriate treatment. The aim of this study was to characterize peripheral blood mononuclear cells (PBMCs) from patients with septic and aseptic non-union and compare with patients with uneventful healing.
Patients were recruited from eight level-one trauma centres in Germany, after appropriate ethical approval. Blood from healed (n=18; HEAL), septic non-union (n=21; S) and aseptic non-union (n=24, AS) patients was taken before surgical revision for routine implant removal or treatment of septic or aseptic non-union respectively. PBMCs were immunophenotyped using high-dimensional mass cytometry with a total of 43 markers. Targeted proteomics was performed on plasma samples using Olink 96-inflammation-panel.
T regulatory cells were increased in both AS (p=0.0118) and S (p=0.0478) compared to HEAL. Furthermore, monocytes and Th1 cells were elevated in S compared to both HEAL (p=0.0004, $p\leq$0.0001) and AS ($p\leq$0.0001; p=0.0023). Activation marker CD38 was decreased in CD4+ T cells in AS compared to HEAL (p=0.0005) and AS (p=0.0332). Exhaustion (PD-1, OX-40 and ICOS) and activation markers (HLA-DR and CD69) showed no significant differences in CD4+ and CD8+ T cells. Proteomics revealed increased IL18 expression in S compared to HEAL (p=0.0090). IL6 was increased in S compared to AS (p=0.0062) and HEAL ($p\leq$0.0001) as well as in AS compared to HEAL (p=0.0014). IL10 abundance was increased in S compared to AS (p=0.0469).
In summary, S patients show an elevated number of monocytes and Th1 cells and a high expression of pro-inflammatory cytokines IL6 and IL18. These findings reflect a state of infection and therefore suggests correlations between protein abundances and elevated cell number in the different groups.
In addition, T helper cell subsets seem to have a fundamental role in non-union patients. These findings bring us one step closer to the final goal of providing distinct biomarkers to distinguishing between non-union patients.

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1891 – P3.05.53

Impact of CD69 on experimental autoimmune encephalomyelitis development

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CD69 is a homodimeric membrane type II C-type lectin, expresses in all leukocytes and platelets upon activation in inflammatory, infectious or stressful processes and is not expressed in erythrocytes. CD69 has a pivotal regulatory role in shaping the immune response, facilitating the circulation of lymphocytes through lymphoid organs, retaining memory T cells, and driving the effector differentiation of T cells through cytokine regulation. In addition, our studies have recently described the regulated expression of CD69 in endothelial vessels and its involvement in stroke pathophysiology.
The sphingosine 1-phosphate receptor 1 (S1P1) is known to modulate human Multiple Sclerosis (MS). We propose to study the role of CD69 in experimental autoimmune encephalitis (EAE) using CD69-deficient mice (CD69-/-), CD69 overexpressing mice (CD69_Hi), and treatments with anti-CD69 monoclonal antibodies (Mab).
Using CD69−/− mice we found that they are higher susceptible to EAE than WT mice. This result was similar to that found in WT mice treated with anti-CD69 and in those expressing human-CD69 treated with anti-mouse-CD69 and anti-huCD69 mAb respectively, which block CD69 expression. Conversely, the transgenic mouse that overexpresses CD69, recently developed in our laboratory, did not develop any symptoms related to the induction of the EAE disease. We present results to investigate whether CD69 plays a physiological role in regulating the encephalitogenic T cells.
Using CD69 knockout (CD69−/−) mice, we discovered that they exhibit increased susceptibility to EAE compared to wild-type (WT) mice. This susceptibility was further heightened when WT mice were treated with antibodies against the mouse CD69 molecule and when transgenic mice expressing human CD69 were treated with antibodies against the human CD69 molecule. These antibodies effectively block the expression of CD69 and internalize it. Notably, in contrast, the transgenic mouse overexpressing CD69, recently generated in our laboratory, did not manifest any symptoms related to the induction of EAE disease. We will discuss the results of our investigation on lymphocyte migration to the spinal medulla and cytokine production, focusing on whether CD69, through its role in promoting lymphocyte retention and regulating cytokines, plays a physiological role in the regulation of encephalitogenic T cells.

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1938 – P3.05.54

T cell aging deciphered: a comprehensive study of aging-related adaptations in murine intestinal T cells

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Background: In the last century, we have seen a dramatic shift in the global demographic toward older ages, a trend known as grey tsunami. Although it is widely accepted that the aging of the immune system contributes to the elderly frailty, currently it is still not clear whether there is a causal link between age-related changes in the gut microbiota and immune-ageing.
Objectives: In this study, we analysed mice aged under well-defined conditions of genotype, sex, diet and environment, to spotlight age-related adaptations, irrespective of infections or cancer.
Methods&Results: Comparable clinical serum parameters and cytokine levels across all analyzed age groups confirmed good overall health status. Moderate changes in the gut microbiota composition were detected in the metatranscriptomic analysis of feces, which further result in age-related alterations in the microbial metabolic functions. Host serum and fecal metabolome were also affected, since metabolomics analyses revealed changes in some microbiota-derived metabolites, thus suggesting possible links between ageing of the host and gut microbiota. Accumulating evidence highlights T cells as important contributors to the multifaceted phenotype of aging. Indeed, unsupervised high-dimensional flow cytometry analysis showed ageing-related adaptations in the colon lamina propria immune cell subset composition. A special focus on colonic $CD4^+$ T cells unraveled accumulation of tissue-resident $Foxp3^+$ regulatory T (Treg) cells, while old $Foxp3^-$ $CD44^+$ memory T (Tmem) cells acquire a more activated and exhausted phenotype. A deeper profiling of these cell types, via ATAC-seq and EM-seq, revealed that colonic Treg cells show only moderate epigenetic age-related changes, while Tmem cells, in line with the flow cytometry data, show remarkable epigenetic changes.
Outlook: Integration of these multi-layer data together with those from scRNA-seq and scTCR-seq will allow us to better investigate the impact of ageing on gut microbiota and host immunity, and to shed light on age-related adaptations in tissue-resident intestinal T cells.

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690 – P3.05.55

Th17 cells requires the DNA repair protein XPC to control oxidative DNA damage

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T helper 17 (Th17) cells play a pivotal role in immune defense against bacterial and fungal infections at mucosal surfaces by producing IL-17A, IL-17F, and IL-22 cytokines. However, dysregulated Th17 responses contribute significantly to the pathogenesis of autoimmune diseases. The differentiation of Th17 cells relies on T cell receptor (TCR) signaling and the presence of cytokines such as TGF-β, IL-6, IL-21, and IL-23, which activate transcription factors promoting Th17 cell differentiation. Endogenous factors, including reactive oxygen species (ROS), affect T cell differentiation and autoimmunity. Oxidative stress is known to threat T cells by driven changes in their differentiation and function. However, the mechanisms by which Th17 cells controls oxidative DNA damage remain elusive. We investigated the involvement of the nucleotide excision repair (NER) pathway, particularly Xeroderma pigmentosum complementation group C (XPC), in controlling oxidative DNA damage and Th17 cell function. Our results demonstrate that Th17 cells upregulate NER genes, including XPC, and accumulate less DNA damage compared to other T helper subsets. XPC deficiency compromises Th17 differentiation and function, leading to increased ROS levels, oxidative DNA damage, and impaired glycolytic capacity. Furthermore, we identified the transcription factor BATF as a regulator of XPC expression in Th17 cells. BATF deficiency results in reduced XPC expression, increased ROS levels, and DNA damage, resembling the phenotype of XPC-deficient Th17 cells. Co-immunoprecipitation experiments confirmed a direct interaction between XPC and BATF, suggesting a functional relationship in DNA repair and genomic integrity maintenance in Th17 cells. These findings highlight the critical role of the NER pathway, particularly XPC, in protecting Th17 cells against oxidative DNA damage and ensuring their differentiation and function. Understanding the molecular mechanisms underlying DNA repair in Th17 cells may offer insights into therapeutic strategies for autoimmune diseases targeting Th17 cell dysregulation.

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1964 – P3.05.56

Unraveling the impact of CD69 modulation on CD8+ T cell responses during viral infection

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Understanding the intricate mechanisms governing immune responses against pathogens is pivotal for developing effective therapeutic strategies. CD69, a transmembrane C-type lectin protein expressed in immune cells, including T cells and dendritic cells, has emerged as a key regulator in orchestrating immune reactions. In this study, our aim was to clarify the influence of CD69 modulation on CD8+ T cell responses during vaccinia virus (VACV) infection. VACV serves as a model pathogen, and prior research on immunodominance against it has outlined a diverse repertoire of peptides recognized by CD8+ T cells.

This study delves into the influence of CD69 on antigen processing and CD8+ T cell responses against different MHC-I epitopes during VACV infection, utilizing CD69-deficient, CD69-overexpressing, and wild-type (WT) mice. Interestingly, in secondary lymphoid organs, CD69-overexpressing mice showed a 90% reduction in CD8+ T cells, while CD69-deficient mice exhibited a 30% increase compared to WT mice.

Surprisingly, CD69-overexpressing mice exhibited a remarkable reduction in CD8+ T cell populations in secondary lymphoid organs, while CD69-deficient mice displayed an increase compared to WT counterparts. Despite these alterations in lymphocyte numbers, CD69-overexpressing mice demonstrated heightened IFN-γ responses against immunodominant VACV peptides.

Further investigation into CD8+ T cell clones specific to VACV peptides revealed a consistent proliferation and interferon production, irrespective of the significant differences in lymphocyte counts among the mice groups. A remarkable reduction in granzyme production is observed in CTLs from CD69-overexpressing mice. Additionally, we present detailed findings outlining the impact of this reduction on both the functionality and regulation by CD69.

These findings underscore the multifaceted role of CD69 in shaping CD8+ T cell responses during viral infections, shedding light on its potential as a modulatory molecule in host-pathogen interactions. The observed alterations in cytokine profiles and cytotoxicity emphasize the intricate balance maintained by CD69 in fine-tuning immune responses.

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1965 – P3.05.57

Modelling drug-sensitisation of human T-cells to predict risk of skin rash in the clinic

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Purpose: Traditional readouts for T-cell antigenicity in response to drugs are capable of detecting T-cell activation by way of proliferation and secretion of cytokines. However, these lack sensitivity and do not distinguish between mild, moderate and severe skin reactions. This gives rise to the problem that more subtle T-cell responses may be missed by these conventional readouts, leading to a false negative diagnosis. Analysis of global protein repertoires in patient T-cells yields greater sensitivity which ultimately can diagnose the occurrence of a skin reaction but also the severity. Therefore, we aim to identify a panel of biomarkers using PBMC samples from known hypersensitive patients with varying degrees of skin adverse reactions.
Methods: PBMC isolated from a drug hypersensitive patient and a healthy, drug-naive, donor were co-incubated (100,000 cells/well) with an offending and non-offending drug at a range of concentrations for 6 days. Following incubation, cells were washed with PBS then snap frozen using liquid nitrogen. Samples were prepared for mass spectromic analysis with 150ng of peptides injected following tryptic digestion and C18 purification. Samples were searched against a library free spectral library and analysed using MsStats with comparisons made between patient and healthy donor samples.
Results: A large number of significant protein groups were detected in the hypersensitive pateint with the offending drug, when compared to the healthy donor, suggesting a drug specific protein change. Interestingly, a large number of significant proteins were also detected with the patient in the presence of the non-offending drug, compared to the healthy donor, suggesting that the patient may also have underlying susceptibility to the non-offending drug, not detected in prior proliferation experiments, warranting further study and optimisation.
Conclusion: The analysis of global protein changes in cells of hypersensitive patients indicates a significant alteration of the protein repertoire in response to drug treatments suggesting this to bear a greater sensitivity than proliferation as an immunological readout.

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2021 – P3.05.58

Neonatal regulatory T cells persist into adulthood in multiple tissues and are enriched in the skin

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Foxp3$^+$ regulatory T-cells (Tregs) are present in lymphoid and non-lymphoid organs to establish immune tolerance and tissue-homeostasis. In mice, Tregs start colonizing these tissues just after birth, participating in long-term immune tolerance. However, the generation kinetics of the adult Treg-pool across different tissues is unknown. Here, we investigated the ontogenesis of Tregs from birth to adulthood in different tissues. In lymphoid organs, the adult Treg-pool is composed of cells generated gradually at different ages. In sharp contrast, in the skin and visceral adipose tissue, a large majority of the adult Treg-pool develops in neonates. The kinetics of Treg development is intermediate in the liver, lungs and colon. Compared to Tregs generated later in life, neonatal Tregs that persist into adulthood have a more activated phenotype, express markers of tissue-Tregs and type 2 immunity. Our study reveals major differences in the Treg-pool generation kinetics between tissues and uncovers a main phenotypic shift between Tregs generated in neonates and afterwards.

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2061 – P3.05.59

Plasmatic extracellular vesicles at the intersection of immune and metabolic dysregulation in obesity and type 2 diabetes

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Purpose. Understanding the pathological mechanisms involved in the development of type 2 diabetes (T2D) is a pressing priority of biomedical research, considering the high individual burden due to T2D cardiovascular complications. Hence, the identification of the cellular and molecular factors at the node of the association between obesity and chronic inflammation, major drivers of the observed T2D epidemic, is endowed with enormous clinical significance. The aim of this work is to test the hypothesis that plasmatic extracellular vesicles (EVs) may mirror the endogenous dysmetabolic conditions, and consequently hamper the function of $CD4^{+}CD25^{high}$ regulatory T (Treg) cells, key players in the immune tolerance maintenance, hence fueling the inflammatory grade and resulting in increased T2D susceptibility.
Methods. To test the work hypothesis, we have recruited lean healthy subjects (body mass index, BMI<25), and overweight/obese individuals (BMI>25) with i) normal glucose sensitivity, ii) impaired glucose tolerance, and iii) diagnosed T2D. In those subjects, plasmatic EVs were isolated by size-exclusion chromatography and analysed for number, size, and molecular cargo (i.e., protein and microRNA profile); in parallel, peripheral blood circulating Treg cell phenotype and proliferative potential were evaluated by FACS analysis *ex vivo*. Additionally, plasmatic EVs are being tested for their biological effect on Treg cell expression profile, activation, and effector function *in vitro*.
Results. By integrating multiple levels of regulation (metabolic, immunological, and environmental), we are dissecting the specific intersection between the plasmatic EV molecular fingerprint, the cytokine/adipokine signature, and Treg cell fitness in the different groups of overweight/obese subjects compared to controls. We found that plasmatic EVs are significantly affected by the individual metabolic status; specifically, EVs represent a concurrent target during T2D development and, at least in part, they may modulate Treg cell immune suppressive capability in different dysmetabolic conditions and chronic inflammation.
Conclusion. In conclusion, a deeper knowledge of the link between plasmatic EV dysregulation and Treg cell impairment in conditions of obesity and T2D is not only necessary to better stratify patients but also to grasp the very pathological mechanism of T2D disease, thus possibly leading to the development of novel strategies to counteract its development and progression.

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2062 – P3.05.60

An integrated plasma circulating protein-miRNA signature in type 1 diabetes: markers and players of disease progression

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Purpose. Type 1 diabetes (T1D) is one of the most common chronic autoimmune diseases of childhood, in which immune cells invade the insulin-producing pancreatic islets, causing inflammation and progressive b-cell destruction. Since the rate of islet loss is associated with diabetes-related complications at later stages, traceable biomarkers predicting the autoimmunity progression and the decline in insulin secretion in T1D children may sensibly improve patient management. Hence, the object of the present work is to identify novel plasmatic molecules able to efficiently anticipate the T1D disease trajectory. Moreover, we aim at evaluating the potential pathological effect of the same molecules on a system of human b-cells *in vitro*.
Methods. We have recruited forty children with T1D, showing either lower insulin-dose adjusted A1c (IDAA1c < 8, n=20) or higher IDAA1c (>10.5, n=20) one year after disease onset. We have purified EVs from the plasma of those subjects retrospectively collected at the time of T1D diagnosis, using size-exclusion chromatography. Isolated EVs are then analyzed for microRNA content by RTqPCR and protein cargo by liquid chromatography-mass spectrometry.
Results. We have previously demonstrated that, at disease onset, the quantity of plasma miR-23~27~24 clusters is significantly higher in T1D patients who will rapidly lose insulin secretion capability, as compared to those mostly preserving it. We are now testing whether plasma EV purification may sensibly augment miR-23~27~24 sensitivity; furthermore, by the integration of miRNA with protein data, we are dissecting a complex EV-molecular signature more efficiently predicting T1D progression. Finally, the *in vitro* functional characterization of T1D plasma circulating EVs on human pancreatic b-cells in terms of glucose responsiveness and overall cell fitness is leading to the identification of the molecular determinants (miR-23~27~24 clusters and associated proteins) potentially linked to the aberrant effect of EVs in accelerating the decline of insulin secretion capability.
Conclusions. In conclusion, the described approach enables to integrate proteomics and miRNA quantitative data for the identification of complex disease-associated signatures. The molecular and functional characterization of a plasma circulating protein-miRNA hallmark, anticipating T1D progression, may possess both a relevant prognostic potential and pinpoint novel pathogenetic components in diabetic children.

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Mass cytometry-based immune profiling of human Peyer's patches in crohn's disease

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Background: Inflammatory bowel disease (IBDs) like Crohn's disease (CD) is characterized by chronic inflammation of the intestinal tract which has been identified as a potential predisposing factor for the development of colorectal cancer. Peyer's Patches, as specialized lymphoid follicles play a crucial role in the development of oral tolerance due to a constant exposition to environmental factors like microbial or food antigens. Murine data indicate an activation and subsequent apoptosis of food-reactive $CD4^+$ T-cells thus maintaining the healthy balance of the mucosal immune system. In IBD, this homeostasis is disturbed, which could drive the course of the disease and potentially promote tumor formation.
Methods: To study the composition of PP in healthy individuals and CD patients, we collected human biopsies of a respective patient cohort in a prospective manner and performed multiplexed mass and flow cytometry for deep immunophenotyping on single cell suspensions. To evaluate the spatial distribution and cellular interactions of immune cell subsets, we used imaging mass cytometry (IMC) on FFPE samples.
Results: Flow cytometry analysis revealed a reduction of activated B cells and an increase of $CD8^+$ effector memory T Cells in active Crohn's disease. For $CD4^+$ T-cells, total numbers were similar, but CD patients showed an increase of central memory and a reduction of effector memory T-cells. Furthermore, $CD4^+$ T-cells of CD patients in PP revealed a significantly reduced apoptotic rate compared to healthy controls. As the IMC data of lymphoid follicles such as PPs presented several challenges regarding their highly condensed cellular structure, several improvements had to be implemented into our analysis pipeline including cell segmentation, cell type identification and sample integration.
Conclusions: Using an IMC analysis pipeline optimized towards the specific features of lymphoid follicles, we were able to resolve differences in immune cell interactions between lamina propria and Peyer's Patch as well as differences of cell-cell interactions between Crohn's disease patients and healthy controls.

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2105 – P3.05.62

Activation of memory and naïve Th cells distinctively affected by myeloid cells with different co-stimulation capabilities

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Purpose: To test the costimulatory capacities of distinct types of monocyte-derived myeloid cells and myeloid leukaemia cells on activation, proliferation, and cytokine expression profiles of naïve (T_N), central memory (T_{CM}), and effector memory (T_{EM}) $CD4^+$ T cells, ex vivo.
Methods: T_N, T_{CM} and T_{EM} $CD4^+$ T cells obtained from healthy donors via FACS based on their expression of CD45RA, CD45RO, and CCR7. Peripheral blood CD14+ monocytes were isolated by MACS and differentiated into immature dendritic cells (iDC), mature dendritic cells (mDC), M0, M1, and M2 macrophage subsets. Myeloid leukemia cell lines at distinct differentiation stages were also used as myeloid cell models. T cells were co-cultured with the myeloid cells under anti-CD3 stimulation (first signal) where the costimulatory (second signal) capacities of distinct myeloid cell types were followed at different periods. Multiple activation markers, Th-related cytokine profiles and proliferation of T cells were assayed by flow cytometry and cytometric bead arrays.
Results: Different maturation stages within myeloid cells result in diverse activation and proliferation statuses in T_N, T_{CM}, and T_{EM} cells. As the maturation state of the myeloid cells increased, they induced higher CD69 expression for 24h. However, in contrast to more immature cells, mature myeloid cells did not highly induced CD154 expression. Especially in T memory cells, CD69 and especially CD154 expression was not as drastic as in T_N. Notably, memory cells display distinctive activation states compared to their naive counterparts. Additionally, other activation-related surface markers as CD27, CD28, CD25, and ICOS showed diverse early-expression patterns between $CD4^+$ T cell subsets. Furthermore, myeloid cells at different maturation levels induced distinct proliferation kinetics in naïve and memory T cells with different miyeloid cell ratio.
Conclusion: Our results provide proof-of-concept for differential capacities of naïve and memory T cells receiving plethora of costimulatory signals derived from distinct subtypes of myeloid cells.

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2164 – P3.05.63

Inflammatory cytokine-producing gamma delta T cells are increased in the inflamed skin of hidradenitis suppurativa patients

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Background: Hidradenitis suppurativa (HS) is a debilitating inflammatory skin disease characterised by painful nodules and abscesses that lead to scarring and disability. Current treatments for the disease, which include biologics, are inadequate for many patients and the development of specific therapies has been hampered by poor understanding of the pathogenesis. Dysregulation of the immune system is known to be involved, with activation of both innate and adaptive immune cells. We have previously identified IL-17-producing T cells as key players in HS pathogenesis. γδ T cells, which are unconventional innate-like T cells which are also capable of producing IL-17. The three main subsets in humans are Vδ1, Vδ2 and Vδ3, with Vδ2 T cells predominantly found in blood, while the other two subsets are primarily found in tissue. To date, no studies have investigated the potential role of γδ T cells in HS, although they are implicated in other skin diseases such as psoriasis.
Aim: This study aimed to analyse the role of γδ T cells in HS pathogenesis by examining the frequency of γδ T cell subsets and cytokine profiles in the blood and skin of HS patients compared to healthy controls.
Methods: We isolated peripheral blood mononuclear cells (PBMC) and isolated cells from skin biopsies from HS patients and healthy volunteers. We labelled these using a panel of cell surface markers and intracellular cytokines to analyse the frequencies of γδ T cell subsets and cytokine production by flow cytometry.
Results: Analysis of γδ T cell subsets in skin revealed an increase in Vδ1 T cells which had increased production of inflammatory cytokines IL-17A and TNF-α compared to healthy controls. Analysis of peripheral blood showed no significant changes between HS and healthy controls.
Conclusion: These results suggest a role for γδ T cells in HS pathogenesis and may present an important source of pathogenic cytokines in HS.

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2198 – P3.05.64

Role of Tiam1 in finetuning intestinal Foxp3+ regulatory T cells

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Purpose: Foxp3$^+$ regulatory T (Treg) cells are crucial to ensure self-tolerance, maintain immune cell homeostasis and control inflammation. Additionally, Treg cells resident within non-lymphoid tissues (tisTreg cells) are important to promote organ homeostasis and tissue repair. The molecular players regulating the homeostasis and functional properties of tisTreg cells are only incompletely understood. Previously, we had identified the guanine nucleotide exchange factor Tiam1 (T-lymphoma invasion and metastasis 1) as one out of eleven proteins that show diverging signaling patterns between Treg cells and conventional T cells (Tconv) upon TCR-mediated activation. Here, we aimed to explore the so far unknown impact of Tiam1 on the phenotype and function of (tis)Treg cells.
Methods: We characterized the CD4$^+$ T cell compartment in various secondary lymphoid organs and non-lymphoid tissues of Tiam1-deficient mice by high-dimensional flow cytometry under homeostatic conditions.
Results: Under steady-state conditions, we observed significantly increased frequencies of tisTreg cells and effector (e)Treg cells within the colon of Tiam1-deficient when compared to wild-type mice. Single-cell (sc) RNAseq of Treg cells from wild-type mice revealed that *Tiam1* expression is rather homogenously distributed among colonic Treg cell subsets, while it is exclusively expressed in a cluster of splenic Treg cells characterized by the expression of major activation markers.
Conclusion: Together, our study suggests that Tiam1 plays a functional role for the diligent adjustment of the homeostasis and phenotype of intestinal Treg cells.

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2254 – P3.05.65

The role of γδTCR in mouse and human γδ T cells

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γδ cells uniquely display traits of innate and adaptive immunity. γδ They are enriched in many extra-lymphoid sites including skin and gut, making critical contributions to tissue homeostasis at steady-state and in the context of stress. Their homing depends on tissue-specific Vγ chains e.g., Vγ5Cγ1$^+$ dendritic epidermal T cells (DETC); Vγ7Cγ1$^+$ intestinal intraepithelial lymphocytes (IEL); and Vγ6Cγ1$^+$ dermal T cells. Such cells can make rapid, responses to tissue challenges, seemingly by deploying innate NK and cytokine receptors. This has fuelled a widely-held view that the primary role of TCRgd is to drive the development of appropriate immunosurveillance compartments, rather than to contribute to real-time innate-like responses to challenge.
To test this idea, we generated an inducible knockout of TCR-Cγ1, used by all tissue-intrinsic γδ cells. Ccrossing to mice with a fluorescent reporter for Cre activity, we can identify erstwhile γδ cells within tissues following TCRgd depletion. By these means, we show that: [1] TCRgd is not needed for the survival of skin γδ cells *in vivo* or *in vitro*; [2] TCRgd loss rapidly leads to multifaceted dysregulation of the signature phenotypes of tissue-intrinsic γδ cells, with real-time impacts on tissue physiology; [3] TCRγδ is absolutely required for cells to make rapid innate-like responses to challenges, including Aldara that induces a γδ-dependent psoriasis-like pathology. Moreover, TCRgd deficiency at steady-state resulted in significantly dysregulated expression of multiple gene-sets.
In sum, there is essential real-time dependence on TCRgd in several settings of innate-like, tissue-intrinsic immunosurveillance, drawing a clear distinction from immunosurveillance by NK and other innate lymphoid cells.
We are now using a gene-editing approach to assess the role of TCRgd in human tissue-resident γδ T cells which are associated with good outcomes in patients with cancer.

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2274 – P3.05.66

Evaluation of the role of mucosal associated invariant T-cells in drug-induced immunotoxicity

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Mucosal associated invariant T (MAIT) cells have emerged as key players in potentiating adaptive immunity and regulating tissue inflammation. They comprise 1-4% of the peripheral blood but up to 40% of the hepatic T-cell population where they are involved within the liver defence systems through ligand presentation *via* major histocompatibility class I related protein (MR1). Natural MR1 ligands are typically related to bacterial and viral infection but recently a more diverse range of ligands have been established including small molecule drugs and drug-metabolites. With a high hepatic prevalence and restriction to MR1 which can bind small molecules, it warrants investigation as to whether the MR1-dependent activation of MAIT cells may play a role in drug-induced hepatotoxicity. Healthy donor peripheral blood and clinical liver isolates were analysed to characterise MAIT cell populations (CD3+/TCRVa7.2+/CD161^{high}) using flow cytometry. Results revealed a distinct population of MAIT cells within the peripheral blood of healthy donors whilst clinical liver samples expressed lower levels of CD161, suggesting a more exhausted phenotype. Thus, peripheral blood mononuclear cells have been used to develop a flow panel incorporating sequential gating for MAIT cells and CD161- populations to interrogate different expression of markers and cytokine secretion in response to drug compounds. MR1 blockade will be incorporated to assess dependency on an MR1-T-cell receptor pathway. Ultimately, this will allow the determination as to whether drug compounds are able to activate MAIT cells within an *in vitro* system. Subsequently if MAIT activation is confirmed for compounds, their involvement within the development of immunotoxicity will be assessed in multi-cellular systems incorporating conventional T-cell subsets and hepatocytes.

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2285 – P3.05.67

Assessment of immunomodulatory effects of compounds on lymphocyte functions in human and rat cell cultures activated with naïve antigens

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Purpose: Given the rising number of therapies that target the immune system, it is becoming more important to develop *in vitro* experimental approaches which might facilitate a more comprehensive analysis of the pharmacology and safety profiles of potential drug candidates. Additionally, development of *in vitro* assays in toxicological species and human enhances potential translatability. In this context, we have developed a functional assay in which lymphocytes are activated *in vitro* with a naïve antigen and exposed to immunomodulating compounds. The objective of the assay is to identify potential modulation of transcriptome, proliferation and cytokine response upon exposure of cells to different compounds. We use robust model immunogens such as keyhole limpet hemocyanin (KLH) for stimulation of human cells and sheep red blood cells (sRBCs) for rat cell culture.
Methods: Human PBMCs or rat splenic cells were isolated, stained with proliferation tracker and plated. These high-density cell cultures were stimulated with KLH (human) or sRBCs (rat) and treated with compounds for 7 days. Post-treatment, multiplex cytokine readout was performed and cell proliferation was analysed using flow cytometry. RNA was isolated from human cells and analyzed using NanoString nCounter platform.
Results: Both, KLH in human and sRBCs in rat cells induced strong proliferation of CD4 T cells. Stimulation of human PBMCs with KLH upregulated a number of genes related to antigen presentation, cell cycle and interferon response. Both, rat and human assays displayed sensitivity to cyclosporin A. In human cell culture, antibodies targeting PD-1/PD-L1 axis led to an increase in CD4 T cell proliferation and significantly enhanced IFN-gamma release. Surprisingly, ipilimumab targeting CTLA-4 showed inhibitory effect on proliferation.
Conclusion: Developed assays in which rat or human T cells are activated with naïve antigens allow for assessment of compounds immunomodulating potential. As efforts to minimize animal use in preclinical testing intensify, such in vitro tests are becoming increasingly vital to evaluate drug safety and efficacy.

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2294 – P3.05.68

Splicing-aware single-cell RNA-Seq resolution of peripheral blood CD4 T cells

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Purpose: Existing single-cell RNA-sequencing (scRNA-seq) analytical approaches do not generally differentiate between spliced and unspliced mRNA. However, correct usage of splicing information in scRNA-seq data analysis could reveal multiple functional facets of cell biology. Here we develop a method called SANSARA (Splicing-Aware scrNa-Seq AppRoAch) for the splicing-aware analysis of scRNA-seq data. We apply this approach to resolve the transcriptional landscape of human peripheral blood CD4 T cells in a splicing-informed manner.
Methods: scRNA-seq of sorted CD4 T cells. Splicing-adjusted Gene Expression (saGEX) matrix calculation based on the classical scRNA-Seq bionformatical pipelines (Cellranger, velocyto, veloVI, Seurat): splitting spliced and unspliced UMI counts, feature selection and splicing estimation, saGEX cell-feature matrix calculation. Data integration, clustering and dimensionality reduction of saGEX data. Differential gene expression testing and CD4 T cell subset states annotation.
Results: In SANSARA, we combine algorhytms developed by the Kharchenko and Yosef teams and classical bioinfromatical tools to transform splicing-unaware single-cell gene expression data into a splicing-aware format. It is then used for clustering, dimensional reduction and downstream data analysis. In peripheral blood CD4 T cells, accounting for splicing paints a distinct picture of transcriptional heterogeneity across subsets of helper T cells, often differing from conventional splicing-unaware scRNA-seq. We demonstrate reciprocal splicing interaction between the master transcription factors FoxP3 and Helios in Tregs, along with the exclusive expression of the spliced form of IL10RA in activated and effector Tregs. For Th1/cytotoxic axis of helper T cells, SANSARA shows highly specific splicing-related patterns for a number of subset-specific genes, including NKG7, PRF1, GNLY, GZMA, CCL5, FGFBP2, ETS1 and HOPX.
Conclusions: Splicing-aware scRNA-seq analysis reveals a complex organisation of the intrinsic heterogeneity of regulatory T cells and the Th1/cytotoxic axis of CD4 T cells. Beyond that, SANSARA offers a straightforward way to enhance conventional scRNA-seq data analysis with splicing information, opening up a new dimension to explore the role of splicing regulation in cellular gene expression programs.

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P3.06 THERAPY IN AUTOIMMUNITY

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24 – P3.06.01

Therapeutic Implications of a Herbal supplement: A Study on Alleviating Adjuvant-Induced Rheumatoid Arthritis in Rats

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Purpose: Nutritional status significantly affect disease activity in rheumatoid arthritis (RA). Assess the effectiveness of the Nutrition Bio-Shield (NBS) supplement in alleviating symptoms and modulating the progression of adjuvant-induced rheumatoid arthritis in a rat model.
Methods: In this experimental investigation, a cohort of twenty-five male Wistar rats was subjected to RA induction via Freud's complete adjuvant. Subsequently, 15 of these rats underwent oral administration of the NBS supplement at varying concentrations (12.5, 25, and 50 mg/kg) over a 30-day period, while the remaining 10 rats constituted the untreated control group. Baseline and terminal blood samples were acquired from all subjects subsequent to the 30-day treatment regimen for the purpose of evaluating serum levels of rheumatoid factor (RF), erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP).
Results: Rats subjected to RA induction exhibited a discernible augmentation in inflammatory markers, namely ESR, CRP, and RF, relative to the control group. However, a one-month administration of the NBS supplement at varying dosages (12.5, 25, and 50 mg/kg) resulted in a statistically significant attenuation of ESR, CRP, and RF levels in the treated rats. Notably, the highest efficacy was noted in the 50 mg/kg dosage group. Rigorous statistical analyses corroborated the substantial disparities in ESR, CRP, and RF levels among the experimental groups, with all NBS supplement dosages exhibiting significantly reduced levels vis-à-vis the untreated control group. Specifically, the mean ESR within the 50 mg/kg cohort demonstrated a marked reduction compared to the 12.5 mg/kg and 25 mg/kg groups. Similarly, the mean CRP in the 25 mg/kg and 50 mg/kg cohorts exhibited a significant decrease relative to the 12.5 mg/kg group. Furthermore, the NBS supplement demonstrated efficacy in restoring RF levels to a normative state across all treated rats, with the most pronounced effects noted at the 50 mg/kg dosage ($p < 0.05$).
Conclusion: The findings posit the potential of the NBS supplement to normalize RF levels and mitigate symptoms associated with RA in animal models. The study advocates for the inclusion of the NBS in the therapeutic paradigm for RA, emphasizing the necessity for further exhaustive analyses to validate its therapeutic efficacy.

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27 – P3.06.02

Delayed-type hypersensitivity component of mouse trinitrobenzenesulfonic acid-induced colitis and collagen-induced arthritis is suppressed by extracellular vesicle-carried miR-150

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Purpose: Despite recent scientific advances the complex pathogenesis of rheumatoid arthritis and Crohn's disease remains incompletely understood. Both diseases are linked by the involvement of Th1 lymphocytes mediating delayed-type hypersensitivity (DTH). Although trinitrobenzenesulfonic acid (TNBSA)-induced colitis in mice is dominated by the autoimmune DTH reaction, its contribution to collagen-induced arthritis (CIA) is unclear. Our previous studies uncovered the immunoregulatory role of suppressor CD8+ T cells releasing extracellular vesicle (EV)-carried miR-150 against effector Th1 lymphocytes in DTH induced by haptens or protein antigens. Therefore, we first attempted to experimentally induce DTH component of CIA and then investigated the immunoregulatory activity of EV-carried miR-150 in both diseases.
Methods: Colitis was induced in CBA mice by rectal administration of TNBSA in 50% ethanol and monitored daily by weighing the mice and scoring diahrrea. Some mice received intraperitoneal injection of EV-carried miR-150 or were transferred with miR-150-treated macrophages. At the endpoint, colons were assessed macroscopically and collected for further analyzes. To induce DTH in CIA, DBA donor mice were injected twice with collagen either in incomplete Freund's adjuvant into tail base skin or in physiological saline into abdominal skin. Twelve or five days later, respectively, DTH effector cells were collected from donor lymph nodes and spleens, partly treated with EV-carried miR-150 and transferred to naive DBA recipients that were intradermally injected with collagen in physiological saline into hind paws, and their swelling was measured 24-72 hours later.
Results: Both, EV-carried miR-150 and miR-150-treated macrophages significantly alleviated active DTH reaction in TNBSA-induced colitis. Moreover, EV-carried miR-150 suppressed adoptively transferred DTH in CIA in both elaborated protocols. These effects were selectively blocked with anti-miR-150.
Conclusion: Currently modified CIA protocol allows for studying DTH reaction induced by adoptive transfer of collagen-induced effector Th1 lymphocytes. Moreover, this reaction is specifically down-regulated by therapeutically-administered miR-150 protected by EVs. Similarly, miR-150 inhibits DTH component of TNBSA-induced colitis and its suppressive activity is greatly increased by macrophages. Altogether, our current findings suggest the possibility of therapeutic use of miR-150 in ameliorating the DTH response in the pathogenesis of various autoimmune diseases.

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141 – P3.06.03

Characterization of HDM3006, a highly potent and selective leading compound targeting STING for the treatment of Systemic Lupus Erythematosus

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Purpose: Systemic lupus erythematosus (SLE) is an autoimmune disorder with pathogenic autoantibodies and immune complex deposition that lead to severe inflammation and tissue injury. Simulator of interferon genes (STING) plays a critical role in recognizing dsDNA and stimulating immune response in innate immunity. The over activation of STING may cause a range of DNA-driven autoimmune disorders such as SLE. In series of the study of STING modulators, we have characterized a highly potent and selective small-molecule antagonist.
Materials and methods: THP-1 cell line, human and mouse primary cells, two different mouse lupus models were utilized to explore the action of STING antagonist, and its ability to regulate disease development both *in vitro* and *in vivo*. All statistical analyses were calculated through GraphPad Prism.
Results: In our study, HDM3006 showed robust down-stream cytokine inhibition through STING blockage in mouse and human monocytes, and cell potency is nanomole. There was no significant effect on the human ether-à-go-go-related gene (hERG) and cytochrome P450. Importantly, HDM3006 blocks STING activity *in vivo* with good oral bioavailability of 67.3% and ameliorates disease development in two mouse models for lupus, with decreased dsDNA secretion, improved histology infiltration and prolonged mouse survival rate.
Conclusion: The preliminary data of HDM3006, a highly potent and selective small molecule leading compound, has shown the potential for treatment against STING-related autoimmune diseases, especially for SLE.

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306 – P3.06.04

NK CD45RA+ and CTLs CD62L+ as potential diagnostic biomarkers of Rheumatoid Arthritis, with increased expression of immunosuppressive immune checkpoints following ex vivo cell therapy

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Purpose: Mesenchymal stem cells (MSCs) are multipotent cells with immunomodulatory and immunosuppressive effect on leukocytes, that have become a potential therapeutic treatment for inflammatory diseases, such as rheumatoid arthritis (RA). RA is a systemic autoimmune disease marked by synovitis resulting in cartilage and bone degradation, diminishing the patients' quality of life. The aim of this study is to analyse variations in the leukocyte expression of different antibodies as potential biomarkers capable of discerning the activity status of RA, and how MSCs could immunomodulate this expression.
Methods: Newly diagnosed RA patients (n=5) and healthy controls (HDs, n=6) were recruited to study their leukocyte phenotype through staining anticoagulated blood samples with different anti-human monoclonal antibodies: CD3, CD4, CD8, CD11a, CD16, CD19, CD28, CD45RA, CD45RO, CD56, CD62L, CD69, CTLA-4 and PD-1 (BD Biosciences) and analysed by spectral flow cytometry (Northern Lights, Cytek). MSCs conditioned medium (CM) was obtained from 10.000 MSCs cultured with DMEM supplemented with 10% FBS, 1% glutamine and 1% antibiotics. After 4 days, the supernatant was frozen at -20ºC. PBMCs from HDs (n=4) and RA patients (n=2) were isolated from anticoagulated blood samples by density gradient centrifugation and cultured in 96-well plates (100.000 cells/well) with different concentrations of MSCs CM (0%, 25%, 50% and 100%). After 5 days of incubation, PBMCs were stained with the previous anti-human monoclonal antibodies and analysed by spectral flow cytometry.
Results: The only statistically significant differences found between HDs and RA were in $CD62L^+$ CTLs ($p<0.01$), $CD45RA^+$ NK cells ($p<0.05$), $CD45RO^+$ granulocytes ($p<0.05$), and $CD45RO^+CD62L^+$ granulocytes ($p<0.0001$). With ROC curve analysis, only $CD62L^+$ CTLs ($p=0.0550$) and $CD45RA^+$ NKs ($p=0.0472$) were able to discern between newly diagnosed patients and HD. On the other hand, the addition of CM from MSCs decreased the expression of $CD62L^+$, $CD69^+$, while increasing CTLA-4^+ and PD-1^+ in RA patients.
Conclusion: In conclusion, the presence of $CD62L^+$ CTLs and $CD45RA^+$ NKs in peripheral blood could be a potential diagnostic biomarker for RA, and MSC-derived CM increases the expression of immunosuppressive immune checkpoints in these patients, favoring a future cellular therapy.

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332 – P3.06.05

Selective CAR-T cell mediated B cell depletion suppresses interferon signature in systemic lupus erythematosus

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Autoimmune diseases persist as a significant obstacle for those affected, lacking definitive prospects of a cure. Despite notable advances in comprehending auto-reactivity in recent times, much remains unknown, and new questions continue to emerge. However, the evolution of single-cell sequencing technologies and therapeutic strategies has rendered tackling these questions more feasible. By employing advanced molecular profiling alongside targeted therapies, there exists the potential to unravel the complexities underlying immune-mediated inflammatory disease like systemic lupus erythematosus (SLE) in human subjects. In this study, we utilize a combination of single-cell RNA sequencing and T/B cell repertoire analysis to conduct a thorough examination of molecular alterations in the immune profile following anti-CD19 CAR T cell-induced B cell depletion in SLE patients in a reverse translational manner. The resultant datasets not only validate the targeted resetting of the B cell response by CAR T cells but also unveil concurrent shifts in the transcriptional landscape of monocyte and T cell subsets, manifesting in a notable reduction in type 1 interferon signalling. Consequently, our present findings furnish compelling evidence for a causal link between the B cell response and the heightened interferon signature observed in SLE. Moreover, they underscore the efficacy of integrating targeted therapies with innovative analytical methodologies to decipher the molecular underpinnings of immune-mediated inflammatory diseases in human populations.

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510 – P3.06.06

Enhanced therapeutic effect of CXCR4/IL10-expressing mesenchymal stromal cells in a preclinical model of inflammatory bowel disease

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Inflammatory bowel disease (IBD) consists of a chronic inflammatory disorder, which mainly involves the intestinal mucosa of the digestive tract. The aetiology of IBD is not clearly established although many aspects of genetic and environmental factors together with immune responses activated against the epithelium and the commensal flora have been described. Nowadays, no curative treatment for IBD has been discovered. Current treatments of IBD aim to decrease inflammation to prevent recurrence and to prolong periods of remission. Many of them have significant side effects with primary non-response or secondary loss of response. The development of new therapeutic treatments for IBD is urgently required. Mesenchymal stromal cells (MSCs) therapy has emerged as an innovative therapeutic alternative for IBD due to its capacity for modulating inflammatory immune responses. A significant number of preclinical studies in IBD have shown that systemic administration of MSCs can reduce intestinal inflammation without adverse effects. Despite the successful preclinical results in IBD, no consensus on the efficacy of MSCs in IBD clinical trials can be yet drawn. To enhance the therapeutic potential of MSC-based therapy, we have genetically modified adipose tissue-derived MSCs with a lentiviral vector carrying the CXCR4 and interleukin 10 genes aiming to enhance the migration of MSCs with improved anti-inflammatory potential to inflamed tissues. The therapeutic efficacy of CXCR4/IL10-expressing MSCs was tested using the dextran sulphate sodium (DSS)-induced colitis model. Compared with non-genetically modified MSCs, a single intraperitoneal dose of CXCR4/IL10-MSCs on day 5 of a 7-day DSS cycle showed a significant reduction in the disease activity index and the incidence of colitis, preserving the colon structure, both in the short and in the long term. Biodistribution studies by IVIS system indicated that, at 24 h, CXCR4/IL10-MSCs showed decreased signal in peripheral blood whereas an enhanced signal was observed in liver, colon and lungs, with respect to non-modified MSCs. Decreased, inflammatory leukocyte infiltration was observed in colon. These results suggest that CXCR4/IL10-expressing MSCs may represent a targeted and potent MSC-based cell therapy product for the treatment of inflammatory bowel disease.

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521 – P3.06.07

Impact of caloric restriction in patients with multiple sclerosis treated with oral disease modifying therapy.

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Recently, several inflammatory and autoimmune diseases, such as multiple sclerosis (MS), have been linked to gut dysbiosis and to an unbalanced diet. Indeed, many research groups have reported differences in the gut microbiota of persons with MS (pwMS) compared to healthy controls. Furthermore, both acute fasting (AF) and caloric restriction (CR) have been shown to be effective and to reduce severity of experimental autoimmune encephalomyelitis (EAE) in animal models. New clinical studies are needed in order to understand if and how diet could supplement MS therapy. The aim of this project is to improve the efficacy of disease-modifying therapies (DMTs) in MS, in influencing the metabolism and immune modulating through CR diet. Patients enrolled in the study were followed for 2 years during which blood for experiments was collected at different timepoints, before the beginning of the therapy, and after 6, 12, 18 and 24 months. Freshly isolated PBMCs from pwMS were stained with different multi-colour flow cytometry panels in order to identify and to characterize the different immune cell populations. Patients were also divided according to the diet in three different cohorts: free diet (FD), caloric restriction (CR) and caloric restriction without gluten and lactose (CRGL). Analyses focused on T lymphocytes phenotype and functionality show the progressive reduction of the absolute numbers of memory T subpopulations and frequencies of pro-inflammatory cytokine (IFN-γ and TNF-α) production during all timepoints in both CR and FD groups. No additional impact was found when a gluten- and lactose-free regimen was followed. Analysis on different immune subsets (B cells and innate immune cells) are still ongoing and may reveal further immune adaptations. Although several dietary regimens have been proposed to provide benefits in pwMS, some of them including the intermittent-fasting diet have been shown to be detrimental in the long run. We propose that a simple diet based on moderate caloric restriction significantly enhances the beneficial effects of a DMT through an additional immunomodulatory effect on inflammatory T cells.

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522 – P3.06.08

Study of B cells in subjects with multiple sclerosis treated with cladribine

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Inflammation is pivotal in relapsing-remitting multiple sclerosis (MS) pathology and involves both T and B cells. Treatment with B cell-depleting monoclonal antibodies is effective in reducing the clinical inflammatory activity of MS, but other drugs with different mechanisms of action also appear to be effective in the treatment of MS. Cladribine is a second-line oral therapy which does not completely deplete all circulating B lymphocytes and impacts mainly memory B cells. However, the exact memory B cell subpopulation involved in disease progression and the most responsive to therapy has not been identified.
We enrolled 40 patients with MS to study how B cell subpopulations change during Cladribine treatment. PBMC were isolated from fresh blood before starting therapy (T0), six months (T1), one (T2), two (T3) and three years later (T4). We monitored B cell reconstitution performing a complete immunophenotyping at baseline and during cladribine treatment with high dimensional flow cytometry. We correlated this dataset with serum neurofilament light-chain dosage, to identify biomarkers of treatment efficacy.
In addition to the reduction of classical memory B cells, changes in atypical subpopulations also emerge. Type 2 Double Negative (DN2) are highly activated unconventional switched (IgD-IgM-) memory B cells peculiarly lacking CD27, associated with chronic immune disorders. Crucially, these cells express the highest levels of HLA-DR among B cell subsets, indicating high potential for antigen-presentation and autoimmune perpetuation through efficient interaction with T cells. Interestingly, MS patients have significantly higher fractions of DN2 at baseline, and we find that these cells are drastically reduced following cladribine treatment. Also IgM+ memory B cells decrease and remain stable for more than two years after treatment.
Meanwhile, naive B cells increase, particularly transitional B cells. The increased presence of transitional B cells, which reach the systemic circulation directly from the bone marrow, compensates for the lack of memory B cells and seem to correlate with a slower progression of the disease.
Our data show a reduction in the circulation of distinct B cell subpopulations together with waves of repopulation by B cells from the bone marrow, which may underlie the efficacy of cladribine in containing neuroinflammation.

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529 – P3.06.09

Innate immunity under high efficacy disease modifying therapy

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Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS), characterised by the formation of demyelinating lesions in the white and grey matter. While its pathogenesis is still unclear, multiple evidence points towards the role of the neuro-immune crosstalk in determining neuronal damage. Neuroinflammation is mediated by dysregulated pro-inflammatory effector T cells and an involvement of B-cells that induce CNS injury in MS. Cladribine, a high efficacy disease modifying therapy, is a prodrug whose phosphorylated form interferes with DNA synthesis and repair leading to both B and T cell death. To verify the efficacy of this treatment, the serum concentration of neurofilaments was determined, and as we expected, they are higher in persons with MS (pwMS) compared to healthy donors (HD), and their levels in pwMS are reduced under treatment. To better understand how this drug works, it is necessary to expand the view on the spared innate immune cells whose role has not yet been deeply investigated. The function and phenotype of innate immune cells was studied in 40 pwMS and 16 HD. Fresh heparinized blood sample of pwMS was collected before (T0) and after pharmacological treatment at 6, 12, 24 months (respectively T1, T2 and T3) and whole blood was stimulated with LPS and R848 stimuli which mimic bacterial or viral infections, respectively. Thanks to multiparametric flow cytometry analysis, we investigated 10 myeloid subpopulations and their production of 7 different cytokines. Our analysis confirm that Cladribine does not induce a change in the amount of innate immune cells, also in rare innate subpopulations, not previously studied. Before starting therapy, the majority of innate immunity cell populations, including classical, non-classical monocytes and dendritic cells are more reactive in pwMS compared to HD, except for the cDC1 population. During the treatment, this reactivity remains stable, and this ensures a first line protection against infection for pwMS. The preserved cytokine production in pwMS under Cladribine could explain this drug's ability to protect against infection, compared with other immune-depleting drugs which have as main side effect a high risk of infection.

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586 – P3.06.10

Characterization of immunomodulatory effects of dental pulp stem cells for therapy in rheumatoid arthritis

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Purpose: We aimed to investigate immunomodulatory effects of dental pulp stem cells (DPSCs) on lymphocytes and monocytes from patients with rheumatoid arthritis (RA), a chronic, autoimmune disease that primarily affects joints leading to cartilage and bone damage.
Methods: We evaluated the effects of DPSCs *ex vivo* on lymphocytes and monocytes obtained from peripheral blood of 8 patients with RA non-responders to conventional synthetic disease-modifying antirheumatic drugs. Lymphocytes were activated with anti-CD3/CD28 beads or with CpG ODN 2006, while monocytes were activated with LPS. After 48 hours of co-culture between DPSCs and activated lymphocytes or monocytes, 14 cytokines were quantified in supernatants by a multiplex bead-based assay and the expression of PD-L1, PD-L2, CD155, CD80, CD86 and 4-1BBL immune checkpoints were analyzed by flow-cytometry on DPSCs. Activated lymphocytes and monocytes as well as DPSCs cultured alone were used as comparison.
Results: After co-culture with DPSCs, levels of sIL-6R, MIP1β and TNFα decreased while levels of MCSF, MCP1 and IL-23 increased in supernatants of CD3/CD28 activated lymphocytes. In ODN 2006 treated lymphocytes, levels of IL-6, MCP1 and IL-21 increased in supernatants. Monocytes activated with LPS showed a decrease in IL-6, sIL6R, IFNγ, IL-8, MCP1, TNFα, BAFF, IL-1β, IL-18 levels while an increase in IP-10 levels in supernatants.
DPSCs showed a basal expression on the cell surface of PD-L1, PD-L2, CD155, while they did not express CD80, CD86 and 4-1BBL. Co-culture between DPSCs and CD3/CD28 activated lymphocytes as well as co-culture between DPSCs and LPS activated monocytes from all patients increased PD-L1, PD-L2 and CD155 expression on DPSCs. Instead, the expression of PD-L1, PD-L2 and CD155 on DPSCs following co-culture with ODN 2006 treated lymphocytes resulted heterogeneous among patients. The expression of CD80, CD86 and 4-1BBL were not induced by any of the co-culture conditions.
Conclusions: DPSCs modulated cytokine levels in supernatants from activated lymphocytes and monocytes. In particular, the effects of LPS stimulation on monocytes appeared to be mainly counteracted by DPSCs. Data suggest that the overexpression of inhibitory immune checkpoints by DPSCs could be involved in DPSCs immunomodulatory activities.

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600 – P3.06.11

Investigating the impact of MSC-EVs on B cell responses

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MSCs are multipotent stem cells found in the bone marrow, and there is much interest in their therapeutic potential in the context of tissue regeneration and immunosuppression. MSCs have been shown to induce a regulatory response in some effector immune cells, and have been shown to have immunomodulatory effects on T and dendritic cells. However, their effect on B cell function remains unclear. When these stem cells are exposed to inflammatory conditions mimicking infection or injury, it is thought that their immunomodulatory properties are enhanced. To bypass the risks associated with using cells as therapeutics, we can utilise the MSC secretome, which mainly consists of nanosized extracellular vesicles. We hypothesize that proinflammatory priming of MSCs will increase the immunomodulatory effect of their secreted EVs on B cells, which may offer insight into their use as modifiers of B cell activity in autoimmune disease. To determine this, we are investigating the effects of EVs isolated from resting and primed MSCs on primary murine B cell activation, proliferation, and antigen presenting capabilities.
We isolated EVs from resting and primed MSCs using ultrafiltration and size exclusion chromatography, allowing the collection of a pure fraction of EVs. The EVs were then characterised and co-cultured with primary murine B cells during various cell-based assays including activation and antigen presentation assays, which were analysed using flow cytometry. These experiments have indicated that EVs from primed MSCs enhance B cell activation *in vitro*, even when the cells are not stimulated to induce activation, and potentially promote B cell survival.
Building on this, we are also looking at the effect of MSC-EVs on B cell differentiation and antigen presenting capacity, with a view to replicate these experiments in vivo to advance our understanding of the therapeutic uses of EVs.

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730 – P3.06.12

Methotrexate hampers induction of antigen-specific CD4 T-cells, while TNF inhibitors impede maintenance of antigen-specific B cells in IMID patients following SARS-CoV-2 mRNA vaccination

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Purpose: Immune-mediated inflammatory disease (IMID) patients are often treated with immunosuppressive medication, such as methotrexate (MTX) and TNF inhibitors (TNFi), to dampen inflammatory immune responses. At the same time, these therapies may impact vaccination responses. Post SARS-CoV-2 vaccination, humoral immune responses exhibited slower kinetics in IMID patients using MTX, and reached lower median antibody titers and decreased faster over time in patients using TNFi, compared to controls. B cells and CD4 T-cells are key players in the formation of high-affinity antibodies, yet the effects of MTX and TNFi on cellular vaccine responses remain largely unclear. Understanding the effect of TNFi and MTX on humoral and cellular vaccine responses may help to design optimal vaccination strategies for IMID patients.
Methods: PBMC samples were collected 1 week and 6 months after two SARS-CoV-2 mRNA vaccinations from rheumatoid arthritis (RA) patients using MTX (n=16), inflammatory bowel disease (IBD) patients using TNFi (n=15), RA and IBD controls (n=20) and healthy controls (n=18). An activation-induced marker assay and Spike-specific probes enabled deep phenotyping of vaccine-induced CD4 T- and B-cell responses.
Results: SARS-CoV-2 vaccination induced ~33% lower Spike-specific CD4 T-cell responses in MTX treated patients compared to untreated controls. Moreover, Spike-specific CD4 T-cells highly correlated with vaccine-induced antibody titers, but solely in MTX treated patients, suggesting an association between the antigen-specific CD4 T-cell reduction and slower humoral kinetics in this group. Furthermore, patients treated with MTX generated robust Spike-specific B cell responses, similar to controls. Interestingly, the opposite was observed in TNFi treated patients. Although these patients mounted normal Spike-specific CD4 T-cell responses, B cell induction was ~50% lower compared to controls. The percentage of Spike-specific B cells decreased even more over time, which is consistent with a strong decline in antibody titers previously observed (Wieske et al 2023).
Conclusion: Our data show that MTX and TNFi both affect cellular immune responses following vaccination in IMID patients. This may explain the altered Spike-specific humoral responses observed in these patients. Consequently, strategies to improve vaccine induced immune responses in these patients, such as pausing ISP during vaccination, should be investigated to ensure optimal and durable protective immunity.

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788 – P3.06.13

Defining the effects of IL-23 inhibitors on local and systemic immune responses in psoriasis

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Purpose: The importance of the IL-23/IL-17 signaling pathway in the pathogenesis of some chronic inflammatory diseases has been clearly established. Therapies targeting IL-23 show high efficacy for the treatment of psoriasis. This work aims at understanding the impact of IL-23 blockade on local and systemic immune responses by dissecting the immune responses before and after treatment initiation in skin and peripheral blood from psoriasis patients.
Methods: Thirty-five psoriasis patients were recruited for this study. Blood samples of all 35 patients and skin biopsies of 7 patients were obtained before and 12 weeks after initiation of anti-IL-23 therapy. To assess the effects of IL-23 inhibition on systemic immune responses, we performed immunophenotyping of circulating immune cells using spectral flow cytometry, and whole blood stimulation assays. For the analysis of local immune responses, cells were extracted from skin biopsies and analyzed by Cellular Indexing of Transcriptome and Epitopes sequencing (CITE-seq).
Results: Investigation of IL-23R expression by circulating immune cells from psoriasis patients showed that MAIT cells are the population with the highest frequency of IL-23R+ cells, followed by NKT cells, and Vδ2+ γδ T cells. Only a small fraction of CD4+, CD8+ T cells, or NK cells express detectable levels of IL-23R. Comparing before and after initiation of IL-23 therapy, whole blood counts showed a statistically significant increase in lymphocyte counts and a trend for reduction of neutrophil and monocyte counts. We noted a decrease of IL-23R-expressing Th17 cells. Transcriptomic analysis of stimulated whole blood cultures showed a significant decrease in expression of inflammatory cytokines genes following 12 weeks of *in vivo* IL-23 inhibition. Our investigation of local immune responses at the skin tissue level, showed a higher CD45+ cell infiltration in lesional compared to non-lesional skin at baseline. This difference was reduced after 12 weeks of treatment.
Conclusion: IL-23 inhibitors impact both local and systemic immune responses in psoriasis patients. The ongoing single cell analysis of skin infiltrating cells may provide insights into the main cell subsets involved in psoriasis local pathogenesis.

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929 – P3.06.14

Mesenchymal stem cells expressing anti-inflammatory and anti-fibrotic neuropeptides: a new advanced therapy for autoimmune myocarditis

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Myocarditis is a significant inflammatory and autoimmune disorder of the cardiovascular system, often progressing to dilated cardiomyopathy and representing a leading cause of mortality in children/young adults. Evidence indicates that immunomodulatory and anti-fibrotic strategies may be useful for its treatment.
We and others demonstrated the efficacy of neuropeptides, like vasoactive intestinal peptide (VIP) or cortistatin, and of mesenchymal stem cells (MSCs) in reducing autoimmune, inflammatory and fibrotic responses in many experimental models of immune-mediated disorders. However, both approaches face with important limitations for their translation into clinic, including the rapid degradation of neuropeptides in body fluids by endopeptidases, and the difficulty to reach in patients the MSC doses (number/body weight ratio) that were found therapeutically effective in murine models.
To overcome these limitations, we transduced adipose-derived MSCs (ASCs) with lentiviral vectors expressing VIP, cortistatin or a latent-cortistatin form (which is solely released/bioactive in fibrotic/inflammatory environments), as a strategy to potentially increase the efficiency of ASCs by acquiring additional neuropeptide-mediated functions, thus allowing a therapeutic dose reduction. At the same time, transduced-ASCs could act as Trojan horses, protecting neuropeptides of degradation and improving their biodistribution to immune/injured organs. We evaluated their therapeutic efficacy in experimental autoimmune myocarditis (EAM) induced by immunization of Balb/c mice with cardiac alpha-myosin-fragments, a well-established model that mirrors important aspects of human disease, and offers a method to study heart-specific autoimmunity/inflammation (effector phase) and the progression towards dilated cardiomyopathy. Systemic administration of low doses (20% of estimated optimal dose) of ASCs expressing VIP, cortistatin or latent-cortistatin during the effector phase of EAM significantly reduced heart hypertrophy and myocardial inflammatory infiltration (measured by heart-to-body weight, histopathology and flow cytometry). These effects were accompanied by a reduction in self-antigen-specific T-cell responses in spleen. However, at the same doses, empty-lentivirus-transduced ASCs failed to improve all these disease parameters. Importantly, treatment with neuropeptide-transduced ASCs, but not empty-ASCs, ameliorated the subsequent late cardiac remodeling/hypertrophy and fibrosis, as evidenced by echocardiography (normalized ejection fraction and fractional shortening) and by decreased myocardial collagen-fibrotic deposits.
Therefore, the improved version of ASC-v.2 expressing neuropeptides emerges as a new advanced therapy for autoimmune myocarditis.

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933 – P3.06.15

Targeted modulation of myeloid regulatory cells with prostaglandin E2 ameliorates experimental autoimmune encephalomyelitis and preserves gut microbiota

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Introduction: Autoimmune diseases, such as multiple sclerosis (MS), are impacted by disregulated myeloid cells and disbiosis in gut microbiota. Previously we identified that prostaglandin (PG)E_2 is critical for the activation of myeloid-derived suppressor cells (MDSCs), and their capacity to ammeliorate neuroinflammation in experimental autoimmune encephalomyelitis (EAE). However, it remained unclear how different approaches for the modulation of myeloid cells with PGE2 in EAE associate with gut microbiota composition.
Methods: The therapeutic potential of MDSCs activated with PGE_2 *in vitro*, and a direct delivery of PGE_2/MOG_{35-55} to myeloid cells via gold nanoparticles (GNPs-MOG_{35-55}-PGE_2), was evaluated in C57BL/6 mice EAE model induced with MOG_{35-55}/CFA/PTx. The treatment included intraperitoneal administration of cells or nanoparticles on days 1, 3, and 5 post-immunization, respectively. Extensive immunophenotyping was performed on cells isolated from target organs and microbiota composition was analyzed based on shotgun sequencing of fecal samples collected prior to EAE induction and at the peak of disease.
Results: Both therapeutic approaches led to a delayed onset and mitigated EAE severity over the 45-days monitoring period. At the peak of disease, reduced serum levels of IL-6, IL-12p40, TNF-α, IFN-γ, and IL-17 were followed by a decrease in a proportion of T-helper 1- (Th1) and Th17-polarized effector and memory cell subsets in lymph nodes and spleen, as well as reduced leukocyte infiltrates in the spinal cord and with an increased proportion of IL-10-producing Tregs. MDSC-PGE_2 and GNPs-MOG_{35-55}-PGE_2 treatments also prevented EAE-induced alteration in microbiota composition. Thereby, the treatment with MDSC-PGE_2 led to an increase in the relative abundances of *Muribaculum sp*. and *Duncaniella sp*., while GNP-MOG_{35-55}-PGE_2 treatment promoted the dominance of *Alistipes sp.*, all of which are associated with immunoregulatory properties and improved clinical symptoms in MS patients.
Conclusion: Our study highlights that delivery of PGE_2 into myeloid cell compartment can effectively attenuate neuroinflammation with a significant impact on gut microbiota composition, which deserves to be further explored as a potential strategy for MS treatment.

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Generation of regulatory T cells from human memory CD4+T cells by upregulation of NKD2 and downregulation of Wnt-β catenin signaling

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Purpose: Regulatory T cells (Tregs) are indispensable for immune homeostasis and tolerance to self-antigens and allergens whereas the imbalance between immune responses and tolerance causes allergic and autoimmune diseases. A promising therapeutic strategy is to support immune tolerance by converting conventional T cells into suppressive regulatory T cells with small molecular weight compounds, an area which is currently underexplored. To address this unmet need, we aimed to identify small molecule compounds that induce Foxp3 upregulation in $CD4^+$T cells *in vitro*.
Methods: A library of 40,000 small molecules was screened for compounds that significantly upregulated the expression of Foxp3, the master transcription factor of Tregs. One candidate molecule (hereafter referred to as IFA005) was selected and further validated using primary human memory $CD4^+$T cells (Tmem). Dose response studies were performed, long-term toxicity, cell proliferation, and the secretion of pro-inflammatory cytokines was analyzed. Furthermore, the TSDR-methylation status and the suppressive capacity of IFA005-induced Tregs (IFA005-iTregs) towards $CD4^+CD25^-$T cells (Tresp) as well as the transcriptional profiles of IFA005-treated memory $CD4^+$T cells were investigated.
Results: We identified a novel quinoxaline derivative (IFA005) that significantly and dose dependently increased Foxp3 expression in Tmem without impairing cell proliferation or viability. IFA005 significantly upregulated CTLA-4, TIGIT, CD39, and ICOS in Tmem, whereas secretion of IL-4, IL-5, IL-13, and IL-2 were significantly reduced. Furthermore, IFA005-iTregs showed robust suppressive activity towards Tresp but did not show conversion to an epigenetically imprinted phenotype corresponding to that of tTregs. IFA005-treated Tmem showed upregulation of naked cuticle homolog 2 (NKD2) on RNA and protein level, which was associated to enhanced phosphorylation of GSK3β resulting in reduced of β-catenin levels and inhibition of Wnt-β-catenin signaling.
Conclusion: IFA005 could be a promising candidate to induce immune tolerance by converting effector T cells into suppressive Tregs through the inhibition of the Wnt-β-catenin pathway.

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996 – P3.06.17

B-cell depletion by a novel “2+1” CD19-targeted T-cell Engager (TCE) for the treatment of B-cell related autoimmune diseases

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Purpose: Autoreactive B cells drives pathogenesis of multiple types of autoimmune diseases, such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), Myasthenia Gravis (MG), Neuromyelitis Optica Spectrum Disorder (NMOSD), and multiple sclerosis (MS). The utilization of B cell depletion therapy has great potential as a therapeutic approach in the treatment of autoimmune disorders. The therapeutic efficacy of B-cell-depleting monoclonal antibodies in autoimmune diseases is restricted, primarily attributed to the incomplete B cell depletion and persistence of autoreactive B cells within tissues. In addition, CD19-targeted CAR-T therapies have exhibited promising early clinical efficacy in the treatment of SLE. Here We report a novel "2+1" CD19 x CD3 bispecific T cell engager (TCE), ATG-201, which effectively depletes B cells with minimal risk of CRS and demonstrates potent in vivo efficacy in SLE and MS mouse models.
Methods: ATG-201 was constructed by introducing a high affinity, novel conformational epitope-targeted anti-CD3 single chain fragment variable (scFv) to the hinge region of one of the heavy chains of a CD19 monoclonal antibody. It was evaluated in a series of preclinical studies for binding affinity, T cell activation, T cell dependent cytotoxicity (TDCC), and cytokine release. The *in vivo* efficacies were investigated in a mouse MS model of myelin oligodendrocyte glycoprotein-induced experimental autoimmune encephalomyelitis (MOG-EAE) and a spontaneous SLE mouse model using a mouse surrogate TCE with the same format.
Results: ATG-201 exhibited a single-digit nM binding affinity to CD19 positive cells, and limited binding capability to CD3+ cells before CD19 crosslinking. It activated T cells in the presence of CD19+cell and mediated potent T cell-dependent B cell depletion. Cytokine release is strictly dependent on the presence of CD19+ target cells. The surrogated CD19xCD3 TCE demonstrated enhanced efficacy in suppressing disease progression than CD20xCD3 bispecific antibody, and CD19 or CD20 monoclonal antibody in MOG-EAE mice. In addition, the surrogated CD19xCD3 TCE showed more potent therapeutic benefits in mouse SLE model with higher B cell depletion compared with controls.
Conclusion: ATG-201 demonstrates CD19-dependent T cell binding and activation with low risk of CRS which warrants further study in clinical trials.

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1295 – P3.06.18

Effect of extracellular vesicles derived from licensed and unlicensed MSCs on B cell function and activation

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Purpose: Autoimmune diseases (ADs) are characterized by B cell function dysregulation. Novel therapies such as antibody-mediated B cell depletion have significant side effects. Mesenchymal stem cells (MSCs) have been proposed as an alternative therapy for ADs, as these cells bear potent immunomodulatory capacities, particularly when exposed to inflammatory conditions known as 'licensing'. MSCs-derived extracellular vesicles (EVs) have been proposed as safer alternative to MSCs. The aim of this study was to evaluate the effect of EVs derived from licensed and unlicensed MSCs on B cell function and activation
Methods: Bone marrow murine MSCs (BM-MSCs) were cultured on tissue culture plastic until 70% confluency. Cells were then incubated in EV-depleted media with IFN-γ and TNF-α to 'license' them, or in standard conditions, for 48 hours. After this time, the conditioned media was collected, and the MSCs-EVs were isolated by size exclusion chromatography. EVs samples' particle concentrations and protein concentration were measured by NTA and BCA, respectively.
For assessing the effect of EVs on B cells, primary B cells were isolated from an allogeneic strain mouse and activated with CD40L, or CpG. The effect of licensed and unlicensed EVs on the expression of activation surface markers and proliferation of B cells was measured by spectral flow cytometry.
Results: 'Licensed' EVs could modulate the expression activation markers MHCII, CD80, CD69 and CD86 on allogeneic murine B cells activated both by CD40L and CpG, in a dose-dependent manner. This effect was seen regardless of if the EVs were added together with the activation factors or 2 hours after activation. EVs were also capable of modulating B cell proliferation tracked by CellTraceViolet dilution**.**
Conclusion: Our findings demonstrate that BM-MSCs-derived EVs can modulate B cell activation and function *in vitro*. Moreover, our study highlights the influence of MSCs' culture conditions on EV effects, suggesting potential therapeutic versatility in diseases characterized by immune dysregulation, such as autoimmune diseases. Further elucidation of the underlying mechanisms is crucial to fully harness the clinical potential of MSCs-derived EVs as immune therapies.

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1352 – P3.06.19

Protective Properties of Dimethyl Fumarate on Synovial Fibroblast Function

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Purpose: Dimethyl fumarate (DMF) has been approved for the treatment of psoriasis and multiple sclerosis and has shown a range of anti-inflammatory properties in various tissues or cell types. Its precise effects on synovial fibroblast phenotype and function are yet to be understood.
Methods: PsA and RA patients underwent key-hole joint arthroscopy and synovial biopsies were obtained. PsA and RA synovial fibroblasts (FLS) were isolated and grown to passage 1-8. PsA and RA FLS were pre-treated with DMF (25 μM or 50 μM) for 2 hr before TNF (1 ng/ml) stimulation for 24hr. FLS function was quantified by ELISA/MSD, RTPCR, wound healing assay, network formation and Seahorse metabolic assay.
Results: DMF dampened pro-inflammatory functions of FLS. IL-6 and MCP-1 gene expression and secretion were decreased in a dose-dependent manner, an effect that was more pronounced for PsA than RA FLS. RANTES secretion was also decreased by DMF treatment ($p<0.05$), and no effect was observed on IL-8 secretion. Moreover, DMF treatment led to a decrease in the secretion of chemokines from both PsA/RA FLS, including TARC, MIP-1α, IP-10, MCP-4, and MDC (all $p<0.05$). FLS migratory capacity was also decreased by DMF ($p<0.05$), as measured by the wound healing assay, however no effect was observed on FLS network formation. Furthermore, basal OCR was decreased by DMF ($p<0.01$), with little effect observed on basal ECAR, suggesting a decrease in the utilisation of energy from the oxidative phosphorylation pathway. DMF also decreased maximal respiration, spare respiratory capacity, and ATP production (all $p<0.05$). Interestingly, gene expression of key glycolytic markers GLUT1, LDHA and PKM2 were increased in response to DMF (all $p<0.05$), suggesting that FLS shift their metabolic reliance to glycolysis. Finally, DMF also decreased secretion of the bone mediator OPG ($p<0.05$), suggesting a role in regulating bone remodelling.
Conclusion: Differential effects on FLS were observed in response to DMF treatment. The inflammatory, migratory, and metabolic profiles of FLS were dampened, in addition to a reduction in bone remodelling protein OPG. Overall, DMF demonstrates a specific protective and immunomodulatory role in FLS, with a stronger effect in PsA compared to RA.

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1353 – P3.06.20

Parameters of thrombosis risk and percentage of CD4+Foxp3+ regulatory T cells (Treg) after the first three months of baricitinib treatment of rheumatoid arthritis (RA) patients

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Purpose: Baricitinib is one of two selective oral Janus kinase inhibitors (JAKi), approved for RA treatment. Increased risk of venous thromboembolism (VTE) in RA, expanded by JAKi treatment is well proved, however knowledge about JAKi influence on thrombosis parameters is sparse. In the present work we compared the $CD4^+Foxp3^+$ Treg phenotype and thrombotic parameters in RA patients before and after three months of baricitinib treatment.
Methods: Eighteen RA patients with high disease activity (DAS28 >5.1), the inefficacy of at least one conventional DMARD, and without a history of cardiac event or VTE were qualified for baricitinib therapy. Peripheral blood mononuclear cells (PBMC) were obtained from the citrated blood taken from healthy controls (HCs) and the patients before and after 3 months of baricitinib treatment. Treg cells phenotype was assessed by FACS analysis while thrombotic parameters were evaluated by turbidimetric method. According to EULAR response criteria, patients were termed as good or moderate responders.
Results: In RA patients 3 months of baricitinib treatment resulted in significant percentage decrease of $CD4^+CD25^+$ cells (46.3 ± 1.9 vs 54.4 ± 2.3; p<0.0001), $CD4^+Foxp3^+$ cells (4.6 ± 0.4 vs 5.6 ± 0.4; p=0.01) and $CD4^+Helios^+$ cells (7.4 ± 0.7 vs 8.6 ± 0.5; p=0.049). At baseline, the RA group had higher fibrinogen and d-dimers compared to HC (410.4±29.5 vs 334.9±19.2; p=0.04 and 1472.3±349.2 vs 450.3±54.5, p=0.0002, respectively). After 3 months of baricitinib treatment a significant increase in homocysteine (10.7±0.6 vs 9.1±0.5; p=0.018) and antithrombin III (119.7±2.7 vs 110.4±3.2; p=0.004) was observed, the latter correlated negatively with DAS28 (r=-0.686, p=0.002). Moderate responders had lower antithrombin III (105.3±3.6 vs 115.1±2.7; p=0.043) and higher d-dimers (1639.2 ± 550.5 vs 450.3 ± 54.5; p<0.0001) at baseline as compared with HC, and lower antithrombin III than good responders at month 3 (113.5±3.2 vs 127.5±3.0; p=0.0029).
Conclusions: Three months BARI treatment resulted in restoration of Treg cells balance to that observed in HC. Interestingly, low levels of antithrombin III together with increased d-dimers seem to predispose to moderate response to baricitinib.

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1405 – P3.06.21

Bruton's tyrosine kinase inhibitors tolebrutinib, evobrutinib and fenebrutinib affect neutrophil functions in vitro: implications for treatment of autoimmune disease

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Purpose: Multiple sclerosis (MS) is a neurodegenerative, autoimmune disease that is still incurable. Nowadays, a variety of new drugs are being developed to prevent excessive inflammation and halt neurodegeneration. Among these are the inhibitors of Bruton's tyrosine kinase (BTK). Being indispensable for B cells, this enzyme became an appealing therapeutic target for autoimmune disease. Recognizing the emerging importance of BTK in myeloid cells, we investigated the impact of upcoming BTK inhibitors on neutrophil functions. Although adaptive immunity in MS has been thoroughly studied, unanswered questions about the pathogenesis can be addressed by studying the effects of candidate MS drugs on innate immune cells such as neutrophils, previously overlooked in MS.
Methods & results: In this study, we used three BTK inhibitors (evobrutinib, fenebrutinib and tolebrutinib), and found that they reduce neutrophil activation by the bacterial peptide N-formylmethionyl-leucyl-phenylalanine and the chemokine interleukin 8/CXCL8. Furthermore, they diminished the production of reactive oxygen species and release of neutrophil extracellular traps. Additionally, the production of CXCL8 and interleukin-1β in response to inflammatory stimuli was decreased. Inhibitory effects of the drugs on neutrophil activation were not related to toxicity. Instead, BTK inhibitors prolonged neutrophil survival in an inflammatory environment. Finally, treatment with BTK inhibitors decreased neutrophil migration towards CXCL8 in a Boyden chamber assay but not in a transendothelial set-up. Also, in vivo CXCL1-induced migration was unaffected by BTK inhibitors.
Conclusion: Collectively, this study provides novel insights into the impact of BTK inhibitors on neutrophil functions. These findings might have important implications for patients' innate immune responses but can also impact excessive neutrophil activation in chronic inflammatory conditions, such as MS.

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1472 – P3.06.22

Comparison of three assays to quantify serum concentrations of infliximab and adalimumab

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Purpose: Infliximab and Adalimumab are monoclonal antibodies that work by preventing the action of TNF alpha, which is used to treat several chronic inflammatory and autoimmune illnesses. Adequate monitoring of serum drug concentrations is crucial for optimising drug dosage and identifying potential therapeutic setbacks prior to the onset of clinical signs. Our gold standard monitoring is performed by ELISA, yet it has significant limitations in clinical practice, primarily in relation to the clinician's response time. As a result, new strategies are being developed to speed up response time.
Methods: Chemiluminescence immunoassay (i-Track10®, THERADIAG), fluorescence immunoassay (AFIAS-10, Boditech Med Inc.), and ELISA (Promonitor assays, GRIFOLS) were used to quantify the serum levels in a group of 40 patients who received Infliximab and 35 patients who received Adalimumab.
Results: Measurement ranges for both drugs, when comparing i-Track10 and AFIAS-10 to the ELISA assay showed a correlation above 0.9. The correlation was higher for AFIAS-10 than for i-Track10 (0.979 vs. 0.921 for Infliximab, and 0.972 vs. 0.949 for Adalimumab). The systematic error in the determination of infliximab was lower in the i-Track10 than in the AFIAS-10 (12.56 vs. 13.42). However, the AFIAS-10 device had a lower systematic error for the determination of Adalimumab (14.81 vs. 23.18). Passing Bablok regression was also conducted. The results indicated slopes and y-intercepts as follows: for infliximab, AFIAS-10 yielded a slope of 0.952 and a y-intercept of 0.603, while i-Track10 yielded a slope of 1.201 and a y-intercept of -0.093. For adalimumab, AFIAS-10 produced a slope of 1.189 with a y-intercept of 0.008, whereas i-Track10 resulted in a slope of 1.613 and a y-intercept of -1.2756.
Conclusion: Both methods demonstrated good correlation with the already in use ELISA approach and were valid for determining serum drug levels. While the AFIAS-10 system yielded higher correlation results a priori, the i-Track's smaller detection range (up to 24 μg/mL) may have contributed to selection bias.

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1622 – P3.06.23

Complex immunomodulation by pomegranate-derived ellagitannins

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Purpose: Pomegranate peel extract (PoPEx) has been shown to have antioxidant and anti-inflammatory properties, but its effect on the adaptive immune system has not been sufficiently investigated. This study aimed to examine the immunomodulatory effects of PoPEx and its main ellagitanin constituents using in vitro models on human immune cells.
Methods: PoPEx was prepared from pomegranate peel powder by using 50% ethanol. The extract was dominantly composed of punicallagin (PG), punicalin (PN), ellagic acid (EA), and gallic acid. The extract and individual ellagitannins were tested in the culture of human peripheral blood mononuclear cells (PBMC) and co-culture of monocyte-derived dendritic cells (MoDCs) and T-cells. Cytotoxicity, autophagy, proliferation, marker expression, and cytokine production were assayed.
Results: The treatment of PBMC with PoPEx (range 6.25-400 μg/mL) resulted in cytotoxicity at concentrations of 100 μg/mL and higher, due to the induction of apoptosis and oxidative stress, whereas autophagy was reduced. Cytotoxicity of ellagitanins was obtained with lower concentrations such that IC50 values (μg/mL) were: EA (7.56), PG (38.52), and PN (69.95). Both PoPEx and ellagitanins inhibited PHA-induced proliferation of PBMC at non-cytotoxic concentrations which was followed by a dose-dependent inhibitory effect on the production of Th1 (IFN-γ), Th17 (IL-17A, IL-17F, and IL-22), Th9 (IL-9), and proinflammatory cytokines (TNF-α and IL-6) in culture supernatants. Lower concentrations of PoPEx upregulated Th2 (IL-5 and IL-13) cytokine production in contrast to ellagitanins. Lower concentrations of PoPEx and EA stimulated the production of IL-10 and increased the frequency of CD4+CD25hiFoxp3+ cells. Both PoPEx and all three ellagitannins inhibited differentiation and maturation of MoDCs, inhibited their potency to induce proliferation of alloreactive T-cells and their Th1 and Th17 polarization properties. All components were able to induce the expression of tolerogenic markers (ILT3, ILT4, and IDO1) on MoDCs upon induction of their maturation with LPS and IFN-γ which was accompanied by an increased frequency of Tregs and Tr1 cells. PG and EA were more potent than PN.
Conclusion: PoPEx exerted potent anti-inflammatory and immunoregulatory effects in vitro. The immunomodulatory effect of the extract is very complex, probably associated with the induction of tolerogenic DCs, and was dominantly attributed to ellagitannins.

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European Journal of Immunology Clinical-Basic-Translational

1768 – P3.06.24

Unique abilities of Acr1 protein of Mycobacterium tuberculosis to elicit Tregs and MDSCs and impart protection against experimental autoimmune encephalomyelitis

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Purpose: Autoimmune diseases (AID) stem from an immune response that goes awry, targeting the body's own antigens by breaching self-tolerance. Th17 cells play a central role in advancing AID progression, while being counterbalanced by Tregs to some extent. Myeloid derived suppressor cells (MDSC) and Tregs have gained wide impetus in alleviating the inflammation in autoimmune diseases. In a somewhat disparate domain, the latent stage of tuberculosis (LTBI) harbors *Mycobacterium tuberculosis (Mtb)*, which composes an immunosuppressive milieu aided by an array of proteins. Intriguingly, recent research has suggested that *Mtb's* presence / BCG vaccination could mitigate the intensity of autoimmune diseases. Keeping this in view, we aimed to use immunosuppressive proteins of *Mtb* to resolve inflammation in AID. Acr1 is highly expressed in LTBI could generate a suppressive phenotype in both T cells and dendritic cells. So, we were curious whether Acr1 can be used to protect against AID.
Methods: We cultured naïve CD4 T cells and dendritic cells with Acr1 and monitored their phenotype and function. FoxP3-GFP mice were immunized with Acr1 to check the generation of MDSC and Tregs and delineate the mechanistic pathway. Further, we adoptively transferred $MDSC^{Acr1}$ into the mouse EAE model (experimental model for multiple sclerosis) to check whether it can suppress the inflammatory response of AID.
Results: We observed that naïve CD4 T cells and dendritic cells treated with Acr1 were skewed towards Tregs and myeloid-derived suppressor cells ($MDSC^{Acr1}$) respectively. These MDSCs showed elevated expression of suppressive molecules (PDL1, TGF-β, etc.) and could stimulate *in-vitro* generation of Treg and suppress Th17 cells. The RNA-seq data showed differentially expressed genes of granulocytic MDSC. Mice immunized with Acr1 (s.c.) showed elicited Tregs and MDSCs population by activating TLR4. EAE group transferred with $MDSC^{Acr1}$ showed an increased population of Tregs, decreased Th17 population alongwith a significant decline in clinical score.
Conclusion: The results suggest that Acr1 can induce suppressive Tregs and generates TGF- β and IL-10 secreting MDSCs both *in-vitro* and *in-vivo* by activating TLR4, further MDSC leads to Tregs generation and ameliorate EAE symptoms. Conclusively, the study provides new insight into immunotherapeutic strategies for curing AID.

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1844 – P3.06.25

Sialic ac a novel approach to promote FVIII-specific tolerance

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DCs are key orchestrator of the adaptive immune response, instructing the balance between tolerance and activation. They thus represent the elected cells to target to promote/restore FVIII-specific tolerance in Haemophilia A, where the insurgence of anti-FVIII antibodies makes the therapy ineffective. We have previously shown that sialic acid activates a tolerogenic pathway in DCs via Siglecs. Here, we investigate the capability of sialic acid to induce antigen-specific tolerance by employing immunodominant sialylated peptides from FVIII. We first set up a pipeline to identify the best FVIII epitopes to be conjugated to sialic acid based on molecular docking, predicted secondary structure and promiscuity of binding to the most common Caucasian HLA haplotypes. Using these sialylated FVIII sequences (Sia), we tested the effects on monocyte differentiation to DC *in vitro*. We observed a reduced secretion of TNFa, IL-6, IL-12 and the induction of the expression of CD86 and PDL-1. The effects observed are dependent on Siglec-3 and Siglec-9, as demonstrated by knockout of the receptors in primary monocytes from healthy donors, immunophophoblots, and binding/uptake assays in monocytic cell lines genetically engineered to express various combination of Siglecs. Next, we showed that moDCs loaded with Sia were capable to inhibit the proliferation of allogenic naïve $CD4^+$ T cells. We further studied the antigen-specific effects by using a FVIII-$CD4^+$ T cell clone from a severe Haemophilia A patient. When the clone was co-cultured with Sia-loaded HLA-matched moDCs, an anergic-like phenotype with high expression of PD-1, LAG3, CD127 was induced upon second encounter with the antigen. Currently, we are investigating the ability of Sia to suppress the formation of anti-FVIII antibodies *in vivo* in a relevant FVIII-KO mouse model. Our results provide a new strategy to modulate pathogenic CD4+ T cells responses in Haemophilia A by targeting Siglec-3 and Siglec-9 on dendritic cells with sialic acid. This approach could prove useful in other autoimmune diseases to promote/restore antigen-specific tolerance.

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1884 – P3.06.26

Anti-citrullinated histone monoclonal antibody CIT-013 to inhibit eosinophil extracellular trap release.

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Purpose: In response to specific stimuli, eosinophils can release decondensed chromatin as eosinophil extracellular traps (EETs) which contain granule contents and citrullinated histones. This programmed form of cell death has been termed cytolysis or EETosis. EETs have been described in various eosinophilic diseases such as eosinophilic esophagitis, eosinophilic granulomatosis with polyangiitis (EGPA) and chronic rhinosinusitis with nasal polyps (CRSwNP). EETs have been described to induce tissue damage and pro-inflammatory cytokine production, resulting in a vicious cycle of inflammation. Currently there are no EET-specific therapies available. We have developed a first-in-class therapeutic monoclonal antibody, CIT-013, specifically targeting citrullinated histone H2A and H4 present in EETs. Previous work has shown CIT-013's inhibitory efficacy for neutrophil extracellular traps. Here, we investigated CIT-013's potency in inhibiting EETosis and as a potential therapy for EET-driven diseases.
Methods: Eosinophils were isolated from healthy volunteers and cultured with disease-relevant stimuli to study EETosis characteristics and underlying pathways. EET release was analyzed with quantitative live imaging immunofluorescent microscopy. High resolution confocal microscopy was used to visualize CIT-013's mechanism of EETosis inhibition.
Results: We have demonstrated that EETosis is associated with PAD4 activity, with high levels of histone citrullination within EETs upon stimulation with A23187, PMA, and platelet activating factor. JBI-589, a specific PAD4 inhibitor, inhibits A23187- and PMA-induced EETosis with an IC50 of 2.7 µM. CIT-013 acts upon the final stage of EETosis, binding to its chromatin epitopes when plasma membrane integrity is compromised and prevents EET release in response to multiple stimuli with an IC_{50} of 2.5 nM. In order to consider CIT-013's safety profile, we show that CIT-013 only binds to eosinophils stimulated to release extracellular traps, not to healthy eosinophils. Furthermore, CIT-013 does not interfere with other eosinophil functions such as IL5-induced EDN degranulation, adhesion, superoxide production and MCP-1 chemokine regulation.
Conclusion: CIT-013 binds to citrullinated H2A and H4 and inhibits pro-inflammatory EET release into the extracellular environment. CIT-013 offers a potential new treatment for patients with eosinophilic diseases high in tissue EETs, such as CRwNP. CIT-013 will enter phase 2 proof-of-concept trials in Reumatoid arthritis and Hidradenitis suppurativa at the end of 2024.

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1904 – P3.06.27

Anti-citrullinated histone antibody CIT-013 is a dual action therapeutic targeting neutrophil extracellular trap-associated pathology in rheumatoid arthritis

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Neutrophil extracellular traps (NETs) are pro-inflammatory mediators that drive pathogenesis by inducing osteoclast formation and bone erosion in joints of rheumatoid arthritis (RA) patients. Though NETosis-targeting therapeutics have shown potential as effective treatments, currently there are no NET-specific therapies available. We have developed CIT-013, a first-in-class monoclonal antibody specifically targeting citrullinated histones H2A and H4 in NETs, that has shown efficacy in multiple pre-clinical models of NET-associated inflammation. In this study, we unravel CIT-013's mechanism of action (MoA) and examine CIT-013's target engagement in humans. Additionally, we investigate CIT-013's potential as treatment for RA.

Neutrophils isolated from human blood, were stimulated to induce NETosis and confocal microscopy was used to study CIT-013's MoA. CIT-013's target engagement was examined in healthy volunteers administered with 2 ng/kg LPS to induce low-grade inflammation. A collagen-induced arthritis (CIA) mouse model was used to investigate CIT-013's potential in RA by assessing its distribution, NET-targeting properties, and therapeutic effects on inflammation-induced bone resorption. Subsequently, serum and synovial tissue from RA patients was used to detect presence of CIT-013's epitope.

We here demonstrate that CIT-013 binds to its epitope during the final stage of NETosis when plasma membrane integrity is compromised and prevents NET release with an IC_{50} of 4.6 nM. Monovalent CIT-013 completely lacks the NET-inhibitory capacity. NETting neutrophils and NETs opsonized with CIT-013 are efficiently cleared by macrophages which is Fc domain dependent. LPS nano-dosing in healthy volunteers induces a significant level of circulating NETs which are completely extinguished with 0.3 and 0.9 mg/kg CIT-013 administration. In CIA mice, we demonstrate that CIT-013 distributes specifically to the inflamed joints which correlates with joint arthritis severity (R^2=0.6233, $p<0.0001$). NETs are significantly reduced within these joints upon therapeutic administration of CIT-013 and that interrupts the progression bone/cartilage loss. In serum and tissue of RA patients, we show elevated CIT-013 epitope levels, which correlates with inflammation grade in synovium.

This data demonstrates that CIT-013 has a unique dual NET-targeting MoA, suppressing the pathological consequences of NETs in inflammatory-mediated immune diseases like RA. This reinforces the position of CIT-013 as a drug for NET-associated diseases with unmet therapeutic needs.

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1992 – P3.06.28

Jakinibs effect on subsets of the innate and adaptative immune system

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Introduction: Rheumatoid arthritis (RA) is a chronic autoimmune disease caused by genetic and environmental factors characterised by joint inflammation. The JAK-STAT inhibitors (jakinibs) are among the therapeutic options.
Aim: To determine the impact of jakinibs in cellular subsets of the immune system in RA patients by flow cytometry.
Materials and methods: Seventy-five patients treated with jakinibs were recruited, 20 healthy donors and, 20 RA patients treated with biological DMARDs, both paired by sex and age with the jakinibs group, were enrolled as controls. Peripheral blood mononuclear cells were isolated and analysed by multiparametric flow cytometry to characterise the immunophenotype of different subsets of the immune system.
Results: Within the jakinib group, 36 (48.00%) patients were treated with baricitinib, 13 (17.33%) tofacitinib, 19 (24.00%) filgotinib and, 8 (10.67%) upadacitinib; in the RA control group 11 patients were treated with tocilizumab and 9 with abatacept. A significant decrease in the percentage of cytotoxic NK Dim (CD56+CD16+) subset was observed in the jakinib group in comparison to the RA group (87.28 vs 92.18, p=0.001). There were significant differences between jakinib group and both healthy and RA patients in the percentage of activated NK Dim expressing Nkp30 (59.51 vs 89.74 and 85.28, respectively; p values: <0.001 and 0.007). In addition, the percentage of intermediate monocytes (CD14+, CD16+) was decreased in jakinib group in comparison with RA and healthy controls (9.84 vs 19.41 and 20.55, p values: <0.001 and 0.001). When comparing the subsets of T helper (Th) cells, the percentage of Th17 (CD3+CD4+CD45RA+CCR6+CXCR3-) and Th1+17 (CD3+CD4+CD45RA+CCR6+CXCR3+) were decreased in the jakinib group in comparison with healthy and RA controls (6.86 vs 9.38 and 10.01, p=0.023 and p=0.012) and (3.43, 10.43 and 8.38, p<0.0001 and p<0.0001).
Discussion: The JAK-STAT inhibition by jakinibs affects innate cells differently than biological DMARDs do. The decrease in both the cytotoxic activated NK and intermediate monocytes could explain some of the side effects caused by these drugs related to intracellular threats such as viral infections or possible neoplasia appearances. Further functional studies should be addressed to better understand the impact of jakinibs.

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European Journal of Immunology
Clinical-Basic-Translational

2025 – P3.06.29

Which came first, the chicken or the egg? Usefulness of determining total anti-Infliximab antibodies for monitoring biological therapy

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Purpose: Infliximab (IFX) is an Anti-TNF approved for the treatment of inflammatory bowel disease (IBD). It is a humanized chimeric biologic antibody that may be immunogenic. The loss of response due to the presence of anti-drugs antibodies (ADA) in 30-46% of treated patients. There are detection methods for these ADA that detect free antibodies vs. others that detect total antibodies (IFX-complex + free). To date, there is not much evidence regarding the difference or advantage of the determination of total antibodies over free antibodies. The objective of the study is to analyze the difference between the determination of free vs total ADA and their correlation with clinical response.
Methods: We performed a retrospective study with the determination of free and total antibodies in 33 samples of 12 patients with IBD from patients treated with IFX by HUVH gastroenterology unit with loss of response due to serum IFX concentrations < 3ug/ml using Time Resolved Fluorescence Lateral Flow Immunoassay (TRF-LFIA) with europium labeling.
Results: Free antibodies were detected in 9 samples from 6 patients and total antibodies in 15 samples from 8 patients. Of these 8 patients, total antibodies were also detected in 6 samples 1-3 months in advance to the appearance of free antibodies in 4 patients.
It was detected the presence of total but not free antibodies in patients with detectable drug concentrations (between 0,7 and 2,4ug/ml).
Conclusion: The determination of total ADAs can be an important tool in the monitoring of patients with IBD. In our results this determination is useful since it precedes the appearance of free ADAs.
In addition, in case of detecting drug levels below the therapeutic range, free ADAs could take time to appear while total ADAs are detectable 1-3 months earlier.

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2050 – P3.06.30

Advantages of the TRF-LFIA monotest method for monitoring biological drugs in clinical practice.

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Purpose: Infliximab (IFX) and Adalimumab (ADL) are anti-TNF monoclonal antibodies approved for the treatment of inflammatory bowel disease (IBD). Between 13-40% of patients treated with anti-TNF lose response during the maintenance treatment period. This loss of response can be primary or secondary, due to low concentration and/or presence of anti-drug antibodies (ADA) respectively. This makes the therapeutic drug monitoring (TDM) a tool of great interest. The aim of this study is to compare two methods for monitoring drug concentrations.
Methods: We analyzed 43 samples from patients treated with IFX and 21 samples from patients treated with ADL by HUVH gastroenterology unit by chemiluminescence immunoassay method (CLIA) vs single test method of Time Resolved Fluorescence Lateral Flow Immunoassay (TRF-LFIA) with europium labeling. The analysis we performed using the Passing Bablok method. Values ranging from 0.2 to 49.67 ug/ml are obtained for IFX and 2.05 to 16.09 ug/ml for ADL
Results: The results obtained by both methods show a good correlation, to IFX pearson´s r=0,984, passing bablok regression showed the following equation: Y= 1,01x – 1,38. The analysis did not show a proportional difference (slope - 1,384 95% CI, 0.9496 - 1.064), but it showed a constant difference given that the 95% CI of the intercept did not contain 0 (intercept 1,007 95% CI, -2.147 -0.6207). To ADL pearson´s r=0,980, passing bablok regression showed Y=1,39X – 2,32. The analysis showed a proportional difference given that the 95% CI of the slope did not contain 1 (slope 1,389 95% CI, 1,213-1,541). And showed a constant difference given that the 95% CI of the intercept did not contain 0 (intercept -2,319, 95% CI, -3,945 -0,6497).
Conclusion: The correlation of both methods is good for both parameters, although they are not interchangeable. The TRF-LFIA monotest method has several advantages. It has a wide laboratory catalog for minority analyses; it is ideal for optimizing savings and waste in low-volume, multiple-test throughput, and short turnaround routines to support immediate clinical decision-making. In addition, the equipment is easy to use, allowing analysis without the need for a highly trained operator and a high degree of flexibility and ease of use.

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2125 – P3.06.31

Gene expression analyses identify biomarkers that distinguish stable and active multiple sclerosis patients treated with fingolimod

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Purpose: Fingolimod is a sphingosine 1-phosphate receptor modulator that inhibits the egress of lymphocytes from the lymph nodes into the peripheral circulation and the central nervous system and reduces relapses and MRI activity in relapsing-remitting multiple sclerosis (RRMS). However, some patients continue to experience disease activity while on treatment. The purpose of this study was to identify genes, for use as predictive biomarkers of relapse on fingolimod treatment.
Methods: Gene expression was measured by Affymetrix Gene 2.0 ST array on RNA extracted from whole blood in an explorative cohort of 40 fingolimod-treated RRMS patients of which 20 were stable and 20 had disease activity with at least one relapse during the first year of treatment. Patients were matched on previous treatment, sex, and age. For validation samples from 109 RRMS patients after their six-months of fingolimod-treatment were included and information on clinical and imaging disease relapse activity was obtained from the Danish MS Registry. Real-time qPCR was used for validation of selected targets using TaqMan chemistry and a gene expression index was calculated. Analyses were performed in GenEx, Qlucore, and SPSS on log-transformed data and unadjusted p-values are reported.
Results: In the explorative cohort gene expression differences were observed between stable and active fingolimod-treated patients for 68 genes (±1.2-fold: ANCOVA, $p<0.01$, with adjustment for age, sex, and previous treatment). From these, ten genes were selected for validation - five high expressed genes in unstable patients; *MIR744*, *IL1R1*, *NEAT1*, *FCGR3B*, *TRPM6* and five genes with lower expression; *SBDS*, *KLRAP1*, *LILRB4*, *CYP4F22*, *SAT2*. To investigate their relationship with risk of relapse on treatment, we used cox-regression analysis and showed *MIR744*, together with sex, and previous treatment, could predict the risk of relapse on treatment (3.97 95%CI 1.62-9.76, p=0.003). A negative binomial analysis of total number of relapses confirmed the *MIR744* association (2.65 95%CI 1.15-6.10, p=0.022).
Conclusion: Of the ten differentially expressed genes tested in an independent cohort *MIR744* was able to predict relapses on fingolimod treatment and further tests should clarify its use as a predictive biomarker. Interestingly, its mature form, miR-744, was previously identified increased in MS patients.

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2231 – P3.06.32

Selective suppression of pathogenic B lymphocytes from Hashimoto's thyroiditis patients by chimeric protein molecules

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Purpose: Hashimoto's thyroiditis is one of the most common endocrine disorders affecting up to 20% of the adult population. No treatment or prevention exists except hormonal substitution of hypothyroidism.
We hypothesize that it may be possible to suppress selectively anti-thyroglobulin (Tg) IgG antibody producing B lymphocytes from HT patients by a chimeric protein molecule containing a monoclonal antibody specific for the human inhibitory receptor CR1, coupled to peptide epitopes derived from Tg protein. We expect that this treatment will down-regulate B cell auto-reactivity by delivering a strong inhibitory signal.
Methods: Three peptides – two epitope-predicted ones derived from Tg and another irrelevant peptide – were synthesized and then coupled with monoclonal anti-human CR1 antibody to construct three chimeric molecules. The binding to CD35 on human B cells and the effects of the chimeric constructs on PBMC and TMC from patients with HT were tested using flow cytometry, ELISpot assay and ELISA methods.
Results: We found that after the chemical conjugation all chimeras retained their receptor-binding capacity and the Tg epitopes could be recognized by anti-Tg autoantibodies in the patients' sera. This treatment down-regulated B cell autoreactivity and cell proliferation, inhibited Tg-specific B cell differentiation to plasmacytes and promoted apoptosis to the targeted cells.
Conclusion: The treatment of PBMCs from HT patients with Tg epitope-carrying chimeric molecules affects the activity of Tg-specific autoreactive B lymphocytes delivering to them a strong suppressive signal.

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P3.07 THERAPY OF ALLERGY AND HYPERSENSITIVITY

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69 – P3.07.01

Treatment of insulin allergy by intranasal Fc-fused preproinsulin

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Insulin allergy (IA) represents a rare yet severe immunological reaction to exogenous insulin treatment, affecting between 0.1% and 3% of patients with type 1 and type 2 diabetes. Symptoms range from type I IgE-mediated or type IV cutaneous reactions, including urticaria to laryngeal angioedema, occasionally escalating to potentially life-threatening anaphylaxis. This condition complicates diabetes management by hampering effective insulin therapy and glucose regulation, with no established cure currently available, often necessitating a shift to an unnecessary insulin pump regimen. Therefore, our goal is to establish a desensitization therapy for this condition.
We have developed a tolerogenic fusion protein that comprises preproinsulin linked to the Fc domain of a human IgG (PPI-Fc). The Fc fragment increases the half-life, bioavailability, and uptake by immune and mucosal epithelial cells by binding to Fc gamma receptors and the neonatal Fc receptor (FcRn).
We evaluated PPI-Fc's effectiveness in treating insulin allergy through non-invasive intranasal delivery. We first verified the expression of FcRn by mouse epithelial cells from the naso-respiratory tract and efficient PPI-Fc transcytosis and bioavailability. Second, we established a mouse model of cutaneous insulin allergy in the Non-Obese Diabetic (NOD) background developing spontaneous type 1 diabetes (T1D), thus simulating the human situation. IA is induced by intradermal sensitizations and revealed by local extravasation, increased IgE titers, insulin-specific Th2 responses, and mast cell degranulation. Third, we demonstrated that PPI-Fc intranasal administrations, after the insulin sensitization phase, significantly decreased the allergic reaction and all associated immune responses, thus providing the first proof-of-concept of the therapeutic efficacy of our strategy.
These encouraging outcomes emphasize the potential of intranasal PPI-Fc as a novel therapy for insulin allergy while also indicating the feasibility of administering Fc fusion proteins through the intranasal route for various allergic conditions. Furthermore, they may open new therapeutic opportunities for T1D treatment as both diseases share the same target insulin antigen.

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246 – P3.07.02

Immunomodulatory impact of the BanLec-Bet v 1 chimera and its variants on antigen-presenting cells

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Employing effective adjuvants and hypoallergenic isoforms is essential for improving the efficacy and safety of allergen-specific immunotherapy (AIT). Banana lectin (BanLec) exhibits immunomodulatory potential, and mutation of histidine at position 84 to threonine ($BanLec_{H84T}$) reduced its mitogenicity. Our study aims to assess the immunomodulatory potential on antigen-presenting cells (APCs) of recombinant chimeras combining the major birch pollen allergen Bet v 1a and its hypoallergenic isoform Bet v 1l with BanLec and $BanLec_{H84T}$. After the production in *Escherichia coli* and purification, chimeras were co-incubated with peripheral blood mononuclear cells (PBMC) from birch pollen-allergic patients. Subsequently, secreted cytokines were analyzed. Antigen uptake was evaluated by co-incubating the treated monocyte-derived dendritic cells (MoDC) or HLA-DR7$^+$ EBV-transformed B cells (EBV-BCL) with T cell receptor (TCR) transgenic (tg) Jurkat reporter cells. The tg TCR recognizes the immunodominant epitope of the Bet v 1, Bet v $1_{142\text{-}153}$ in the context of HLA-DR7 ($Jurkat_{tg}$). MoDC were analyzed for the upregulation of activation marker expression (CD80, CD86, HLA-DR) and secreted cytokines (IL-6, IL-8, IL-10, IL-12p40, IL-23, TNF- α, IFN-γ). Furthermore, chimera's uptake by APCs was modulated by incubation with different uptake pathway inhibitors/enhancers during antigen loading, and co-culture with $Jurkat_{tg}$. We found that the co-incubation of PBMC with Bet v 1a-BanLec prompted the secretion of the anti-inflammatory cytokine IL-10 and augmented the IFN-γ/IL-4 ratio. Co-incubation of chimeras with EBV-BCL or MoDC revealed that all chimeras strongly activated the allergen-specific $Jurkat_{tg}$. Additionally, activation marker expression and secreted cytokines of MoDC co-incubated with the respective chimeras indicated that they led to the activation of these cells. Supplementation of media with low concentrations of glucose stimulated antigen uptake and subsequent $Jurkat_{tg}$ activation. In contrast, antigen uptake was inhibited by sucrose, suggesting interference with the macropinocytotic uptake pathway. Our results highlight the potential of chimeras to modulate allergen-specific immune responses, enhancing anti-inflammatory cytokine secretion and promoting allergen-specific T-cell activation thereby demonstrating their potential in AIT.

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1005 – P3.07.03

Long-term immune changes in patients allergic to bee venom undergoing immunotherapy

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Purpose: Hymenoptera venom allergy is usually manifested with local symptoms, but systemic reactions, including cardiac or respiratory arrest are not uncommonly described after a bee sting. The only available healing treatment to prevent these potentially life-threatening reactions is venom immunotherapy (IT). The mechanisms which govern the achieving of tolerance are not completely known yet. The aim of this study was to analyse changes in the immune system of patients allergic to bee venom who underwent IT.
Methods: A total of 17 allergic patients to bee venom were recruited at the Reina Sofía University Hospital (Córdoba, Spain). Blood samples were collected before (T0) and after 1 (T1), 3 (T3) and 5 (T5) years of IT, when sting challenges were conducted. Basophil activation test (BAT) was performed using 0.1 and 1 μg/mL of *Apis mellifera* venom extract at each time. Circulating T-cell subpopulations were also analysed by flow cytometry.
Results: The study population included 13 (76%) men and 4 (24%) women (mean age 50 ±13). Sting challenges demonstrated full effectiveness in the 16 tested patients. Regarding BAT, the percentage of degranulated basophils ($CD63^+$) using 1 μg/mL of whole bee venom extract was significantly reduced over the course of IT (T0: 53.3%; T1: 22.1%; T3: 8.7% and T5: 10.2%; $p<0.05$). When considering T-regulatory (Treg) cells, those $CD39^+$ significantly increased in T3 (T0: 25.4% vs. T3: 32.4%; $p<0.05$), whereas those $Ki67^+$ significantly increased throughout the follow-up time (T0: 1.6% vs. T3: 6.8% and T5: 8.8%; $p<0.05$). Furthermore, $CTLA\text{-}4^+$ Tregs significantly decreased at T5 (T0: 10.9% vs. T5: 1.3%; $p<0.05$). No significant differences were found with regard to the remaining studied populations.
Conclusion: IT with bee venom promotes the desensitisation of basophils and changes in the proliferative and tolerogenic properties of Tregs subpopulations, which may be implied in achieving immunotolerance. These cellular biomarkers could be considered as candidate for monitoring the tolerance state.

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P3.08 TRANSPLANTATION IMMUNOLOGY

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217 – P3.08.01

Influence of the Glutathione S-transferase theta 1 antibodies on renal function rates after kidney transplant

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Introduction: The glutathione S-transferase theta 1 *(GSTT1)* is a protein that may function as a minor histocompatibility antigen. Specific antibodies could result an alloimmune reaction in *GSTT1-null* recipients receiving a positive graft. In kidney transplantation, *GSTT1* donor-recipient mismatch is associated with productions of anti-GSTT1 antibodies. *GSTT1* is encoded by a single polymorphic gene with two alleles, *GSTT1* (positive) and *GSTT1*0* (null). The detection of *anti-GSTT1* antibodies is frequent in renal transplant patients with *GSTT1-null* allele who receive organs from donors with the *GSTT1-positive* allele. However, its impact on the evolution of renal grafts is not currently defined.
Purpose: To determine if the presence of *anti-GSTT1* antibodies influence the renal function of the transplanted patient's graft.
Methods: We analyzed the presence of *anti-GSTT1* antibodies in serum, pre- and post-transplant of 42 recipients with the condition *"GSTT1* positive Donors / *GSTT1-null* Receptor". We conducted this analysis using Luminex technology (Anti-no HLA LIFECODES Non-HLA Antibody Kit). To assess the renal function of the graft, CKD-EPI formula was used. The influence of these antibodies was evaluated at the end of the follow-up (median follow-up of 54.3 months post-transplant) by multivariable linear regression. We performed data analysis using the statistical program SPSS 26.0, with the quantification of antibodies measured as median fluorescence intensity (MFI) treated as a quantitative variable.
Results: *anti-GSTT1* antibodies were detected in 16 out of 42 recipients (38%), with a mean MFI of 11,700 (standard deviation 6,360). Among these, 12 (29%) tested positive pre-transplantation and the rest (9%) tested positive at a median follow-up of 34.5 months. Patients with *anti-GSTT1* antibodies exhibiting high MFI values tended to have lower glomerular filtration rates at the end of follow-up compared to those with lower MFI values. However, the results did not reach statistical significance (B: -1.9, 95% confidence interval: -3.9 to 0.0, p=0.054).
Conclusion: There is a negative correlation between MFI values of *anti-GSTT1* antibodies and the glomerular filtration rates of the graft following kidney transplantation. However, further studies with a larger sample size would be necessary to confirm these findings on a greater alteration of renal function.

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231 – P3.08.02

SLC7A5 deletion in T cells or L-leucine restriction in diet efficiently control acute graft-versus-host disease mouse model

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Background: Acute graft-versus-host disease (aGVHD) remains the principal obstacle to successful allogenic hematopoietic cell transplantation. The disease is triggered by the activation and expansion of donor T cells that recognize the genetically incompatible host as foreign, inducing multiple-organ damage to the transplant recipient. The amino acid transporter SLC7A5 (LAT1), which mediates the uptake of L-Leu in activated T cells as well as in malignant cells, has become an important target in the control of inflammation and cancer. However, its role in aGVHD has not been explored before.
Methods: We assessed the role of SLC7A5 expression in T cells in a mouse model of aGVHD. In addition, we evaluated the effect of dietary L-Leu restriction in mice subjected to aGVHD, to control SLC7A5 function in allogenic T cells. Metabolomic experiments were performed to assess the role of L-Leu in aGVHD.
Results: SLC7A5 deletion in donor T cells completely prevented aGVHD-induced gut inflammation and lethality in mice, thus enhancing the replenishment of non-allogenic T cells. Moreover, SLC7A5 deletion prevented proliferation and promoted apoptosis of activated T cells, reducing T-bet and IFNγ expression. Treg differentiation was increased by the SLC7A5 deficiency. The administration of a L-Leu-free diet prevented allogenic T cell expansion and aGVHD associated mortality in mice, thus resembling the results obtained by deletion of SLC7A5 in T cells. Dietary L-Leu restriction modifies serum amino acid profile and prevents the increase of metabolites derived from tricarboxylic acid cycle, ketogenesis, and urea cycle, induced by aGVHD. Our data identify L-Leu as the preferred substrate oxidized to ketone bodies during aGVHD.
Conclusion: This study demonstrates for the first time that targeting SLC7A5-mediated L-Leu uptake in allogeneic T cells by genetic deletion or amino acid restriction diet effectively prevents allogenic T cell expansion in an experimental model of aGVHD.

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257 – P3.08.03

Reduced ceramides are associated with acute rejection in liver transplant patients and skin graft and hepatocyte transplant mice, reducing tolerogenic dendritic cells

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We set up this study to understand the underlying mechanisms of reduced ceramides on immune cells in acute rejection. The concentrations of ceramides and sphingomyelins were measured in the sera from hepatic transplant patients, skin graft mice and hepatocyte transplant mice by LC-MS/MS. Serum concentrations of C24 ceramide, C24:1 ceramide, C16:0 sphingomyelin, and C18:1 sphingomyelin were lower in liver transplant (LT) recipients with than without acute rejection (AR). Comparisons with the results of LT patients with infection and cardiac transplant patients with cardiac allograft vasculopathy in humans and in mouse skin graft and hepatocyte transplant models suggested that the reduced C24 and C24:1 ceramides were specifically involved in AR. A ceramide synthase inhibitor, fumonisin B_1 exacerbated allogeneic immune responses *in vitro* and *in vivo*, and reduced tolerogenic dendritic cells (tDCs), while increased P3-like plasmacytoid DCs (pDCs) in the draining lymph nodes from allogeneic skin graft mice. The results of mixed lymphocyte reactions (MLR) with ceranib-2, an inhibitor of ceramidase, and C24 ceramide also support that increasing ceramide concentrations could benefit transplant recipients with AR. The results suggest increasing ceramides as novel therapeutic target for AR, where reduced ceramides were associated with the changes in DC subsets, in particular tDCs.

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374 – P3.08.04

Following autologous hematopoietic stem cell transplantation decidual-like NK cell subsets are expanded in oncologic patients

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Purpose and methods: Early immune reconstitution after autologous hematopoietic stem cell transplantation (autoHSCT) is associated with a better outcome in a variety of cancers. Natural killer (NK) cells have a role in anti-tumoral responses and they are the first lymphocyte subset recovering after autoHSCT. In order to understand the biology and physiopathology of NK cells after autoHSCT, we have studied them by multiparametric flow cytometry and analyzed relevant cytokines in the plasma of adult patients with different hematological cancers before and after autoHSCT (over 6 months). We have also performed in vitro experiments using flow cytometry and scRNA-sequencing in order to better characterize the different NK cell subsets.
Results: Circulating NK cell subsets significantly changed early after autoHSCT. There was a transient acquisition of a decidual-like phenotype, characterized by a significant expansion of CD9+CD151+ cells. Furthermore, plasma levels of some cytokines, such as IL-15 and GDF-15, with relevant roles in NK cell function were significantly increased, while TGF-β levels were unchanged. In vitro flow cytometry and scRNA-sequencing experiments and correlation analysis showed that the combination of IL-15 and TGF-β, among other factors, could explain the acquisition of this distinctive decidual-like phenotype and transcriptome early after autoHSCT. Furthermore, we have observed a correlation between the frequencies of certain NK cell subsets with relapse, which could be used as prognosis biomarkers.
Conclusion: In conclusion, NK cell phenotype and transcriptome are significantly altered early after autoHSCT, resembling NK cells from the decidua. IL-15 and TGF-β may have a significant role in the acquisition of this phenotype.

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407 – P3.08.05

BSA dilutions as a helpful tool to treat background in single antigen luminex

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Purpose: Anti-HLA antibodies are one of the major causes of graft rejection in kidney transplantation. Single Antigen bead (SAB) assays have improved the sensibility of anti-HLA antibodies detection. Nevertheless, some treatments or autoimmune diseases could interfere with this analysis, increasing the background and conducting to a high median fluorescence intensity (MFI) of the negative control bead (NCB) and false positive results. This could extend the time in the waiting list or disabling the opportunity of transplantation for these patients, especially in hypersensitized with elevated cPRA. The aim of this study is to evaluate a protocol of sera pretreatment to eliminate background in sera with SAB with values of MFI >250 in NCB.
Methods: We conducted a prospective study of 21 sera with a NCB >250 MFI or increased background in SAB (1/2/EDTA-pretreated) from patients included in waiting list for kidney and bone marrow transplantation. These sera and negative and positive pools were retested by SAB assay using Luminex after EDTA-0.6%/BSA-5%/PBS pre-treatment at 1/2, 1/4 and 1/8 dilutions.
Results: Negative and positive pools maintained stable after pretreatment at the three dilutions. The MFI value of positive bead control of the 21 pretreated sera remained in correct range for the three dilutions. The MFI value of NCB decreased to correct range for the 21 pretreated sera, but the dilution which reach the correct MFI range vary for each patient. 1/8 dilution was the only one with no NCB over 250 MFI. Before pretreatment, several patients held anti-HLA antibodies against self HLA molecules, but after pretreatment 1/8 no one held them. Four hundred and ninety-one less anti-HLA reactivities in those 21 patients were present (average 23 less). cPRA greatly decreased in the majority of the patients. Ten patients underwent transplantation thanks to this pretreatment without complications in their follow up.
Conclusion: Sera pretreatment with EDTA-0.6%/BSA-5%/PBS at 1/8 dilution is a good option to reduce background and false positive results, increasing transplantation opportunities for those patients with interferences in SAB.

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408 – P3.08.06

Quantifying HLA mismatches at epitope level in haploidentical hematopoietic stem cell transplnatation: impact in the outcome in strategies using PTCy

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Purpose: Allogenic stem cell transplantation is one of the main curative therapies for haematological malignancies and some immune disorders. The HLA system remains one of the main barriers facing the success of this process. Only 25% of the patients who receive an allogeneic hematopoietic stem cell transplantation (allo-HSCT) from a related donor is HLA identical; thus, new alternative donors like haploidentical HSCT (haplo-HSCT) with post-transplant cyclophosphamide (PTCy) are increasing in clinical practice. The aim of this study is to evaluate the immunogenicity of the HLA mismatches in haplo-HSCT with PTCy accordingly to relapse and GRFS.
Methods: A cohort of 145 haplo-HSCT patients and donors, were retrospectively analysed. HLA-A, -B, -C, -DRB1, -DQB1 and -DPB1 typing of the pairs was performed by PCR-SSOP and/or NGS, when appropriate. HLA compatibility was evaluated by the *Predicted Indirectly Recognizable HLA-Epitopes* (PIRCHE) algorithm and PIRCHE Score (PS).
Results: The median age of the patients was 55 years, enriched in man (55.9%). Acute Myeloid Leukemia (AML) and Myelodysplastic syndromes (MDS) were the main indication for haplo-HSCT (52%). The followed up was 36 months.
PIRCHE algorithm showed that patients with relapse presented an augmented PS in host versus graft (HvG) direction (66, IQR: 50-85 vs 44, IQR: 29-69; p=0.002). ROC curve analysis showed that patients with PS >50 presented relapses earlier (HR: 4.66, p=0.001). The same data was observed analysing incompatible ABO haplo-HSCT (HR: 2.66, p=0.014). In a multivariate analysis both parameters remain as independent risk factors for relapse.
The analysis of PS and GRFS showed that patients with events included in GRFS presented an augmented PS HvG (60, IQR 36-84 vs 43, IQR: 29-56; p=0.001). Patients with PS>49 (calculated by ROC) presented a reduced GRFS (HR: 2.19, p=0.001). Similar results were obtained examining the comorbidity index (HCT-CI)>3 (HR: 1.84, p<0.001). The multivariate analysis demonstrated that both parameters were independent risk factors for a diminished GRFS.
Conclusion: HLA disparities at epitope level calculated accordingly by PIRCHE-algorithm, especially PS-I+II, influence the clinical outcome of haplo-HSCT with PTCy and should be included in the donor selection algorithm.

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583 – P3.08.07

Pharmacodynamic effect of mTOR inhibition-based immunosuppressive therapy on T and B cell subsets after renal transplantation

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Objective: Kidney transplantation is currently the best treatment option for terminal kidney failure. After transplantation, a tailored immunosuppression balancing the protection from infections and allograft survival is of utmost importance. Today, the immunosuppression by mTOR inhibitors (mTORi) is solely pharmacokinetically monitored, not necessarily reflecting the efficacy of the blockade of the PI3K-Akt-mTOR pathway which may lead to a potential under- or over-immunosuppression. The close correlation between the mTOR activity and the p70S6 kinase (p70S6K) phosphorylation renders the latter as a promising target to monitor pharmacodynamic effects of mTORi.
Design: In this cross-sectional study, phosphoflow cytometry was used to determine the efficacy of mTOR inhibition in peripheral T and B lymphocyte subsets by assessing p70S6k phosphorylation in renal transplant recipients upon treatment with a combination of either mTORi and calcineurin inhibitors or mTORi with mycophenolic acid. Dialysis patients with end-stage renal disease and healthy age-matched volunteers served as controls.
Results: mTORi-treatment reduced p70S6K phosphorylation in $CD4^+$, $CD8^+$ T and $CD19^+$ B cells compared to healthy controls. An analysis of select subpopulations of $CD4^+$ T cells and B cells revealed a significant reduction of p70S6K phosphorylation levels in helper T cells, transitional B cells, memory B cells, and naive B cells upon mTORi treatment, while regulatory T cells and plasmablasts were not affected. Compared to mTORi+MPA therapy, mTORi+CNI treatment exhibited an even stronger inhibition of p70S6K phosphorylation in helper T cells and cytotoxic $CD8^+$ T cells. However, trough levels of mTORi displayed no correlation with p70S6K phosphorylation.
Conclusion: mTORi selectively inhibited p70S6K phosphorylation in select lymphocyte subtypes. Assessing p70S6K phosphorylation by phosphoflow cytometry may serve as an approach to understand cell subset specific effects of mTOR inhibition and providing detailed pharmacodynamic information for an individual tailoring of the mTORi therapy.

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654 – P3.08.08

Withaferin A suppresses the activation of CD4+ T cells and the following differentiation of B cells

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The generation of class-switched donor-specific antibodies (DSAs), pivotal in chronic antibody-mediated allograft rejection (AMR), is mediated by a T cell-dependent mechanism, wherein $CD4^+$ T cells promote the differentiation of naive B cells into memory B cells and long-lived plasma cells. Withaferin A (WA), a steroidal lactone from Withania somnifera, recognized for its immunosuppressive properties by inhibiting lymphocyte proliferation and exhibiting anticancer and anti-inflammatory effects, remains unexplored as an immunosuppressant to mitigate AMR by attenuating $CD4^+$ T cell activation and subsequent B cell differentiation. Here, human peripheral blood mononuclear cells (PBMCs) were employed to evaluate whether WA can impede B cell differentiation via the attenuation of helper T ($CD4^+$) cell activation. Our findings indicate that WA effectively suppresses $CD4^+$ T cell proliferation without inducing cell death, evidenced by reduced expression of activation markers (CD25, CD69, CD71, and CD54) and diminished interleukin-2 (IL-2) secretion, critical for T cell proliferation and differentiation into plasma cells (PC). Additionally, WA attenuates B cell activation, as shown by decreased expression of CD80, CD86, and MHC-II, along with reduced interleukin-6 (IL-6) secretion, pivotal for PC progression and high-affinity antibody production. Moreover, WA disrupts the $CD4^+$ T cell-dependent differentiation of B cells, underscoring its potential as an immunomodulatory agent in preventing antibody-mediated responses. In conclusion, WA demonstrates the capability to inhibit B cell activation and differentiation into PCs by suppressing both the proliferation and activation of $CD4^+$ T cells. These findings suggest that WA could serve as a potential therapeutic agent for managing AMR by specifically modulating the interaction between $CD4^+$ T and B cells.

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944 – P3.08.09

Enhanced Renal Tissue Regeneration and Functional Recovery with IL-15 Treatment in Acute Kidney Injury

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During transplantation, temporary deprivation of blood supply to the organ followed by reperfusion can lead to ischemia-reperfusion injury (IRI), which negatively impacts graft function and viability. Post-kidney transplantation, IRI can result in acute kidney injury (AKI), a complex clinical condition characterized by a rapid decline in renal function and extensive tubular epithelial cell (TEC) injury. Despite advances in understanding the pathophysiology of AKI, effective therapies to protect the donor organ from IRI remain limited. IL-15 was identified as a survival factor for TECs and $CD8^+$ T cells. This study investigates the potential of low-dose rIL-15 treatment in a mouse model of AKI.
Male C57Bl6/J and $CD8\alpha^{-/-}$ mice, 8 weeks old, received either a low-dose of rIL-15 treatment or vehicle. After 7 days, both groups were subjected to bilateral renal IRI, with their body temperature continuously maintained throughout the 20-minute ischemia period. Following a reperfusion period of 20 hours, the mice were sacrificed for subsequent analysis. IL-15 treatment resulted in improved renal function, as indicated by reduced blood urea nitrogen and creatinine levels in treated wild-type mice post-IRI. Histological assessment showed a comparable level of tubular injury between groups, but the IL-15-treated group exhibited reduced tubular atrophy and a higher presence of recovering TECs. Metabolomic analysis of kidney tissue revealed that IL-15 treatment partially reverted the metabolic phenotype of IRI, with upregulation of metabolites counteracting oxidative stress and improving mitochondrial function and energy regulation. Interestingly, IL-15 treatment increased kidney-infiltrating $CD8^+$ memory T cells expressing regulatory-associated markers CD122 and Ly49. IL-15 treatment failed to protect $CD8\alpha^{-/-}$mice from AKI.
Collectively, our findings suggest that IL-15 pre-treatment enhances the regeneration of renal TECs following IRI and ameliorates AKI, presenting an innovative way we can approach donor organ treatment prior to transplantation. Further research is warranted to unravel the mechanisms underlying IL-15-mediated improvement of AKI and ascertain the potential role played by $CD8^+$ T cells in this process.

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983 – P3.08.10

Immunosenescent T-lymphocyte subpopulations are greatly expanded in long-term kidney transplant recipients.

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Purpose: This ongoing study aims to assess the effect of long-term kidney transplantation (KTx) on the T-lymphocyte series and especially subpopulations with an immunosenescent phenotype.
Methods: The study population consisted of a group of long-term KTx patients (>17 years) and a group of recently transplanted patients (1 year). Peripheral blood samples were examined by flow cytometry and the subpopulations of total lymphocytes, CD4+, CD8+, Natural Killer (NK), CD4+CD28null, CD8+CD28null and CD4+CD25+FOXP3 (Tregs) T-cells were evaluated.
Results: Twenty-five long-term and 38 recently transplanted patients took part in the study. Patient age (p=0,714), sex (p=0,304), eGFR (p=0,153), rejection episodes (p=0,154), compatible HLA antigens (p=0,496 for class I and p=0,334 for class II), DGF episodes (p=0,522) preemptive transplantations (p=0,092) and CMV infection history (p=0,168) did not differ between groups, while long-term KT recipients exhibited a higher ratio of DSAs (4, 6, 6% vs 0, 0% p=0,017) and living donor transplants (16, 64% vs 15, 39,5%, p=0,049), more infection episodes (22, 88% vs 3, 7,9%, p<0,001), as well as a lower percentage of patients treated with basiliximab (11, 57,9% vs. 35, 92,1%, p=0,004) and lower dialysis vintage (30:2-90, vs 75,5:0-156, p=0,028). In the long-term group, a significantly higher number of total lymphocytes (2300: 1245-3900 /μL vs 1600: 800-2900 /μL, p=0,003), CD4+ (1266 ± 591 vs 781 ± 320/μL, p<0,001), CD4+CD28null (125: 26-2232/μL vs 41: 2-254/μL, p<0,001), and CD8+CD28null (441: 55-922/μL vs 241: 34-1601/μL, p=0,021) T-cells, a greater percentage of CD4+ T-cells (52,2 ± 13% vs 44,8 ± 11,6%, p=0,024) and a smaller percentage of Tregs (2,8: 0-7,5% vs 4,2: 1,1-7,8%, p<0,0001) was observed, while there was no significant difference concerning the ratio of total lymphocytes (p=0,183), CD8+ T-cells (p=0,171), NK cells (p=0,174), and the number of NK cells (p=0,653) and Tregs (p=0923).
Conclusions: Long-term KTx is associated with significant alterations in the T-lymphocyte series, mainly due to the expansion of immunosenescent T-cell subpopulations.

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1028 – P3.08.11

QuantiFERON-CMV assay by chemiluminescence immunoassay: Is it more suitable for real-live monitoring of transplant patients?

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Purpose: Interferon-gamma release assay is used to monitor CMV-specific cell-mediated immunity (IGRA-CMV) in immunocompromised patients. IGRA-CMV is an *in vitro* diagnostic test that quantifies the interferon-gamma (IFNG) released by CD8+ T cells after stimulation with CMV antigens by the enzyme-linked immunosorbent assay (ELISA). However, ELISA has some disadvantages for clinical implementation such as its slow processing speed. A fully automated chemiluminescent immunoassay (CLIA) detection technique has recently been developed to quantify IFNG to detect latent tuberculosis infection (CLIA-TB). The aim of this work is to compare the results of IGRA-CMV by ELISA with those obtained by CLIA in an automated analyzer using CLIA-TB reagents.
Methods: The IGRA-CMV assay was performed by ELISA in kidney and lung transplant patients between July 2019 and April 2023 on an automated platform at the IMIBIC/Reina Sofía Hospital (Cordoba, Spain). In brief, 1 mL of heparinized whole blood was collected in three collection tubes (nil, CMV peptides and mitogen) and incubated for 16–24 h at 37 °C. Supernatants were analyzed for IFNG by ELISA. Samples were considered "Positive" when the IFNG level ≥0.2 IU/mL. The remaining supernatants were subsequently preserved at -80 °C. For the present study, the IFNG levels in the same samples were determined by CLIA on an automated analyzer and the results were compared.
Results: One hundred and three IGRA-CMV supernatants from kidney (n = 50) and lung (n = 53) transplant patients were selected. An agreement of 87.4% (kappa coefficient 0.788) between CLIA and ELISA was observed. Thirteen (12.6%) discrepant results were detected, mainly in borderline ELISA results. Some borderline Negative results by ELISA (<0.2 IU/mL) were above the 0.2 IU/mL cut-off by CLIA and then were Positive (range 0.21–0.31 IU/mL).
Conclusions: CLIA shows substantial concordance with ELISA and acceptable discrepancies. This, together with some advantages of CLIA, such as full automation, reproducibility, faster processing or its continuous-load system, make it a good alternative for monitoring CMV response in transplant patients in clinical routine. However, the possible higher sensitivity of CLIA returns a higher number of Positive results, which entails potential clinical consequences.

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1087 – P3.08.12

HLA compatibility in renal transplantation: Next Generation Sequencing versus Low/Intermediate resolution methods in our Centre

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Introduction: HLA typing is an essential step in the selection of receptors for kidney transplant. In recent years, Next Generation Sequencing has become a technique present in all histocompatibility laboratories, making high-resolution typing more accessible to all. Histocompatibility of a higher resolution could lead to a more accurate patient selection and a better immunological follow-up after transplant.
Purpose: To compare the HLA compatibility between kidney transplant donors and receptors studied through low resolution techniques versus high resolution in our cohort
Methods: A retrospective observational study comparing HLA compatibility of kidney transplant of 58 patients and their donors, measured through low resolution methods (SSO, qPCR test) with the same compatibility measured with Next Generation Sequencing (NGS) in our centre. Patient data (age, sex, blood type…) was collected from the available clinical records. Panel Reactive Antibody (PRA) was calculated with the results obtained through Luminex testing of patient sera. Compatibility was evaluated using the alleles for HLA-A, HLA-B and HLA-DRB1. All transplants were performed among patients of the same blood type
Results: A total of 39 donors and 58 corresponding receptors were typed. From the evaluated receptors: there were 34 men and 28 women; divided by age groups, 10 patients were under 50 years, while 48 were older than 50 years. Divided by blood type, there were 28 type A patients, 3 type B, 5 type AB and 22 type 0. A total of 4 patients were excluded due to insufficient data. After reevaluating the compatibility with their donors through NGS typing, a total of 32 patients showed worse compatibility than what was initially analysed through low/intermediate resolution testing.
Conclusions: Considering that parameters most frequently used for pairing donor and receptor include blood type, age disparity, immune sensitivity and HLA compatibility, high resolution testing can impact receptor selection and immunological follow-up, as compared to low/intermediate resolution methods.

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1143 – P3.08.13

Assessment of clinical-laboratory parameters in patients with autoimmune renal disease following transplantation

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Background: Rare autoimmune disorders, such as lupus nephritis (LN), granulomatosis with polyangiitis (GPA), and anti-glomerular basement membrane (anti-GBM) disease, can progress to end-stage renal disease (ESRD). Renal transplantation (RT) is the most effective replacement therapy, improving survival and quality of life. Despite the low prevalence of recurring nephritis in allografts, clinical and laboratory surveillance is necessary for successful management of both grafts and patients.
Purpose: The aim of this study was to record and present the data from clinical and laboratory monitoring of patients with autoimmune-mediated kidney diseaseafter RT.
Results: Five patients who had undergone RT due to auto-immune mediated ESRD were included in the present study. Specifically, we enrolled 2 females (aged 50 and 37) with SLE and elevated levels of anti-dsDNA antibodies (Abs), 2 males (aged 40 and 53) with GPA and positive anti-C-ANCA Abs, and 1 male (aged 43) with positive anti-GBM Abs. Immunosuppressive agents, such as tacrolimus, mycophenolic acid, and corticosteroids, were administered to all patients. In the 5-year follow-up period after RT, we recorded good rates of graft survival and no case of antibody or T-cell mediated rejection. Importantly, no recipient has developed donor-specific Abs. There was no documented case of disease recurrence, and Abs followed a downward trend over time until normalization. A single patient passed away five years after transplantation from necrotizing CMV colitis, maintaining normal graft function until the time of death.
Conclusions: Given our small sample size, we acknowledge the limitations of our study. However, it appears that RT is the definitive treatment option for ESRD of autoimmune origin. Despite the low prevalence of post-RT disease recurrence, recipients should be monitored closely, enabling timely diagnosis and early management to prevent transplant rejection.

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1199 – P3.08.14

Kidney transplantation with several and repeatedly positive crossmatch: making a leap into the void

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Purpose: The aim of this study is to find an explanation for five positive crossmatch results in a year and a half period, from a patient on kidney transplant waiting list, who had been tested for HLA class I and II DSA and MICA antibodies several times, being negative against all the potential donors HLA-alleles. Besides, this patient has not been diagnosed so far with any autoinmune disorder which could lead to a positive crossmatch.
Methods: Anti-HLA class I and II IgG and MICA antibodies and anti-HLA class I and II of the IgM isotype antibodies were examined and came out negative. Taking this into account, and finding no other explanation for the positive crossmatch results, we decided to test 60 different autoantibodies (non HLA related), that could be related to post-trasplantant rejection. The selection of autoantibodies was based on studies of acute accelerated rejection in renal transplantation between HLA identical siblings.
Results: 58 out of 60 non HLA tested autoantibodies were positive. Nevertheless, none of the most positive ones, such as ENO1, HSPB1, FIBRONECTIN1, PLA2R1, MYOSIN, SNRPB2, Thyroglobulin, LPHN1, SNRPN and COLLAGEN III, were associated to a positive crossmatch outcome. Thus, Nephrology and Immunology units decided to transplant the patient at the next opportunity regardless of the crossmatch result. The patient was finally transplantated, sharing one HLA-DR and one HLA-A allele with the donor, with a new positive crossmatch result again, as expected. The surgery was succesfully, with only some post-surgery non-graft related complications. No signs of any type of rejection have been observed until today, in a 6 months follow up period.
Conclusion: In our experience, those patients negative for DSA and MICA specific antibodies, and with a history of positive crossmatches that cannot be explained by any other event/condition, should be considered candidates for kidney transplant as long as they do not show any other clinical contraindications. Thus, we believe that the autoantibodies panel mentioned above, can be helpful to rule out any other posible interferences in the positive crossmatch results, making the complicated decision to transplant patients in a similar situation easier and safer.

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1374 – P3.08.15

Human leucocyte antigen and eplets – A trimolecular docking method for donor selection in renal transplantation

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Within the past 5 decades, the studies focusing on human leucocyte antigen (HLA) for identifying a perfect donor for renal transplantaion have found its way to new hieghts. HLA, itself being an antigen and a complex gene set plays a strong role in transplantation. The present study has been focused to ascertain an ideal strategy for selecting unrelated individuals as potential donors for renal transplantation by analyzing the number of eplet mismatches and HLA structural variations, that each individual possesses, thus eliminating the possibility of an undesired immune reaction observed in Indian population.

The study was conducted from a cohort of 1144 transplant patients and donors (n=572 pair) collected from 2 hospitals in India, within a period of 50 months. The mechanism of antigen presentation by class II HLA protein was simulated using the molecular modeling and docking studies. Required structural optimizations on HLA, and other moleclues were carried out with the aid of Schrodinger Maestro. The HLA-antigen interaction studies were carried out by PRODIGY web server. The study is proposed as a new approach, which combines transplantation immunology and immunoinformatics which aided in identifying an appreciable correlation between the acquired clinical data, eplet mismatch analysis and HLA structural information in comparison with the corresponding molecular models. Here, the difference obtained in the $\Delta G/K_d$ values in case of eplet matched and mismatched HLA DRB pairs substantiates the inference. There was an appreciable number of interfacial contacts between the eplet matched HLA DRB structures which were comparable.

The study establishes that class II HLA DRB structures which share same eplets between each other can present an antigen peptide in a similar energy ($\Delta G/K_d$) pattern despite of the peptide involved. The comparison between eplet matched and mismatched class II HLA DR using bi-molecular and tri-molecular protein docking, the results revealed that the HLA-Ag complexes with similar eplets showed same degree of energy in antigen presentation to TCR. The outcome of our study was parallel with those patients who faced graft rejection. Therefore, the study bridges the gap between eplet number and graft survival by structural biological analysis.

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1375 – P3.08.16

Analysis of the relative immunogenicity of mismatched HLA class I eplets in renal transplantation

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Purpose: It is now universally accepted that HLA matching affects transplant outcome and that HLA antibodies are primary causes of transplant rejection. HLA antibodies recognize epitopes, which can be structurally defined by eplets. Understanding the immunogenic potential of HLA epitopes/eplets is increasingly relevant in the field of donor-recipient compatibility as it allows for the identification of acceptable mismatches for sensitized patients and the development of permissible mismatch strategies for non-sensitized patients. The fundamental objective of this study is to analyze differences in the immunogenicity of the HLA eplets described to date in the context of renal transplantation.
Methods: The study population consists of 118 consecutive renal transplant patients who have experienced graft loss and have developed DSA detected through Single Antigen studies performed at least 3 months after complete cessation of immunosuppression. Based on high-resolution typing, mismatched donor eplets were identified using the MatchMaker module of the HLA-Fusion software. The relative immunogenicity of the eplets (I-score) was determined by analyzing the frequencies of antibody response to each of them. All eplets, collected in the HLA Epletregistry database for HLA-A and HLA-B were analyzed; they are categorized into antibody-verified and non-verified. Statistical analysis was performed using SPSS v26 software, and a p-value < 0.05 was considered statistically significant.
Results: Eighty-five verified eplets were observed at least once. Of these, 64 (75%) were observed more than 15 times. The mean I-score was 0.188 [0.026-0.826] Antibody reactivity was not observed against 12 out of the 64 (18.7%).
Regarding non-verified eplets, 106 out of 161 (65.8%)were observed more than 15 times, and 59 of these (55.6%) were found not reactive. The mean I-score was 0.101 [0.032-0.250].Some of the antibody reactivities were explained only by non-verified eplets.
The eplets located in the alpha-2 domain of the molecule showed higher I-score than those located in the alpha-1 domain (0.182 vs 0.108; p=0.05)
Conclusion: The degree of relative immunogenicity of eplets is highly variable. There are eplets with a very low or probably null ability to generate antibodies, so they could be considered as "permissible" incompatibilities. Eplets now categorized as non-verified or theoretical may have clinical relevance.

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1467 – P3.08.17

Donor organ age correlates with NLRP1 inflammasome activation in immune cells in kidney transplantation

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Purpose: Inflammation causes a wide range of health disorders. In this process, the formation of inflammasome complexes plays a key role. While inflammasomes have been extensively studied during kidney disease, their role in kidney transplantation has not been fully elucidated. We evaluate the gene and protein expression of several components of the inflammasome pathway before and at several time points after kidney transplantation in a cohort of patients of different ages and receiving an organ from older or younger donors.
Methods: PBMC were extracted from blood samples were collected at various time points: prior to transplantation, as well as at 24 hours, 72 hours, 7 days, 3 months, and 6 months post-transplantation. Expression of NLRP1, NLRP3, ASC and caspase 1 was analysed by quantitative PCR. Mann-Whitney test was used to compare data between 2 groups. All results are expressed as mean ± SD of 3 independent experiments and a p-value<0.05 was considered as statistically significant.
Results: Our results showed that donor age correlated with inflammasome expression levels. Receiving an older kidney increased NLRP1 expression over time, while receiving a younger kidney had the opposite effect. We also checked NLRP3 expression which followed a similar pattern but decreased faster in all groups. Meanwhile, ASC and caspase-1 both increased in the first few days after surgery, but their levels stabilized at later times. For all studied genes, initial expression levels were lower in young patients than in older ones. This difference in starting expression could be due to previous renal failure and/or the inflammaging process. Regardless of that, the presented results suggested the potential activation of the NLRP1 inflammasome in all patients.
Conclusion: Our findings indicate that NLRP1 inflammasome activation is the primary effector in renal transplant, and its level increases gradually in patients who receive an older organ, whereas it has the opposite effect on older patients who receive a younger organ. Despite treatment with immunosuppressants, inflammation persists in some patients. These results suggest that the donor's age is a critical factor in post-transplant inflammasome activation and that specific inflammasome inhibitors should be considered to increase the success of kidney transplantation.

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1469 – P3.08.18

Donor Age Have Effects on Senescence Biomarkers in Kidney-Transplanted Patients

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Purpose: Young patients who undergo renal transplantation often face a scarcity of kidneys from donors of similar age, resulting in the transplantation of older organs. Young patients who receive older kidneys have a higher chance of graft rejection and various problems compared to older individuals who receive kidneys from donors who are similar in age or younger. This study focuses on studying different senescence biomarkers in donors and patients who received kidneys from various age ranges. Our hypothesis is based on a well-known phenomenon known as the transfer of the senescence phenotype.
Methods: The senescence biomarkers examined include gene expression of P16 and P21, the presence of γH2AX histone. We selected individuals from different age groups and divided them into three categories: older donor than the patient, younger donor than the patient, and similar age.
Results: First, we conducted an immunofluorescence assay on pre-transplant donor kidney biopsies, focusing on the γH2AX histone. Biopsies from donors spanning various age groups were chosen, including individuals aged 23, 43, 57, and 80 years. Our findings demonstrated a gradual elevation in γH2AX expression corresponding to advancing age.
Then, we studied the expression levels of two main genes and proteins associated with senescence and aging in the patients: p16Ink4a and p21. Our findings revealed a correlation between donor age and p16Ink4a expression levels. Young patients who received an older kidney showed an increase in p16 expression over time, while those who received a younger kidney experienced the opposite effect. However, in the final time point, the old donor group exhibited a reduction in p16 gene and protein expression. Moreover, p21 gene expression remained high across all groups and time points, although protein expression increased over time in the Old Donor and Matched groups.
Conclusion: Receiving older organs from young patients induces a pro-senescence state that may cause difficulties later. When older patients receive younger organs, their initial senescent phenotype gradually improves. These results may open new avenues for therapeutic targets, such as senolytics, to reduce the presence of senescent cells and mitigate the complications associated with the transplantation of older organs in young patients.

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1483 – P3.08.19

Identification and characterization of ten novel HLA alleles. Evaluation of their role in optimal donor selection in transplantation

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HLA mismatch is a critical negative factor in Hematopoietic Stem Cell Transplantation (HSCT) and therefore, finding a suitable donor with an acceptable HLA match is essential for successful transplant outcomes. Although advances in HLA typing have improved transplantation success rates, the search for the ideal matched donor remains a major challenge in daily clinical practice.
Purpose: The objective of this study is to present ten novel HLA alleles and evaluate their significance in the search and selection of the most suitable donor for transplantation.
Methods: In the period 2021-2023 2,340 samples consisting of patients undergoing HSCT and their respective unrelated donors were analyzed. Genotyping at 11 (HLA-A,-B,-C,-DRB1,-DRB3/4/5,-DQA1,-DQB1,-DPA1,-DPB1) or 6 (HLA--A,-B,-C,-DRB1,-DQB1,-DPB1) loci was performed using commercial locus-specific primers supplied by CareDX (AlloSeq Tx17 kit) and GenDx (NGSgo®-MX6-1 kit), respectively. Sequencing was carried out on the MiSeq platform. The raw sequencing data were analyzed by AlloSeq Assign analysis software Tx17.1 v1.0.5 (CareDX) and NGSengine analysis software v.2.31.0 (GenDx) with references from the IPD-IMGT/HLA Database v3.53.0.
Results: During this study, novel HLA allele sequences are deposited into the GenBank database and new HLA allele assignments were obtained from WHO Nomenclature Committee for Factors of the HLA system. Particularly, one 5' UTR variant (HLA-A*02:01:01:243_GenBank accession number OQ357854), four intronic variants (HLA-B*51:01:01:109_OQ357857, HLA-A*02:05:01:23_OP889296, HLA-B*18:01:01:73_OP889297, HLA-A*02:09:01:04_OP795782), one synonymous mutation (HLA-DPB1*02:01:68_OP019279) that had silent base substitution with no change in amino acid sequence when compared with the most similar allele, and four non-synonymous mutations (HLA-C*07:1052_OP889298, HLA-A*24:587_OP654751, HLA-B*51:380_OQ357852, HLA-A*01:426_OP712622) that were single amino acid substitution variants when compared with the most similar allele, were discovered. Variations in amino acids have the potential to influence peptide folding and protein interactions, thereby potentially altering its immunogenic properties.
Conclusion: This study emphasizes the importance of NGS-based HLA typing, where higher resolution HLA typing may elucidate the clinical significance of variation in coding and non-coding regions. By defining novel HLA alleles associated with different ethnicities, our work provides further insight into HLA polymorphism. Moreover, by exploring their potential involvement in allo-recognition and their impact on transplant success, our findings aid in the precise selection of donor-recipient pairs, further optimizing transplant outcomes.

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1498 – P3.08.20

Improving lymphocyte purity in flow cytometric crossmatch assays: a comparison of cell isolation methods

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Purpose: The flow cytometric crossmatch (FCXM) assay is used to determine whether donor specific HLA antibodies (HLA-DSA) are present in recipient sera. Isolated cell purity is a widely recognized critical factor in the test outcome. For that reason, our goal was to compare the performance of two cell isolation methods in FCXM assays carried out with peripheral blood from living donors for kidney transplant.

Methods: Hematological cell fractions from living donor whole blood samples were evaluated before and after being processed by erythrocyte aggregation and total lymphocyte enrichment through negative magnetic selection (EA&TLE). Results from FCXM assays carried out with EA&TLE and a conventional cell isolation technique by gradient density centrifugation (Ficoll) were compared and correlated with HLA-DSA detection using the Luminex single-antigen bead assay (LSA).
Results: The mean lymphocyte percentage of samples processed using EA&TLE was 94% (95% confidence interval [95% CI] 89% to 97%), with 24 to 50% of the lymphocytes present before isolation retained and a lymphocyte count after enrichment of 3 to 10 million cells/mL. Analysis of 65 FCXM assays performed with EA&TLE and 66 with Ficoll demonstrated that EA&TLE results in higher sensibility (67% EA&TLE vs 43% Ficoll) and higher kappa correlation coefficient (0.61, 95% IC 0.318 to 0.90 EA&TLE vs 0.40, 95% IC 0.01 to 0.82 Ficoll) with HLA-DSA detection by LSA. Furthermore, FCXM assays performed with EA&TLE showed a lower detection threshold for class II HLA-DSA in comparison to FCXM assays performed with Ficoll (lowest mean fluorescence intensity of 5193 for EA&TLE vs 10289 for Ficoll).
Conclusion: Implementation of EA&TLE for cell isolation from whole blood increased lymphocyte purity in the samples, improved FCXM assay sensitivity and resulted in a greater correlation with HLA-DSA identification by LSA. Thus, EA&TLE can increase cell isolation efficiency and cost-effectiveness in FCXM assays.

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1554 – P3.08.21

A novel calculated panel reactive antibody score calculator incorporating HLA-DQB1/DQA1 and DPB1/DPA1 associations: enhancing allocation for organ transplantation in the spanish population

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Purpose: Calculated panel reactive antibody (cPRA) score represents the possibility of encountering an incompatible donor for organ transplant candidates. This study aims to evaluate a novel calculator designed to improve compatibility assessment in the Spanish population and by considering HLA-DQB1/DQA1 or DPB1/DPA1 associations not accounted for in existing calculators.
Methods: A cohort of 1000 deceased donors from Spain was analysed, focusing on HLA-A, B, C, DRB1, DQB1, DQA1, DPB1, DPA1, and DRB345 loci typing. The HLA genotype was compared with Single Antigen Bead (SAB) assay results from 398 patients within the kidney transplant waiting list. A custom computer script written in R language was created for identifying any unacceptable antigen matches between patients and donors. The cPRA represented the percentage of donors with incompatible HLA typing relative to patient serum profiles. Notably, the calculator incorporated distinctions between DQB1/DQA1 and DPB1/DPA1 associations to enhance estimation precision compared to existing calculators.
Results: Our calculator exhibited a high Lin's concordance correlation coefficient (rho = 0.92, 95% CI 0.91-0.93) when compared to the Eurotransplant Reference Laboratory (ERL) calculator. A limit of agreement was established to identify samples requiring further investigation, with 24 samples falling below this threshold. These samples exhibited positive reactions to certain DQ or DP beads but lacked specificity for individual DQB1, DQA1, DPB1, or DPA1 alleles. Mean difference between these samples was –55.39 ($p < 0.001$), so our calculator increased the apparent compatibility in these patients with positivity to single DQB1/DQA1 and DPB1/DPA1 associations.
Conclusions: The novel calculator demonstrates optimised performance in predicting donor-recipient compatibility within the Spanish population, offering additionally improved precision through the consideration of single HLA-DQ or DP associations. Further validation and refinement may enhance its utility in clinical practice, ultimately benefiting organ allocation outcomes.

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1834 – P3.08.22

Mononuclear phagocyte system readily responds to allogeneic platelets in a murine model of platelet transfusion refractoriness

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Purpose: Platelet transfusion is a lifesaving procedure to prevent or to stop actual hemorrhage. However, transfusing patients who are immune to HLA-I molecules can result in transfusion failure, known as platelet transfusion refractoriness (PTR). Through mechanisms that are not yet fully understood, alloantibodies cause the rapid clearance of transfused platelets from the bloodstream. Moreover, the variability in transfusion efficacy in immunized patients could be explained by a different immune signature, which may or may not favor PTR.
We aim to analyze the recipient's immune response and the fate of transfused platelets in a mouse model of MHC-I-alloimmune PTR.
Methods: PTR was mimicked by transfusing allogeneic eGFP$^+$-H2^b-platelets into a recipient H2^d-mouse previously immunized against H2^b-platelets. Blood, spleen and liver were sampled 30 min after platelet transfusion. Monocytes, neutrophils, splenic and liver immune cells were analyzed by flow cytometry (FC).The relative importance of spleen and liver was studied *in vivo* by evaluating PTR either in splenectomized/sham mice or in mice treated with asialofetuin/fetuin to block asialoglycoprotein receptors (n=3).
Results: Transfused platelets were eliminated from circulation within the first 30 min only in alloimmune mice, revealing a PTR. At this time point, FC showed a significant decrease ($p<0.05$) of circulating Ly6C^{high} monocytes and an increase in the proportion of CD11b$^+$ cells in the liver of refractory mice, suggesting a recruitment of circulating cells during refractoriness. Analysis of organs revealed that (61.7±7.7)% of hepatic- and (18.7±3.0)% of splenic-F4/80$^+$CD11b$^+$ cells are positive for allogeneic platelets, indicating the involvement of macrophages from both organs in platelet clearance during PTR. However, splenectomized alloimmune mice remained in a refractory state similar to the sham control mice (0.8±0.4)% vs (1.0±1.0)% of total platelets, respectively, indicating that the spleen is dispensable for PTR. Furthermore, treatment with asialofetuin before transfusion did not impact platelet clearance ((0.8±0.4)% vs (0.7±0.4)%) suggesting that the mechanism of platelet internalization is not influenced by desialylation.
Conclusion: Allogeneic platelets are eliminated by hepatic and splenic phagocytic cells during refractoriness. In combination with the compelling shift in Ly6C^{high} circulating monocytes and CD11b+ cells in the liver, these changes point to an overall immunomodulation taking place during PTR.

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1868 – P3.08.23

Impact of end-stage lung diseases on the immune cell composition in lung parenchyma

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Purpose: Lung transplantation is the ultimate option for patients suffering from severe end stage lung diseases such as emphysema, pulmonary arterial hypertension or fibrosis. Yet, little is known about the immune cell composition in these diseased lungs that may be critically shaped by the underlying lung disease. Hence, we investigated the immune cell distribution of lung parenchyma obtained from different diseased lungs after explantation in the course of lung transplantation (LTx).
Methods: Explanted lung parenchyma (n=57) of patients that underwent LTx was enzymatically digested and immune cell distribution was determined by flow cytometry. Lung tissue derived from patients with chronic obstructive pulmonary disease (COPD), idiopathic pulmonary fibrosis (IPF) and PAH was compared to immune cell composition of healthy control parenchyma derived from non-tumor lung tissue.
Results: Leukocyte proportions of in lung parenchyma were generally enriched in diseased lungs (with >50% in COPD), indicating their role in these diseases. Lymphocytes constituted the main leukocyte subset in healthy and COPD lungs, while granulocytes represented the majority in IPF and PAH lungs. In all diseases, T cells, primarily tissue-resident memory (TRM), constituted the major lymphocyte population with particularly elevated levels in COPD and lowest levels in PAH and IPF tissue. COPD lungs showed highest proportions of $CD4^+$ T cells translating into highest $CD4^+/CD8^+$ ratio. Regulatory T cells were enriched in IPF lung parenchyma compared to other diseases and lowest in COPD lungs. Subtle differences were found in naïve vs. memory phenotypes of T cells.
Conclusion: Our data conclusively prove that end-stage lung diseases imprint the immune repertoire – and probably vice versa – that populated the lung parenchyma, demonstrating that each lung disorder is characterized by a unique immune cell component. A better understanding of the immune mechanisms involved in these lung disorders will be essential for an adequate management of these diseases in the future.

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1932 – P3.08.24

Selective Deletion of HLA Class I Molecules for Improved Skin Graft Compatibility

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Treatment for organ failure typically involves transplantation. However, compatibility issues between donors and recipients often arise due to the vast diversity of human leukocyte antigens (HLA) class I molecules, leading to strong immune reactions and organ rejection. To address this, we propose generating skin grafts from cells with selective deletion of HLA class I molecules, except for the prevalent HLA-A allele. We deleted HLA-B and -C genes from cells with a common HLA-A allele using CRISPR technology and also established a humanized murine model to evaluate the feasibility of this approach in vivo. In this study, we developed a humanized skin graft model using mice transgenic for HLA class I, enabling us to closely examine the intricate HLA-mediated immune response in skin transplantation. Mice expressing HLA-A*02:01 allele alone or in combination with HLA-B*07:02 do not reject the skin of mice expressing only HLA-A*02:01 molecule. Additionally, we developed a humanized skin rejection model by transplanting skin fragments from HLA-A02:01/B07:02 mice onto HLA-A*02:01 mice, revealing rejection of incompatible skin and demonstrating HLA-restricted specificity in rejection by specific CD8+ T cells. However, skin from double HLA-A*02:01/B*07:02 transgenic mice transplanted into HLA-A*02:01 mice is rejected and triggers a strong specific CD8+ T cell response against HLA-B*07:02 allele. This mouse model was used to analyze a human lymphoblastoid cell line in which HLA-B and -C genes were knocked out by a one-step CRISPR-Cas9 strategy while retaining expression of the most common HLA class I allele, HLA-A*02:01. Human cell clones lacking HLA-B and -C genes showed hypoimmunogenicity in a skin allograft model, suggesting potential for universal HLA-compatible grafts. In contrast to the parental line, the edited cells did not elicit specific CD8+ T cell response in HLA-A*02:01 transgenic mice transplanted with HLA-A*02:01/B*07:02 skin. This findings demonstrate a promising approach to overcoming the challenges of organ transplantation and improving patient outcomes. We propose a rapid, simple, and scalable HLA-compatible skin graft model that will improve clinical practice in burn units worldwide.

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1957 – P3.08.25

Longitudinal dynamics of SARS-COV-2 specific humoral and cellular immune responses in patients on waiting list and after lung transplantation

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Purpose: Patients with end-stage lung diseases on the MHH waiting list for lung transplantation (WL-LTx) have been vaccinated against the SARS-CoV-2 spike protein with usually three doses of the mRNA vaccine prior to transplantation without the influence of immunosuppression. Therefore, we hypothesized the induction of high spike-specific IgG levels, T cell responses, and protection against SARS-CoV-2 infection and severe COVID-19.
Methods: Peripheral blood samples of pre- (n=100) and matched post-LTx (n=66) of WL-LTx patients were analyzed. Luminex-based multiplex assays were used to quantify SARS-CoV-2-specific IgG antibodies against RBD, S1, S2 domains of spike and N antigens in plasma. Electrochemiluminescence multiplex assays were utilized for surrogate neutralization by antibodies in plasma. Peripheral blood mononuclear cells (PBMC) were used to analyze changes in immune cell components.
Results: 97% of pre- and post-LTx patients mounted spike-specific IgG, but only few patients had neutralizing capacity against wild-type, and even less against omicron variants. Using paired samples pre vs. post-LTx, we observed three different patterns for spike-IgG, with either decreased, stable, or increased levels. Relevant factors for increased IgG levels post-LTx were the absence of infection before Tx, infection or vaccination after Tx and interval between vaccination and Tx of ≤5 months. Immune cell composition at pre- and post-LTx are not likely to have a substantial and direct influence on SARS-CoV2 antibody levels after vaccination.
Conclusion: We demonstrated that LTx patients in the early post-Tx period did benefit from booster SARS-CoV-2 vaccinations, showing even increased IgG levels post- compared to pre-LTx. Based on the poor neutralizing capacity, we propose omicron-adapted vaccination for LTx recipients.

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1972 – P3.08.26

Donor T and NK cells with a special tissue-resident memory phenotype migrate into the periphery of lung transplant recipients – a potential feature for tolerance development

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Purpose: Subsequent to lung transplantation (LuTx), the migration of lymphocytes from the transplanted lung into the periphery induces a transient chimerism of donor passenger cells in recipient blood. We characterized the phenotype of donor T/NK cells to investigate whether they might represent tissue-resident memory (TRM) cells.
Methods: Lymphocyte dynamics in recipient blood were determined in n=97 LuTx patients directly (T0), 24 hours (T24) and 3 (wks) weeks after LuTx using flow cytometry. Donor cells were analyzed by HLA class I allele-specific mAb in n=44 LuTx recipients. The same markers were used to determine the phenotype of lymphocytes present in organ storage solution (perfusate, n=111), recipient explanted lung parenchyma (n=28) and donor trachea (n=17). Single cell mRNA sequencing of explant lung parenchyma was conducted (n=16).
Results: In peripheral blood of all recipients, donor-derived T/NK cells were detected at T0, T24 and 3 wks after LuTx and had higher CD69 expression compared to recipient cells, and were mostly $CCR7^-$ memory cells. This phenotype was similar to T/NK cells in corresponding perfusates. In recipient parenchyma and donor trachea, most $CD69^+$ T/NK cells showed coexpression of other tissue residency markers (i.e. CD103, CD49a, PD-1). These markers were not found in circulating donor lymphocytes and perfusates, indicating they represent distinct memory T and NK subsets. Sc-mRNA sequencing confirmed distinct TRM T cell subsets in lung parenchyma. Patients with high frequencies of donor T cells showed a trend towards chronic lung allograft dysfunction- (CLAD-)free survival 2 years post LuTx.
Conclusion: Our results demonstrate that donor T/NK cells found in the periphery of lung transplant recipients are a distinct subset from circulating lymphocytes and TRM cells present in lung tissue, since they express CD69 but lack expression of other classical TRM markers. Donor T cells might be clinically relevant for tolerance induction and long-term survival after transplantation due to their unique features.

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2005 – P3.08.27

Analysis of the relative immunogenicity of mismatched HLA class I eplets in renal transplantation

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Purpose: It is now universally accepted that HLA matching affects transplant outcome and that HLA antibodies are primary causes of transplant rejection. HLA antibodies recognize epitopes, which can be structurally defined by eplets. Understanding the immunogenic potential of HLA epitopes/eplets is increasingly relevant in the field of donor-recipient compatibility as it allows for the identification of acceptable mismatches for sensitized patients and the development of permissible mismatch strategies for non-sensitized patients. The fundamental objective of this study is to analyze differences in the immunogenicity of the HLA eplets described to date in the context of renal transplantation.
Methods: The study population consists of 118 consecutive renal transplant patients who have experienced graft loss and have developed DSA detected through Single Antigen studies performed at least 3 months after complete cessation of immunosuppression. Based on high-resolution typing, mismatched donor eplets were identified using the MatchMaker module of the HLA-Fusion software. The relative immunogenicity of the eplets (I-score) was determined by analyzing the frequencies of antibody response to each of them. All eplets, collected in the HLA Epletregistry database for HLA-A and HLA-B were analyzed; they are categorized into antibody-verified and non-verified. Statistical analysis was performed using SPSS v26 software, and a p-value < 0.05 was considered statistically significant.
Results: Eighty-five verified eplets were observed at least once. Of these, 64 (75%) were observed more than 15 times. The mean I-score was 0.188 [0.026-0.826] Antibody reactivity was not observed against 12 out of the 64 (18.7%).
Regarding non-verified eplets, 106 out of 161 (65.8%)were observed more than 15 times, and 59 of these (55.6%) were found not reactive. The mean I-score was 0.101 [0.032-0.250].Some of the antibody reactivities were explained only by non-verified eplets. The eplets located in the alpha-2 domain of the molecule showed higher I-score than those located in the alpha-1 domain (0.182 vs 0.108; p=0.05)
Conclusion: The degree of relative immunogenicity of eplets is highly variable. There are eplets with a very low or probably null ability to generate antibodies, so they could be considered as "permissible" incompatibilities. Eplets now categorized as non-verified or theoretical may have clinical relevance.

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2073 – P3.08.28

Booster dose of Tozimameran, in renal transplant recipients, is followed by T-cell activation, but not senescence or exhaustion

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Background and Aims: Potential detrimental effect of multiple vaccinations on the immune system aging process has not been proved. The aim of our research was to examine whether response to anti-SARS-CoV-2 vaccination with Tozinameran is associated with immunoscenecence and immunoexhaustion in Kidney Ttransplant recipients (KTRs).
Method: In this prospective, observational study, of 39 adult KTRs on stable immunosuppression, naïve to SARS-CoV-2, CD4+ and CD8+ subpopulations [comprising CD45RA+CCR7+ (naïve), CD45RA-CCR7+ (central memory -CM-), CD45RA-CCR7- (effector memory -EM-) and CD45RA+CCR7- (effector memory re-expressing CD45RA -EMRA-, senescent) CD4+/CD8+, CD28- expressing (CD8+) and CD28-null (CD8-, senescent) CD4+/CD8+ and CD3+PD1+ (exhausted) T-cells] were evaluated at time points: T1 (48 hours prior to the 3rd Tozinameran dose) and T2 (3 weeks after the 3rd Tozinameran dose). Results: CD4-CM and CD8-EM were increased, while naïve CD4+ and CD8+ proportions were reduced in the whole cohort of patients, but particularly in responders at T1 and T2.
At T2, responders compared to non-responders had higher concentrations of CD4+CD28+ and CD8+CD28+ [756.34(368) vs. 446.57(925) and 227.15(166) vs. 131.44(121) cells/μL, p: 0.026 and 0.036 respectively] and reduced proportions of CD4+CD28- [6.1(5.5)% vs. 20.7(25)%, p: 0.04]. There was a negative association between age and naïve CD4+ and CD8+ and CD8+CD28+ concentrations at T1 (p: 0.042, 0.042 and 0.006, r: -0.346, -0.345 and -0.444 respectively) and a positive correlation between exhausted T-cell (CD3+PD1+) numbers prior to the first vaccination and TEM / TEMRA CD4+ concentrations at T1 (p: 0.001 and 0.001, r: 0.558 and 0.578 respectively), as well as TEMRA CD8+ counts at T2 (p: 0.037, p: 0.396).
Conclusion: In KTRs, response to vaccination is not associated with an expansion of senescent and exhausted T-cell concentrations, but rather with a switch from naïve to differentiated-activated T-cell forms. Age, T-cell exhaustion status at baseline and eGFR also to be correlated to the differentiation senescence process.

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2094 – P3.08.29

Calcineurin blockade modifies dendritic cell differentiation and induces a tolerogenic phenotype

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The Nuclear Factor of Activated T Cells (NFATs) is a family of transcription factors activated by the phosphatase Calcineurin (CN). This pathway holds significant clinical relevance, as drugs inhibiting the CN-NFAT axis exert a potent immunosuppressive effect and are utilized to manage organ transplant rejection reactions. Our research group revealed that NFATs are activated in dendritic cells (DCs), leading to terminal differentiation and apoptosis. Given the immunosuppressive role played by certain types of myeloid cells in pathological contexts, we aim to determine the involvement of the CN-NFAT pathway in early DC differentiation and its potential contribution to the development of an immunosuppressive phenotype in myeloid cells.
To explore this, we transduced a growth factor-dependent splenic dendritic cell (DC) line with a CN inhibitor peptide, generating DC-iCN. To determine the effects of NFATs inhibition in DC-iCN, we conducted proteomic analyses, as well as functional and metabolic assays. The potential immunosuppressive activity of the DC-iCN was determined by Mixed Leukocyte Reaction assay. We analyzed the potential effects of inhibiting NFAT *in vivo* through the intravenous administration of nanoparticles carrying the iCN peptide to investigate whether this would modify the hematopoietic process leading to the formation of DCs.
Blocking the CN-NFAT pathway in DCs increased their growth rate and modified the cell cycle, prolonging the G2/M phase and shortening the G1 phase. Metabolically, NFAT inhibition induced a marked Warburg effect, characteristic of rapidly proliferating cells. Moreover, DC-iCN demonstrated a highly immunosuppressive phenotype, differentiating naïve T cells into FOXP3+ regulatory T cells in MLR assays. Interestingly, inhibiting the CN-NFAT pathway *in vivo* expanded the granulocyte-monocyte progenitor involved in DC ontogeny.
In summary, blocking the CN-NFAT pathway modifies early DC differentiation, resulting in the acquisition of a highly tolerogenic phenotype and traits resembling undifferentiated cells. This sheds light on the potential use of tolerogenic hematopoietic precursors as a cellular therapy for autoimmune diseases and transplant rejection. Further human studies will enhance these findings.

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2182 – P3.08.30

Immune signature profiling in patients with graft-versus-host disease following hematopoietic stem cell transplantation

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Purpose: Allogenic hematopoietic stem cell transplantation (allo-HSCT) is the best treatment option for many patients suffering from an hematological malignancy. However, about 20-70% of allo-HSCT recipients develop a life-threatening complication in the form of chronic graft-versus-host disease (GvHD). GvHD occurs when the donor immune cells contained in the graft identify the host tissues as foreign and trigger an immune response against them. In the current project, we aimed at identifying cell subsets predicting the occurrence of future GvHD onset using a systems immunology approach of high-parameter flow cytometry and machine learning.
Methods: PBMCs prospectively collected 6 months after allo-HSCT in 63 patients, of whom 32 patients developed GvHD in follow-up and 31 patients did not. The adaptive and innate immune system were characterized in detail using high parameter flow cytometry.
Results: Preliminary analyses revealed a higher proportion of naïve CD4+ T cells, CD28+ CD127+ RORgT+ Naïve CD8+ T cells, and classical monocytes in patients who went on to develop GvHD compared to HSCT recipients who remained GvHD-free. In contrast, patients who did not develop GvHD had higher proportions of effector memory CD8+ T cells and higher proportions of non-classical monocytes.
Conclusion: Contrary to what is most often reported in literature, these preliminary results suggest that a higher proportion of naïve T cells are associated with GvHD while higher proportions of effector CD8+ T cells are associated with a GvHD-free state. One potential cause could be attributed to a more extensive immune reconstitution needed if a larger proportion of T cells is naïve, creating more GvHD potential. These first analyses could point towards a specific immune signature that can help to identify patients who might develop GvHD within the first year of follow-up after receiving HSCT. This immune signature could be used as a predictive biomarker in the clinic to identify patients at a high risk of developing GvHD and adjust drug doses preventatively.

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2214 – P3.08.31

Non-killer cell immunoglobulin-like receptor profile following allogeneic stem cell transplantation

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Purpose: Natural killer (NK) cells are the first lymphocyte subgroup that appear in peripheral blood following allogeneic stem cell transplantation. They have roles in both graft-versus-leukemia effect and prevention of graft-versus-host disease; however, the mechanisms and receptor profiles are not well known. The purpose of this study is to examine the change in non-Killer Cell Immunoglobulin-like Receptor (KIR) NK cell receptor profile following allogeneic stem cell transplantation.
Methods: In this prospective cohort study; peripheral blood samples from patients who were diagnosed with haematological malignancy and underwent allogeneic stem cell transplantation were collected before (Day 0) and after (Day 30, 100, 180) transplantation. Peripheral blood mononuclear cells were isolated and NK cells were identified by flow cytometry. Non-KIR NK cell receptor profiles (NKG2A, NKG2C, NKG3D, NKp30, NKp44, NKp46, ILT-2, ILT-4) were examined by further flow cytometric analyses.
Results: 15 patients (7 acute myeloid leukemia, 5 acute lymphoblastic leukemia, 3 myelodysplastic syndrome) were included in the study. All patients were in remission before transplantation. NKG2A levels decreased over time, statistically significant changes were between days 30 and 100 (p=0,01) and days 30 and 180 (p=0,01). NKG2C levels increased over time, statistically significant change was observed between days 30 and 180 (p=0,040). NKG2D levels decreased over time, statistically significant change was observed between days 100 and 180 (p=0,032). NKp30 levels increased over time, significant change was observed between days 30 and 100 (p=0,042). NKp44 levels decreased over time, changes between days 0 and 100 (p=0,048) and days 0 and 180 (p=0,002) were statistically significant. NKp46 levels and ILT-2 levels decreased over time, however these changes were not statistically significant. ILT-4 levels decreased over time, statistically significant change was observed between days 0 and 180 (p=0,004).
Conclusion: Non-KIR NK cell receptor profile changes after allogeneic stem cell transplantation. According to our study; NKG2C and NKp30 levels increase, while other receptor levels decrease. Further studies are needed to support our findings and determine whether there is an association between the functions of non-KIR receptors and graft-versus-leukemia effect or graft-versus-host disease.

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P3.09 TUMOR MICROENVIRONMENT

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8 – P3.09.01

B cell anti-tumor immune responses in renal cell carcinoma patients

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Renal cell carcinoma (RCC) is one of the most prevalent cancer types worldwide, in which clear cell RCC (ccRCC) is the most common subtype that accounts for over 75% of all RCC. The tumor microenvironment of ccRCC is highly infiltrated with multiple subtypes of immune cells, including a minority fraction of B lymphocytes. Notably, high infiltration of B cells is associated with poor prognosis as tumor-educated B cells play a role in promoting renal cancer metastasis. However, the existence of tertiary lymphoid structures (TLSs), enriched with B cells, has shown a positive association with prognosis in various cancer types. Moreover, tumor-infiltrating B cells exhibit the potential to produce tumor-binding antibodies, opening avenues for therapeutic interventions. In this study we aim to detect and analyze the origin, functionality and target specificity of novel tumor-reactive antibodies derived from ccRCC patients. We used single cell RNA sequencing to characterize the specific subsets of B lymphocytes and examine the B cell immunoglobulin repertoire in ccRCC tumors. Based on these transcriptomics, we cloned and generated monoclonal antibodies originating from highly mutated and clonally expanded cells. We found that class-switched plasma cells residing within the tumors can produce antibodies with tumor-binding capabilities. These findings of tumor derived monoclonal antibodies could have the potential to reveal new targets for the therapeutics and treatment of RCC patients.

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108 – P3.09.02

Oral squamous carcinoma cells differentiate monocytes into immunosuppressive CD25+CD163+CD206+ macrophages

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Introduction: Oral squamous carcinoma (OSC) is one of the top 10 cancers in prevalence and mortality. Tumor-associated macrophages (TAMs) plays an important role regulating OSC progression. Most TAMs derive from circulating monocytes that differentiate *in situ* mostly into M2-like macrophages possessing pro-tumoral functions, enabling immunosuppression, vasculogenesis, tumor progression and metastasis. Understanding how OSC control macrophage differentiation can lead to developing effective anti-tumoral interventions.
Objectives: Study the effect of OSC cells (OSCCs) on macrophage differentiation.
Materials and Methods: We have cultured primary monocytes from healthy donors' peripheral blood for 5 days in the presence of conditioned media derived from two days culture of H413 and TR146 OSCC lines. The phenotype of these monocyte-derived macrophages (moMΦ) was analyzed by flow cytometry. The transcriptomic profile of these conditioned moMΦ was also analyzed by RNAseq. We have studied the stimulation of allogeneic T cells by moMΦ conditioned by OSCC lines.
Results: OSCCs imprint an immunosuppressive phenotype on moMΦ related to M2 macrophages, judged by the lower expression of HLA-DR, CD86, CD11c and increase of CD163 and CD206. In addition, moMΦ differentiated by H143 CM were unable to activate allogeneic T cells, and inhibited T cell activation and proliferation upon CD3/CD28 stimulation.
A signature expression profile involving cytokine and cytokine receptors in the conditioned moMΦ was identified by RNAseq, which surprisingly included *IL2RA* (CD25). We confirmed CD25 expression by flow cytometry in around 20% of $CD163^{+}CD206^{+}$ moMΦ differentiated using H413 CM. We consulted the Single-cell Portal database to identify that approximately the 25% of the TAMs from different tumors express IL2RA. CD25 binds to IL-2, and it is highly express by regulatory T cells, contributing to their immune suppressive functions by sequestering IL-2 required by effector T cells. However, the expression of this marker has been poorly studied before in TAMs.
Conclusion: Our data indicate that OSCCs promote the differentiation of immunosuppressive monocytes into an M2-like moMΦ, which may facilitate tumor progression. Investigating the role of $CD25^{+}$ TAMs *in vivo*, may offer the chance to explore new therapeutic approaches.

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133 – P3.09.03

Novel human monoclonal antibodies specific to the A33 glycoprotein recognizes prostate cancer

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Purpose: Herein, we aim to evaluate GPA33 protein expression, using two new anti-GPA33 monoclonal antibodies (G72 and G36) produced by the hybridoma technique in normal, benign and cancerous prostate tissue.
Methods: The study was carried out in 4 normal (NP), 11 benign prostatic hyperplastic (BPH) and 18 cancerous human prostates (PC). Immunohistochemical analysis was performed to study the expression of GPA33 using two new anti-GPA33 monoclonal antibodies (G72 and G36). Serum levels of PSA were assayed by an immulite autoanalyzer. Fisher, One way ANOVA and Spearman tests were used for statistical analysis.
Results: Our results show GPA33 protein expression either by the anti-GPA33 monoclonal antibody G72 in the totality of prostate samples analyzed, and by the G36 antibody in the majority of prostate samples analyzed. Taking immunostaining intensity into account, no differences could be shown between assessment of GPA33 expression by monoclonal antibody G72 or by antibody G36 in the three prostate groups. The highest intensity of GPA33 expression detected by G72 or G36 was found in cancer patients. Indeed, the GPA33 immunoreaction detected by both G72 and G36 clones increased gradually from normal to benign to cancerous tissues. This similarity in GPA33 immunoreaction intensity between clones G72 and G36 does not reflect a similarity in intracellular localization. Indeed, a difference in GPA33 localization was revealed if the protein was detected by G72 rather than by G36. Like PSA, GPA33 protein expression increases gradually with Gleason score, whatever the anti-GPA33 antibody used. Interestingly, in cancerous prostate tissue, GPA33 is preferentially localized in the stromal component.
Conclusion: Together, GPA33 expression was demonstrated for the first time in normal, benign and cancerous prostate tissue. Thus, the two monoclonal antibodies, newly produced by the hybridoma technique and previously validated by immunofluorescence and flow cytometry techniques, are applicable for GPA33 detection by immunohistochemistry in prostate tissue. Our work argues in favor of a pro-tumoral role for GPA33 in the prostate tumor process. As a follow-up to this work, it would be important to determine the nature of the immune cells infiltrating the prostate tumor and expressing the GPA33 protein.

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142 – P3.09.04

Preferential deployment of DNA repair pathways: The Tumor associated macrophages acting as a coordinator in response to cisplatin treatment in ovarian cancer.

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Introduction- Deeper understanding and analysis of the tumor immune microenvironment ultimately leads to identification of biomarkers and possible cellular and molecular reasons for therapy resistance. Since Tumor Associated Macrophages (TAMs) exhibit dual role in cancer progression, chemoresistance and metastasis. More in-depth investigation is required to reveal the cellular crosstalk with cancer cells and its significance in therapeutic efficacy.
Materials and methods –Western blot, real-time PCR, Cytokine array, flow cytometry, immunocytochemistry, *in-vivo* experiments. All the statistical analysis were performed with GraphPad Prism 8 software.
Results and discussions- *in-vitro* experiments were performed with human ovarian cancer cell lines where cocultured cancer cells with tumor macrophages (THP1 cell line derived) showed elevated expression of Y family DNA polymerases like Polymerase eta (Pol H), REV1 and other TLS (Translesion DNA Synthesis) related genes like RAD18, ubiquityl-PCNA as compared to control. The expression level further increases with cisplatin treatment(20uM). The elevated expression of TLS pathway is known to contribute in DNA lesion bypass, mutagenesis and enhancement of stemness properties which was confirmed by enrichment of CD44+, CD117+ double positive sub-population of cancer cells. On the other hand, there was a downregulation of NER (Nucleotide excision repair) pathway which was confirmed by XPC, XPD, XPA, XPB, XRCC1, XPG expression in cocultured condition. These leads to error prone DNA damage response with downregulated phospho-γH2AX, Cleaved-caspase3 expression and better survival capacity of the cancer cells. Infact, similar experiments were repeated with mice Bone Marrow Derived Macrophages and ID8 mice cell line, which echoed the same differential expression level of both the pathways. With *in-vivo* xenograft tumor model, Immunohistochemistry and Immunofluorescence, flow cytometry analysis of tumor tissues highlighted the similar trend in Pol H, XPC level and related genes of the two pathways. Flow cytometry analysis identified the majority of the TAMs to exhibit M2 phenotype which induces enhanced NFκB expression and IL6 production in cocultured cancer cells which still increases with cisplatin treatment.
Conclusions- TAMs actively modulate the DNA repair pathways of ovarian cancer cells. Which sets a preferential precondition to upregulate TLS pathway and downregulate NER, thus facilitating mutational burden, cancer stemness and chemoresistance.

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147 – P3.09.05

Differential predictive value of resident memory CD8 T cell subpopulations in non-small cell lung cancer patients treated by immunotherapy

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Purpose: CD8+ resident memory T cells (T_{RM}) represent a heterogeneous T cell memory population that patrols tissues. In the setting of infection or malignancy, TRM harbour cytotoxic functions and recruit other immune effectors to control infection or tumor growth. Although heterogeneity exists, CD8 TRM are identified by the expression of tissue retention markers : CD103, CD69, CD49a, and a reduced expression of migration and tissue egress markers (CCR7, CD62L). CD8 T_{RM} tumor infiltration is correlated with a favorable clinical outcome in cancer patients treated by immunotherapy. However, in preclinical models of lung and head and neck cancer, the efficacy of cancer vaccine is supported by a subset of TRM induced by the mucosal route of immunization. In this work, we aimed to better characterize these different T_{RM} subpopulations in mice and humans and to determine whether these T_{RM} subpopulations play a differential role in predicting response to immunotherapy in patients with non-small cell lung cancer

Results: We identified 2 main T_{RM} subpopulations induced after intra-nasal vaccination in mice, and in tumor infiltrating lymphocytes (TILs) from non-small cell lung cancer patients (NSCLC). One subset co-expresses CD103 and CD49a (DP) and the other only expresses CD49a (MP). Using flow cytometry, immunoassay and single cell analysis, we have shown that the DP population displays greater functionality than the MP population despite higher expression of inhibitory receptors. Analysis of their TCRs and the expression of a stemness marker (TCF1) showed that these 2 populations shared many TCRs and that the MP population appeared to be more progenitor-like. In 2 cohorts of NSCLC treated with anti-PD-1 in 1st or 2nd line, multivariate analysis showed that intratumoral CD8 CD103+CD49a+ was highly predictive of response to immunotherapy whereas CD8 CD49a+ was not irrespective of tumour location.

Conclusions: This study demonstrated that not all CD8 T_{RM} populations are equivalent and need to be distinguished to better define their functional role and value as biomarkers of response to immunotherapy.

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266 – P3.09.07

Monocytic- and early stage-myeloid derived suppressor cells are expanded in patients with hepatocellular carcinoma and associate with tumor progression

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Purpose: Myeloid-derived suppressor cells (MDSC) are a heterogeneous and immature cell population with immunosuppressive capacity, contributing to growth and metastatization of tumoral cells. They associate with poor prognosis. MDSC have been scarcely studied in patients with hepatocellular carcinoma (HCC). Here we assess the myeloid compartment in HCC patients and its association with clinical characteristics.
Methods: Peripheral blood was obtained from 22 HCC patients prior to tumoral resection or liver transplantation and from 18 healthy donors (HD). We characterized four myeloid populations ($CD33^+CD11b^+$) by flow cytometry: monocytes: $CD14^+$HLA-DR^{high}, monocytic MDSC (M-MDSC): $CD15^-CD14^+$HLA-$DR^{-/low}$, polimorphonuclear MDSC (PMN-MDSC): $CD15^+CD14^-$HLA-DR^- and early-stage (eMDSC): $CD15^-CD14^-$HLA-DR^-. We assessed their immunosuppressive function *in vitro* in proliferation assays.
Results: All three proportions of MDSC were significantly increased in HCC patients vs HD: M-MDSC (7.8% vs 0.4%), PMN-MDSC (4.75% vs 1.71%) and eMDSC (6% vs 2.5%) (all $p<0.03$). By contrast, monocytes were significantly decreased in HCC patients (36.4% vs 47.5%, p=0.02). A significant proportion of eMDSC expressed CD25. CD25+ eMDSC were higher in HCC than in HD (73.83% vs 44.4%, p=0.008), and the CD25 *per cell* expression in eMDSC was also significantly augmented in HCC vs HD (MFI 1051.5 vs 832 p=0.02). HCC-myeloid cells ($CD33^+$) showed higher suppression of $CD4^+$ and $CD8^+$ T-cell proliferation than HD-myeloid cells (p=0.001 and p=0.0006 respectively). Inhibition of proliferation directly correlated with the number of nodules and M-MDSC and was inverse with HLA-DR expression on $CD14^+$ cells and CD25 expression in eMDSC. HCC patients with > 2 vs ≤ 2 nodules presented higher eMDSC (7.24% vs 4.2%, p=0.02) and CD25+ eMDSC (4.34% vs 1.65%, p=0.0004). Likewise, when compared with patients with less advanced TNM stages (0/I, T1N0M0), patients with TNM stage II/III (T2N0M0 or T3N0M0) exhibited significant higher M-MDSC (0.44% vs 3.67%, $p<0.0001$), eMDSC (0.58% vs 6.48%, p=0.0001) and CD25+ eMDSC (0.3% vs 3.55%, p=0.003). HCC patients with serum alpha-fetoprotein (aFP) levels above median had higher M-MDSC than those with aFP below median (2.98% vs 0.17%, p=0.03).
Conclusion: HCC patients with more advanced disease had an expansion of M-MDSC and eMDSC. Therapies aimed at depleting of MDSC could favor a better outcome.

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459 – P3.09.09

To B or not to B in NSCLC: Functional characterisation of tumor infiltrating B cells in NSCLC

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Purpose: The study aimed to characterize the phenotypic and functional diversity of tumor-infiltrating B cells within the tumor microenvironment (TME) of non-small cell lung cancer (NSCLC) patients. Understanding the roles of these B cell subpopulations could provide insights into modulating B-cell responses for enhanced anti-tumor immunity.
Methods: Integration of high-dimensional protein and RNA expression data from B cells obtained from lung, blood, and tumor samples was performed. Clinical samples underwent surface marker screening via flow cytometry, with validation of differential protein expression. Single-cell RNA sequencing was conducted on sorted B cells from matched tumor and lung samples. Multiplex immunofluorescence (mIF) was employed to assess spatial B cell localization in tumor and adjacent lung regions of formalin-fixed paraffin-embedded (FFPE) patient samples, focusing on selected protein markers.
Results: Four distinct B cell subtypes were identified within the TME of NSCLC patients: Plasma cells, memory B cells, germinal center (GC) B cells, and naive B cells. Differential expression of 11 markers on TME B cells, including CD55, was successfully detected and validated compared to lung and/or peripheral blood mononuclear cells (PBMCs). Notably, CD55 exhibited differential expression at both mRNA and protein levels, with mIF data indicating increased expression localized around the GC area of tertiary lymphoid structures (TLSs).
Conclusion: The study elucidates the heterogeneity of B cell populations within the TME of NSCLC, providing insights into their phenotypic and functional diversity. These findings contribute to a deeper understanding of the complex interplay between B cells and tumors, highlighting that the B cell phenotypes present are highly dependent of the surrounding TME.

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507 – P3.09.10

Rafoxanide negatively modulates STAT3 and NF-κB activity and inflammation-associated colon tumorigenesis.

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Purpose In the colorectal cancer (CRC) niche, the transcription factors signal transducer and activator of transcription 3 (STAT3) and nuclear factor-κB (NF-κB) are hyperactivated in both malignant cells and tumor-infiltrating leukocytes (TILs) and cooperate to maintain cancer cell proliferation/survival and drive pro-tumor inflammation. Through drug repositioning studies, the anthelmintic drug rafoxanide has recently emerged as a potent and selective antitumor molecule for different types of cancer, including CRC. Here, we investigate whether rafoxanide could negatively modulate STAT3/NF-κB and inflammation-associated CRC.
The antineoplastic effect of rafoxanide was explored in a murine model of CRC resembling colitis-associated disease. Cell proliferation and/or STAT3/NF-κB activation were evaluated in colon tissues taken from mice with colitis-associated CRC, human CRC cells, CRC patient-derived explants and organoids after treatment with rafoxanide. STAT3/NF-κB activation and cytokine production/secretion were assessed in TILs isolated from CRC specimens and treated with rafoxanide. Finally, we investigated the effects of TIL-derived supernatants cultured with or without rafoxanide on CRC cell proliferation and STAT3/NF-κB activation.
Results Rafoxanide restrains STAT3/NF-κB activation and inflammation-associated colon tumorigenesis in vivo without apparent effects on normal intestinal cells. Rafoxanide markedly reduces STAT3/NF-κB activation in cultured CRC cells, CRC-derived explants/organoids, and TILs. Finally, rafoxanide treatment impairs the ability of TILs to produce pro-tumor cytokines and promote CRC cell proliferation.
Conclusions We report the novel observation that rafoxanide negatively affects STAT3/NF-κB oncogenic activity at multiple levels in the CRC microenvironment. Our data suggest that rafoxanide could potentially be deployed as an anti-cancer drug in inflammation-associated CRC.

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563 – P3.09.11

High frequency of CD16+ ILT2hi monocytes in colorectal cancer: Friend of foe?

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The CD16+ monocyte population is a population of mononuclear cells to which both pro-(angiogenesis, immunosuppression) and anti-tumor functions (metastasis prevention, antibody-mediated cytotoxicity, NKs recruitment) are attributed (Olingy et al. 2019), so it is of particular interest to study this population in the context of cancer.
In this work, we have evaluated the frequency of CD16+ peripheral blood monocytes (PBMo) and the expression of different markers associated with their function by flow cytometry in 23 patients with colorectal cancer (CRC) and 14 healthy donors (HD).
We found an increase in the CD16+ PBMo population (13.8%±6.5) compared to the control population (7.6%±3.6; p=0.0015 t-test), to the detriment of the CD16- population (82.8%±9.2 vs 89.6%±5.0, p=0.0008 t-test), affecting the CD16+/CD16- ratio (0.17±0.11 in CRC vs 0.07±0.04 in HD, p=0.0022 t-test). We determined that CD16+ PBMo have higher ILT2, ligand of class-I HLA molecules, expression (MFI=376.0±174.4) compared to CD16- PBMo (144.6 ± 60.0, p<0.0001, paired and unpaired t-test) in patients with CRC. We also confirmed the presence of CD16+ and ILT2+ cells in CRC tumors by IHC.
The results herein shown describe an increase in the CD16+ PBMo of CRC, which would be equivalent to the non-classical (CD14- CD16+) or intermediate (CD14+ CD16+) monocyte population. Moreover, CD16+ population presents higher levels of the ILT2 receptor, with a suppressor activity, which could imply a high regulation of CD16+ PBMo through ILT2 ligands such as HLA-G and other HLA class I molecules. ILT2 signals through SHP proteins, that have been related to inflammatory properties of macrophages in different contexts. Further research in analyzing the effect of ILT2 signaling in the context of CRC may seed light to the involvement of CD16+ ILT2+ monocytes in this pathology.

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568 – P3.09.12

Myeloperoxidase alters T cell function and plays an immunosuppressive role in non-small cell lung cancer

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Purpose: The tumor microenvironment (TME) of non-small cell lung cancer (NSCLC) involves high infiltration of immune cells, including neutrophils. These neutrophils contribute to the complexity of the TME by releasing myeloperoxidase (MPO) upon activation and degranulation. In the presence of H_2O_2, MPO generates HOCl, a highly reactive molecule that can cause damage to proteins, lipids and DNA. In this study, we investigated the functional role of MPO in the NSCLC and its effect on T cells within the TME. We hypothesize that MPO in the TME may alter T cell activation and function, ultimately leading to an immunosuppressive TME.
Methods: We studied MPO knock-out mice in a flank tumor mouse model. Additionally, we conducted in vitro experiments using recombinant MPO treatments to analyze the impact of MPO on T cells.
Results: MPO knock-out mice exhibited reduced tumor growth compared to WT controls. This decrease in tumor growth was accompanied by an increase in lymphocyte populations, including natural killer cells (NKs) and $CD8^+$ T cells. Furthermore, MPO knock-out mice demonstrated enhanced expression of IFN-γ by T cells. In vitro experiments also revealed that $CD8^+$ T cells treated with MPO exhibited reduced proliferation and production of IFN-γ.
Conclusion: Our findings indicate that the deletion of MPO promotes an anti-tumorigenic immune environment in a mouse tumor model, characterized by an increase in $CD8^+$ T cells and heightened expression of IFN-γ. Additionally, MPO negatively affects function of anti-tumor T cells, supported by in vitro experiments demonstrating decreased proliferation and IFN-γ expression of $CD8^+$ T cells after MPO treatment. These results suggest that MPO contributes to tumor growth and exhibits an immunosuppressive role in NSCLC. Consequently, MPO might serve as a potential target for lung cancer therapies, aiming to counteract its immunosuppressive effects in NSCLC.

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577 – P3.09.13

Dysregulated lipid metabolism is a key driver of immunosuppression in ovarian cancer

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Ovarian cancer is the most lethal cancer in women worldwide, with over 70% of patients presenting with metastasis upon diagnosis. Build up of ascites fluid in the abdomen promotes metastasis and is associated with relapse and poor survival. Immunotherapy trials to date have failed, and thus there is urgent need to better understand the immunology of ovarian cancer. In this study we analysed the function and metabolism of NK cells from 24 ovarian cancer patients, including samples from up to 6 matched sites of disease. We showed that NK cell dysfunction is evident not only in the primary ovarian tumour, but across all sites of disease, including in secondary tumours and in ascites fluid. Many of the dysfunctional and metabolic features observed in tumour and ascites-infiltrating NK cells were also observed in other lymphocyte subsets, including CD4+ and CD8+ T cells, and innate-like T cells. As ascites plays a key role in promoting the dissemination of ovarian cancer, we developed an *in vitro* model to study the impact of the ascites microenvironment on NK cell metabolism. Using LC-MS based metabolomic and lipidomic analyses, we showed that lipid metabolism is the dominant pathway affected in NK cells exposed to ascites. This was characterised by high uptake of polar lipids and defects in their lipid handling capacity. Treatment with polar lipids *in vitro* recapitulated the phenotype. Importantly, depleting ascites of lipids or blocking the uptake of lipids through FATP2 protected NK cell and T cell anti-tumours functions. Overall, we show that polar lipids are key driver of NK cell dysfunction in ovarian cancer for the first time. These findings have important implications for the design of future immunotherapies for ovarian cancer patients.

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659 – P3.09.14

Immune checkpoint modulation induced by inflammatory cytokines in colon cancer cells

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Purpose: To evaluate the effects of inflammatory cytokines on immune checkpoint expression in colon cancer cells.
Methods: The colon cancer cell lines HT-29 and HCT116 were treated with the following cytokines: IFNγ (10 ng/mL), TNFα (50 ng/mL), IL-1β (50 ng/mL), IL-6 + soluble IL-6R (10 ng/mL + 100 ng/mL), IL-15 (50 ng/mL) and TGFβ (10 ng/mL). The expression of the immune checkpoint PD-L1, PD-L2, Galectin 9 (GAL9), CD155, ICOSL, and 4-1BBL were evaluated after 24 and 48 hours of treatment using flow cytometry. The soluble forms of the same immune checkpoints were quantified in the cell supernatants by multiplex bead-based assays.
Results: Both colon carcinoma cell lines showed a low basal expression of PD-L1, which increased after treatment with IFNγ, TNFα, and IL-1β for 24 and 48 hours, compared to the untreated samples. Intracellular and plasma membrane GAL9 was present in HT-29 and HCT116 cells and increased after treatment with TNFα for 48 hours. On the HT-29 cells, GAL9 also increased after treatment with IFNγ. Both HT-29 and HCT116 cells expressed CD155, but its expression did not change after treatments. HCT116 cells showed a low expression of 4-1BBL which decreased after treatment with TNFα and IL-1β for 24 hours, while HT-29 cells did not express 4-1BBL. PD-L2 and ICOSL were not detected on the cell surface of both cell lines and they were not induced by treatments. IL-6 + soluble IL-6R, IL-15 and TGFβ did not affect the immune checkpoint expression on these cell lines. Low levels of soluble ICOSL were detected in the cell supernatants after treatment with TNFα for 48 hours. The soluble forms of the other investigated immune checkpoints were not detected.
Conclusion: Our findings suggest that the presence of IFNγ, TNFα, and IL-1β in the tumor microenvironment can increase the expression of inhibitory immune checkpoints (PD-L1, GAL9) and decrease the presence of stimulatory ones (4-1BBL), thus favoring immune escape of the colon cancer cells.

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694 – P3.09.15

Modelling macrophages-glioblastoma cross-talk in tumor microenvironment

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Purpose: Glioblastoma (GBM) is the most common malignant primary brain tumor in adults: treatment relies on surgery, even if its highly infiltrative nature frequently prevents an optimal resection, thus recurrence is inevitable and prognosis remains poor. GBM subsets include isocitratedehydrogenase (IDH)-wild type (Wt), and IDH-mutant tumors, the latter with more favourable prognosis. Among infiltrating immune cells of the tumor microenvironment (TME), tumor-associated macrophages (TAMs) are the most abundant, and their abundance has been shown to correlate with clinical outcomes. Since biological behaviour of residual tumor cells at the GBM resection margin (''edge'') determines the clinical course of the disease a better understanding of the role of macrophages in this context becomes fundamental to drive the second line treatment for the urgent medical need of recurrence.
Methods: We set-up a co-culture system using the U87 GBM cell line, both IDH-Wt and mutants, and monocytes-derived macrophages. In this setting, we also modelled distinguish interactions occurring at the tumor edge *vs* the tumor core by exposing cells to normoxic and hypoxic conditions with different O_2 tension (20% *vs* 1% O_2). Then, $CD45^+$ macrophages were FACS-sorted and their transcriptomic profile were obtained from RNAseq analysis. Responses to hypoxia was assessed by evaluating the expression of selected hypoxia target genes.

Results: Results show that this *in vitro* model is a reliable approach to study the effects of tumor oxygen levels on the interplay between macrophages and tumor cells. In particular, we demonstrate that hypoxia induces its target genes in a cell-specific manner, with significant differences between Wt and IDH-mutant U87 co-cultures. Interestingly, addition of macrophages shapes tumor cells responses to hypoxia. Preliminary results on transcriptomic profiles show that cell interactions are the major driver to shape macrophages phenotype in our system, since we observe a higher number of pathways modulated in comparison to metabolic conditions and IDH status.

Conclusion: Additional studies on macrophages phenotypes in all experimental conditions are in progress to better characterized signature genes/pathways (immunomodulation, angiogenesis, proliferation, matrix remodelling) and to integrate them with mathematical models to elaborate strategies to drive clinical practice.

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747 – P3.09.16

Profiling the immune populations in paediatric brain tumours: the MIMIC program

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Background: Brain tumours are the most common solid cancers in childhood, compromising 25% of the paediatric cancers, and are the leading cause of cancer-related death in children. The combination of aggressive tumours in a delicate environment limits the therapeutic options. Myeloid cells, particularly tumour-associated macrophages (TAMs) in the tumour-microenvironment (TME), exhibit potent immunosuppressive features inhibiting effective anti-tumour T-cell responses. Targeting these cells holds promise to improve outcomes, however the underlying mechanisms of immune dysfunction remain poorly understood.
Aim: This study aims to comprehensively profile the various immune cell populations in paediatric brain tumours (pBT) and investigate their functional phenotypes.
Methods: Spectral-flow cytometry was performed on primary tumour biopsies and tumour fragments obtained via cavitron ultrasonic surgical aspiration (CUSA) from pBT patients (high-grade glioma, ependymoma, medulloblastoma, pilocytic astrocytoma and rosette-forming glioneuronal tumour) enrolled in our immune-monitoring program (MIMIC) at the Princess Máxima Centrum. This allowed the quantification and comprehensive phenotyping of the immune cell populations within the TME.
Results: The pBT TME exhibited a low abundance of immune cells across various tumour types, with myeloid cells comprising the predominant proportion. TAMs were detected in both tumour biopsies and CUSA tumour fragments in similar proportions. However, resident TAMs were enriched in the tumour fragments obtained from CUSA. Additionally, invading TAMs displayed a mixed "M1"/"M2"-like polarization phenotype, with higher expression of the chemokine receptor CCR2 and HLA-DR, as well as the immune-inhibitory receptors SIRP1A/B, CD33, and TIM3, compared to resident TAMs. T-cell infiltration in the TME was relatively low, however $CD8^+$ T-cells retained the capacity to produce Granzyme-B, despite the high expression of the immune-inhibitory receptor PD-1.
Conclusions: The apparent T-cell cytotoxic capacity in the TME indicates their potential to combat the cancer cells, on the other hand invading TAMs in pBT evidence a high expression of immune-inhibitory receptors suggesting an immunosuppressive phenotype, potentially facilitating tumour growth, infiltration and escape to T-cell immunity. Overall, CUSA-derived samples are representative of the TME characteristics observed in tumour biopsies, however a few differences were noted. Understanding the immune dynamics and their functional activity in the TME is crucial for advancing therapeutic efficacy in pBT.

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752 – P3.09.17

Ferroptosis induces immunogenic cell death and reshapes the tumor microenvironment towards improved immunotherapy in hepatocellular carcinoma

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Purpose: Immune-checkpoint-inhibitor (ICI) therapy has revolutionized cancer treatment but response rates in hepatocellular carcinoma (HCC) are still unsatisfactory. Immunogenic cell death (ICD) has been proposed as a possible way to overcome uninflamed tumor microenvironments (TME) and improve ICI therapy efficacy. Ferroptosis, an iron-dependent programmed cell death characterized by lipid peroxidation, is dysregulated in many cancers and emerging studies indicate that ferroptosis might be immunogenic. Here, we study how ferroptosis influences the TME of HCC and how this can be leveraged to increase efficacy of ICI therapy.
Methods: Mouse and human HCC cell lines were subjected to multiple ferroptosis inducers and the molecular changes analyzed by spectral flow cytometry and multiplex immunofluorescence staining. Primary immune cells were cocultured with ferroptotic cancer cells or their supernatant and characterized for changes in their activation status. HCC tumor-bearing fully immunocompetent mice were treated with buthionine sulfoximine (BSO) to induce ferroptosis and changes in the TME analyzed by spectral flow cytometry and multiplex immunofluorescence staining. Tumor growth was monitored with longitudinal magnetic resonance imaging. Liver sections of HCC patients were stained for markers of ferroptosis and ICD.
Results: Both human and murine HCC cells were sensitive to ferroptosis induction in vitro and displayed a time-dependent translocation of calreticulin to the outer membrane with a downregulation of CD47, both indicating immunogenicity. This was associated with upregulation of PD-L1. Coculture of ferroptotic HCC cells or their supernatant with primary immune cells resulted in activation of both myeloid cells and T cells. Multiplex immunofluorescence revealed that untreated human and murine HCC tumors displayed cold TME with low immune cell infiltration. BSO treatment of HCC-bearing mice resulted in enhanced immune cell infiltration, delayed tumor growth and increased survival compared to untreated mice.
Conclusion: Ferroptosis induction in HCC cells triggers immunogenic cell death and reshapes the TME towards immune cell infiltration and activation. Upregulation of PD-L1 provides a strong rationale that the ferroptosis induction might induce response to ICI therapy and combination therapies should be explored in further studies.

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757 – P3.09.18

Smoldering inflammation is revealed by the collagen scaffold adaptation and remodeling

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Increased deposition and remodeling of collagen in the microenvironment of tissues and tumors depend on chronic inflammation, a leading factor addressing cancer establishment and evolution. However, even minor changes in immune activation inside a tissue can induce structural remodeling if persisting. Reciprocally, collagen accumulation can affect the local immunity e.g. by interaction with LAIR-1 receptor on immune cells. We have shown the gut colonization of germ-free (GF) mice with intestinal microflora from conventional mice (CV) quickly modifies the mucosal scaffold and influences the systemic immunity. In vivo in rat, the induction of either chronic colitis (dextran sodium sulphate – DSS) or colon carcinogenesis (azoxymethane – AOM) resulted to sustain persistent inflammation and remodeling of the collagen scaffold organization, even when the mucosa appears recovered, at one month after acute induction. The collagen scaffold remodeling associated to persistent pro-inflammatory cytokine activities was documented by multi-photon confocal microscopy (second harmonic generation). This was found both after bacterial (GF CV) and chemical (DSS or AOM) stimulations, making the structural changes mirroring the microenvironment immunological activation. These results suggest a mucosal "inflammatory threshold (IT)", i.e. a regulatory limit for tolerating inflammatory signals and maintaining the tissue homeostasis. The collagen scaffold quickly adapts to the immune microenvironment conditions. A dis-balance between pro-inflammatory and regulatory signals can overcome IT even under apparently normal or reduced levels of microenvironmental cytokines, allowing a smoldering inflammation. The scaffold structure alteration can identify either cancer niche or chronic colitis depending on the local cytokine proportions (IL-6, IFN-γ, IL-1, TGF-β). Furthermore, in a mouse pancreatic cancer model different IL-17 expression differently addressed the profibrotic collagen organization. Concluding, cytokine levels and collagen scaffold remodeling measured in the tissue may represent a new diagnostic tool.

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758 – P3.09.19

Lymphoma B cells express SIRPα and increased SIRPα+ B cells are associated with inferior clinical outcomes in B-cell non-Hodgkin lymphoma

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Purpose: Signal regulatory protein-α (SIRPα) is a key member of the "do-not-eat-me" signaling pathway and expressed on intratumoral monocytes in B-cell non-Hodgkin lymphoma (NHL). In this study, we measured SIRPα expression on lymphoma B cells and determined its biological and clinical significance in B-cell NHL.
Methods: Assays including flow cytometry, PCR, thymidine incorporation, CyTOF, CITE-Seq, and imaging were utilized in this study to measure SIRPα expression, upregulation, and function on B cells.
Results: Using biopsy specimens from follicular (FL) and marginal zone (MZL) lymphoma, we observed that a portion of lymphoma B cells (16.29%, range: 0.2-68%, n=80, FL; 22.8%, range: 0.8-78%, n=30, MZL) expressed SIRPα at a low level. PCR and imaging assays showed that a variety of types of lymphoma B cell lines including diffuse large B-cell lymphoma (SUDHL2, SUDHL6 and OCI-LY3), mantle cell lymphoma (Jeko-1), MZL (Karpas1718), Waldenström's macroglobulinemia (MWCL-1) expressed SIRPα mRNA or protein. Activation with LPS or CpG substantially upregulated SIRPα expression on normal B cells or B cell lines. We observed that SIRPα$^+$ lymphoma B cells exhibited a phenotype similar to plasma cells, as SIRPα$^+$ cells expressed high level of CD38 and CD27 and low level of HLA-DR and IgD. SIRPα$^+$ B cells activated with anti-IgM showed increased proliferation compared to SIRPα$^-$ B cells, as indicated by increased thymidine uptake. Ligation of SIRPα on lymphoma OCI-LY3 B-cells using recombinant human CD47 protein resulted in enhanced BrdU incorporation and increased numbers of cells in S-phase when SIRPα$^+$ OCI-LY3 cells were compared to SIRPα$^-$ cells. The increased proliferation of SIRPα$^+$ cells was associated with increased phosphorylation of SHP-1. Clinically, we observed that a higher number of CD19$^+$SIRPα$^+$ B-cells in the diagnostic tumor biopsies of FL patients was associated with a significantly poorer overall survival.
Conclusion: Our results indicate that a subset of lymphoma B cells express SIRPα, and activation induces SIRPα expression on B-cells. SIRPα$^+$ lymphoma B cells exhibit a plasma cell-like phenotype and are more proliferative in response to activation. Increased numbers of SIRPα$^+$ B-cells in lymphoma patients are associated with a poor prognosis and targeting SIRPα$^+$ B-cells in lymphoma may be a promising therapeutic strategy.

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794 – P3.09.20

Expression patterns of transforming growth factor beta - related genes in metastatic brain tumours

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Purpose: The main aim of the study was to evaluate the local gene expression at the mRNA level of key TGFβ-related genes among cases with metastatic brain tumours.

Methods: Tissue specimens of metastatic brain tumours were collected from 22 patients with a mean age of 64.9 ± 7.7 years. Brain metastasis (BM) originates from primary lung cancer (n=15 cases); colorectal cancer (n=3); melamome (n=2), breast cancer (n=1), and bladder cancer (n=1). For 7 of these patients, peri-tumour samples were available and used as a calibrator. Gene expression analysis was performed by TaqMan-based assay for qPCR (7500 Real-Time PCR System; Applied Biosystems, Foster City, CA, USA). To quantify the results obtained by qPCR for the target genes *TGFB1*, *TGFBR2*, *FOXP3* and *VEGF* after normalization to reference gene *PPIA*, a relative quantification by comparative Ct method was used. The results are presented as a fold of change of the target genes compared to the calibrator.

Results: All studied target genes were significantly upregulated in the brain metastatic tissue compared to peri-tumoral tissue ($p<0.001$). The *VEGF* mRNA was at the highest level (55.6-fold), followed by *FOXP3* (41.5-fold), *TGFBR2* (40.7-fold), and *TGFB1* (24.2-fold). The *TGFBR2* and *TGFB1* mRNA was up to a 2-fold increase among the majority of BM samples (81.8%; 18 of 22). Similar data were obtained exploring cases of BM originating from lung primary cancer (n=15). Among them, the *VEGF* mRNA was at the highest level (64.9-fold), followed by *FOXP3* (53.2-fold), *TGFBR2* (47.4-fold), and *TGFB1* (29.5-fold). In addition, there was a very strong positive correlation between the mRNA level of *TGFB1* and *TGFBR2* ($r=0.857$; $p<0.001$) and *FOXP3* ($r=0.877$; $p<0.001$) in tumour tissues. Similar relations were detected in peri-tumoral samples. The *VEGF* expression was in strong positive relations with the *FOXP3*, *TGFBR2*, and *TGFB1* expression ($r=0.7$; $p=0.05$) in tumoral tissue in contrast to peri-tumoral tissue ($r<0.2$; $p>0.05$).

Conclusion:
Our findings support the crucial role of TGFβ-related genes (*TGFB1*, *TGFBR2*, and *FOXP3*) in local tumour suppressor activity in brain metastasis. Contrary to tumour-surrounding tissues, the *VEGF* expression strongly correlated to TGFβ-related gene expression in the brain metastasis.

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879 – P3.09.21

Effect of IFN-gamma on MIF-CD74-Related Immune Pathways in Triple Negative Breast Cancer Cell Lines

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IFN-γ is a pleiotropic cytokine involved in inflammation-mediated tumorigenesis processes as well as tumor immune regulation. After stimulation with MIF, CD74 molecule forms complexes with CD44, CXCR2 and CXCR4 molecules and activates signals involved in cancer growth and metastasis such as COX2 and PGE2. In this study, the effect of IFN-γ on MHC-II-mediated or MIF/CD74/CXCR and/or COX2 signaling pathways in cancer cell survival were investigated. MCF-10A and MDA-MB-231 cells were incubated with COX2 inhibitor celecoxib, PGE2 synthetase inhibitor MF63 and MIF antagonist ISO-1 molecules (48 hours, 37°C, 5% CO_2) in the presence or absence of 200 ng/ml INF-γ. After culturing, cells were stained with fluorescently labeled CD74, CD44, HLA-DR2, CXCR2, CXCR4, CXCR7, PDL-1 and PDL-2 monoclonal antibodies and evaluated on a spectral flow cytometry.
Our results showed that IFN-γ stimulation increased the expression of CD74, HLA-DR2, PDL-1 and PDL-2 molecules in MCF-10A and MDA-MB-231 cell lines. Celecoxib and ISO-1 molecules suppressed CD74 expression in both cell lines and MF63 molecule suppressed CD74 expression which increased by IFN-γ in MDA-MB-231 cell line. IFN-γ stimulation decreased CD44 expression in MDA-MB-231 cells, although not significantly compared to the unstimulated condition, while MF63+IFN-γ suppressed it with a synergistic effect. MF63 and ISO-1 molecules in MCF-10A cells and MF63+IFN-γ, celecoxib and celecoxib+IFN-γ conditions in MDA-MB-231 cells decreased PDL-2 expression compared to IFN-γ stimulation. When MCF-10 and MDA-MB-231 cells were compared, it was shown that MDA-MB-231 cells expressed PDL-1, CXCR7, CXCR4, CXCR2 molecules at a higher level. In MCF-10A cells, HLA-DR2 expression was found to be lower in unstimulated condition compared to MDA-MB-231 cells but higher in IFN-γ containing conditions.
Our findings indicates that IFN-γ cytokine promotes tumor cell growth by increasing CD74, PD-L1 and PD-L2 expression. Celecoxib, MF63 and ISO-1 molecules suppress CD74, however PDL-2 expression increased by IFN-γ. In addition, MF63 molecule suppresses CD44 expression when used together with IFN-γ, suggesting that MF63 molecule affects CD74/CD44 complex in two different ways. These findings suggest that celecoxib, MF63 and ISO-1 molecules may be potential agents that may suppress the MIF/CD74-mediated pro-tumoral effect of IFN-γ.

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927 – P3.09.22

Tumor specific T cells in Acute Myeloid Leukemia (AML) : illusion or reality ?

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Acute myeloid leukemia (AML) is a heterogeneous group of blood cancer. The standard of care treatment has changed from chemotherapy or hypomethylating agents to the association with Venetoclax or FLT3 or IDH1/2 targeting agents but a large majority of patients still relapse and succumb to the disease, urging the need for better therapeutic approaches. New observations suggest that patients with AML could be good candidates for immunotherapies, but treatment efficacy could be improved by the identification of appropriate therapeutic targets and a better delineation of the patient subgroups. Here, we explore whether tumor-specific T cells response can be identified in AML patients and ultimately determine if a subgroup of patients could be candidates for immunotherapies treatment targeting T cells.
Using mass-cytometry to deeply characterize T cells population in paired blood and bone marrow (BM), we were able to identify a population of $CD8^+$ T cells only present in the BM tissue. These BM-specific cells show the hallmark of activation (i.e. PD-1^+) with a unique cytokine profile. Interestingly, this population is only present in a subgroup of AML patient. Next, we investigated the TCR repertoire of the BM-specific $CD8^+$ T population at the single-cell level to determine whether this population is mono- or polyclonal. Our data indicate the presence of several expanded TCR clonotypes that are specific to this BM cluster and not expressed by other $CD8^+$ T populations.
Taken together, these data highlight that a unique population of $CD8^+$ T cells having the hallmark of activated and expanded T cells is present in AML patient.
Finally, our next step will be to confirm the tumor-specificity of this population. To this end, we will investigate the antigen specificity of these cells using an MHC class I tetramer screening approach for neoantigens derived from splicing variants.

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997 – P3.09.23

Oral colistin improves the therapeutic response of tumors to anti-PD1 treatment through an IFN-γ-dependent mechanism

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Gut dysbiosis plays an important role in the response to cancer immunotherapy and antibiotic treatment is associated with a poorer response to immunotherapy. Here, we investigated how narrow-spectrum antibiotic (colistin) affects the outcome of anti-PD1 cancer therapy.
We exposed mice bearing MC-38 tumor and treated with anti-PD1 to colistin. The effect of colistin on the microbiota was analyzed by sequencing, immune cells in the mesenteric lymph nodes and tumors were analyzed by flow cytometry and the significance of specific immune factors was investigated using IFN-γ-deficient mice or IL-17A and CD4 depleting antibodies.
Colistin enhanced rather than impaired the efficacy of anti-PD1 on MC-38 adenocarcinomas. It only slightly altered the composition of the gut microbiota, but it increased tumor infiltration with IFN-γ and IL-17-producing T cells. Depletion of IL-17A did not alter the effect of colistin, but the effect was lost in IFN-γ-deficient mice. Depletion of CD4 further enhanced the antitumor effect of anti-PD1 treatment.
Some antibiotics, such as colistin, enhance the anti-PD1 effect in MC-38 adenocarcinomas by increasing the antitumor response in the tumor microenvironment. This effect is controlled by IFN-γ, but it is not dependent on IL-17A.

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1044 – P3.09.24

Regulatory macrophages in a murine model of lung carcinoma.

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Purpose: Regulatory macrophages (Mreg) play a pivotal role in asthma regulation through interleukin-10 (IL-10) production. However, their significance in tumorigenesis remains poorly understood. Herein, we investigate their involvement in murine lung squamous-cell carcinoma using luciferase-expressing Lewis lung carcinoma (LLC) cells.
Methods and results: Tumor growth was monitored during 28 days via luciferase imaging following intrathecal injection of LLC cells. Our findings reveal significant infiltration of Mreg into the tumor, with a notable increase in CD16.2-expressing Mreg. Interestingly, the phenotypic profile of Mreg shifts within the tumor milieu, characterized by enhanced CD64 expression and diminished MHC-Cl2 expression. We performed single-cell RNA sequencing to delineate a more precise phenotype of myeloid cells within this tumor model. Given Mreg's hallmark secretion of the immunosuppressive cytokine IL-10, we utilized IL-10 reporter mice (ITIB) to confirm a heightened proportion of IL-10-producing Mreg within the tumor microenvironment. Intriguingly, in vitro experiments demonstrated that LLC-conditioned medium augments IL-10 production specifically in Mreg, without affecting monocytes or alveolar macrophages.
Conclusion: In summary, our study sheds light on the dynamic change of Mreg in the context of lung squamous-cell carcinoma. We provide evidence of their increased presence within the tumor microenvironment, coupled with distinct phenotypic alterations with an increased IL-10 production. We will evaluate the influence of Mreg-produced IL-10 on tumor growth by utilizing mice with myeloid cell-specific IL-10 production deficiency.

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1062 – P3.09.25

Unleashing NK cell anti-tumor response in prostate cancer by targeting the CXCR4/CXCL12 axis

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Prostate cancer (PCa) is the second most common cancer in men worldwide. Recent studies have reported the crucial role of the tumor microenvironment (TME) in each stage of cancer progression, from tumor initiation to advanced disease. Among tumor-infiltrating immune cells, Natural Killer (NK) cells play a crucial role, exerting the ability to directly eliminate cancer cells while also coordinating innate and adaptive immune responses. However, in various cancer types, including PCa, their efficacy is compromised.

In the current project, our objective is to explore the mechanisms underlying the inhibition of NK cells in cancer, with the aim of uncovering innovative therapeutic approaches. We applied single cell RNA sequencing (scRNA-seq) to immune cells infiltrating human advanced PCa that revealed a significant alteration of tumor-infiltrating NK cells (TINKs) in terms of abundance and maturation status, with a decline in the cytotoxic subset. Importantly, TINKs exhibit a profound alteration in the autophagic pathway, as confirmed by FACS analysis in human patients and transplantable and genetic PCa models. To investigate the mechanisms underlying autophagy deregulation, we performed a scRNA-seq analysis to profile $CD45^+$ and $CD45^-$ cells in a transgenic model of PCa ($Pten^{pc-/-}$). The dissection of cellular interactions within the TME by CellPhoneDB and NicheNet algorithms identified the CXCR4/CXCL12 axis among the most significant interactions between tumor and TINKs. FACS analysis confirmed the upregulation of CXCR4 in NK cells in both human and mouse settings. Mechanistically, we demonstrated that the impairment of autophagy in TINKs is mediated by CXCR4 engagement by its ligand, CXCL12. Accordingly, the blockade of CXCR4/CXCL12 pathway determines the recovery of the autophagic defect. Finally, in view of the interconnection between autophagy and NK cell effector functions, inhibition of the axis determines the restoration of NK cell effector functions, which were negatively affected by tumor secretome.

In summary, our findings highlight that the observed autophagy impairment in TINKs is mediated by CXCR4 engagement. Importantly, the inhibition of CXCR4/CXCL12 axis positively regulates autophagy, consequently restoring NK cell anti-tumor functions. These results unveil a novel mechanism underlying NK cell dysfunction that can be targeted to enhance the efficacy of NK cell-based cancer immunotherapy.

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1077 – P3.09.27

Gene electrotransfer of plasmid DNA encoding chemokines CCL5 and CCL17 combined with irradiation induces immunomodulatory effects in murine tumors

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Purpose: Chemokines regulate immune cell migration. The degree and the type of immune cells in the tumor affects disease progression and correlates with the efficacy and outcome of immunotherapies. Similarly, beneficial immunomodulatory effects were also observed after irradiation. Therefore, we sought to investigate gene electrotransfer (GET) of proinflammatory chemokines CCL5 or CCL17 in combination with irradiation, as a potential therapeutic strategy for cancer therapy.
Methods: Tumor models were chosen to correspond to an inflamed (CT26 murine colon cancer) or immunosuppressive (4T1 murine breast cancer) immunophenotype. First, chemotactic properties of investigated chemokines were examined *in vitro*. Next, the potential of chemokines to induce the extravasation of fluorescently labelled splenocytes was determined using intravital microscopy of tumors in dorsal window chamber model (DWC). The antitumor effectiveness of combined therapy utilizing GET of chemokines and two irradiation regimes (single dose of 10 Gy and fractionated dose of 3x 5 Gy) was then determined *in vivo*. Lastly, qRT-PCR was used to evaluate gene expression of several cytokines in tumors after the therapies, while changes in the abundance of CD4+, CD8+ cells and vasculature (CD31+ cells) were determined with immunofluorescent staining.
Results: Both CCL5 and CCL17 induced the migration of murine macrophages RAW264.7 *in vitro*. In tumor models, GET of chemokines increased splenocyte retention in DWCs, and combined therapy led to significant delays in CT26 tumor growth and even tumor cures. However, in 4T1 tumors, combined therapy resulted in significant tumor growth delay but not cures. Gene expression analysis revealed increased expression of chemokines post-therapy, along with elevated expression of CXCL9 and CXCL10, potent chemoattractants for cytotoxic CD8+ T lymphocytes. Immunofluorescent staining indicated increased infiltration of CD4+ and CD8+ T lymphocytes post-GET of chemokines, albeit with decreased numbers upon irradiation.
Conclusion: Combining GET of chemokines with irradiation elicited an antitumor immune response in inflamed tumors (CT26) and partially in immunosuppressive tumors (4T1), highlighting the potential of chemokines in cancer immunotherapy.

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Spectral cytometry reveals distinct immunological signature discriminating PDAC from HD patients

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Purpose: PDAC is the deadliest solid malignancy with a 5-year overall survival of about 12%. We have recently described intratumoral $CD8^+ROR\gamma t^+$ T cells which promoted PDAC progression, particularly through IL17A and TNF. Subsequently, we aimed to characterize this cell population more precisely and resolve whether it can be detected in the peripheral blood of PDAC patients. As pancreatitis is a known risk factor for PDAC, we decided to compare PDAC with pancreatitis patients, and healthy donors (HD).
Methods: Blood samples from 22 PDAC, 22 pancreatitis patients, and 20 HD were collected and peripheral blood mononuclear cells (PBMC) were isolated. A panel for characterization of different T cell subpopulations, including 36 markers, was designed for flow cytometric staining of PBMC. Afterward, various subpopulations were characterized using high-dimensional bioinformatic analyses, and differences between the groups were visualized.
Results: For PDAC patients we revealed a decreased MAIT17 frequency in the periphery, while MAIT1 frequency was increased. Moreover, prolonged overall survival was associated with higher MAIT17 frequencies. To assess if MAIT cells are present in PDAC tissue we analyzed scRNA-Seq data obtained from 9 treatment-naïve PDAC patients published previously. Within cells expressing *CD3ε* and *CD8α,* we identified 5 clusters, which we classified as exhausted and pre-exhausted T cells, γδ T cells, and a big cluster of *KLRB1* (CD161) expressing MAIT cells accounting for more than 50% of intratumoral T cells. As MAIT cells are restricted to antigen presentation via monomorphic MHC class I-related protein 1 (MR1), which presents microbial derivatives of the riboflavin synthesis, we investigated the role of MR1. Thereby, we discovered the ability of fibroblasts and pancreatic tumor cells to express MR1, which makes them potential intratumoral activators of MAIT cells. Analysis of public data revealed increased expression of MR1 correlated with shorter survival, higher staging, and increased $CD8^+$ infiltration into the tumor.
Conclusion: Based on this data, we believe that the previously described intratumoral $CD8^+ROR\gamma t^+$ T cells might be MAIT17 cells, immigrating into the tumor due to increased MR1 expression triggered by changed microbial composition in PDAC. By elucidating the interplay of involved cells we aim to develop novel therapy options.

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1174 – P3.09.29

Neutrophil plasticity in the tumor microenvironment is dependent on HPV status in irradiated head and neck cancer

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Purpose: Neutrophils are the most abundant type of innate immune cell in humans and form the first line of defense against harmful invaders. Due to their short lifespan, they have been largely overlooked in cancer research. However, the presence of neutrophils or neutrophil-derived mediators in the tumor microenvironment has been associated with poor prognosis in several types of solid tumors, including head and neck cancer. Therefore, neutrophils have been reported to be differentially polarized depending on the HPV status of the tumor. However, the effect of cancer therapies such as radiotherapy in combination with HPV status on the tumor microenvironment and neutrophils in particular is not well understood.
Methods: Using an in vitro head and neck cancer model, we aim to analyze the effect of irradiation on HPV+ and HPV- tumor cell survival, immunogenicity and secretome. In addition, we will study the effect of human head and neck cancer secretomes on neutrophils ex vivo to analyze their lifespan, surface molecule expression, effector functions, and ability to migrate out of the bloodstream. We also aim to characterize changes in neutrophil metabolism and gene expression using multiplexed analysis approaches.
Results: Radiation does not differentially affect the viability and cell death modes of HPV+ and HPV- tumor cells. Upregulation of common checkpoint molecules is also independent of HPV status on the head and neck cancer cells tested. Neutrophils, however, show increased survival upon stimulation with the secretome of only irradiated HPV- head and neck cancer cells. Stimulation with secretomes from irradiated and untreated HPV+ head and neck cancer cells does not differentially affect neutrophil survival. Preliminary data also indicate that the effector functions of neutrophils and their ability to migrate are differentially affected by HPV+ and HPV- tumor cells.
Conclusion: We conclude that HPV status does not significantly affect the response of tumor cells to irradiation. Subsequently, however, the secretomes of irradiated HPV+ and HPV- head and neck cancers affect neutrophils differently, potentially affecting the overall anti-tumor immune response. Altered neutrophil migratory behavior may also affect disease outcome by altering the tumor microenvironment.

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Spatial immunoprofiling of the tumor tissue in patients with leiomyosarcomas

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Leiomyosarcoma (LMS) is an extremely rare cancer with a poor prognosis and very limited treatment options at metastatic stages. While immune checkpoint inhibitors (ICI) showed encouraging results for many cancers, their efficacy varies within LMS. The tumor microenvironment significantly affects patient prognosis and responsiveness to ICI in many solid cancers. Our goal was to gain novel insights into the intratumoral heterogeneity and possible mechanisms of non-responsiveness to ICI in LMS.
Retroperitoneal LMS might exhibit a large size. Therefore, we investigated the intratumoral heterogeneity in four large-scaled treatment-naive tumors (ten samples per tumor). We defined four regions: vital and necrotic centers, organ-adjacent and free margins, where we evaluated immune cells with emphasis on T cells, macrophages and immune checkpoint molecules using flow cytometry and immunohistochemistry. Moreover, we analyzed cytokine secretion after anti-CD3/CD28 stimulation at these tumor regions.
The infiltration of immune cells and the expression of immune checkpoint molecules significantly varied between each patient and also within individual samples. The most profound discrepancy was observed within two distinct areas of the same tumor where $CD8^+TIM\text{-}3^+$ T cells ranged from 7% to 79%. Significantly higher expression of PD-1 and/or LAG-3 on non-T cells and almost significant LAG-3 expression on $CD4^+$ T cells was detected in the free margin in comparison to organ-adjacent margin. In contrast to other analyzed cytokines, the stimulation increased IL-17 secretion in cells isolated from organ-adjacent margin compared to cells from free margin. Following the findings about LAG-3 expression, we tested the cells reactivity to anti-LAG-3 blockade. We have observed decreased cytokine secretion and similar reaction pattern across all analyzed locations. Interestingly, IL-8 secretion was enhanced in necrotic center compared to other locations.
These results highlight the intratumoral heterogeneity of LMS, potentially explaining response to ICI therapy despite low or absent expression of these molecules and vice versa. As the presence of immune checkpoint molecules affects patient's eligibility for ICI administration, our data suggest that multiple biopsies are suitable for analyzing tumor-infiltrating immune cells in large-scaled tumors. This approach may provide higher diagnostic value and better patient selection for the optimal treatment strategy.

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1273 – P3.09.31

Exploring the role of mutant Kras-mediated inflammation in endometriosis development using murine models

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Purpose: Endometriosis is a common gynecological disease characterized by the presence of ectopic endometrial-like epithelium and stroma outside of the uterine cavity. Previous research has demonstrated that mutated Kirsten rats sarcoma viral oncogene *(KRAS)* can be identified in normal human endometrium and several types of endometrial malignancies. Studies suggest that *KRAS* mutations may foster an immune-suppressive microenvironment in malignancies, hindering effective immune cell infiltration while recruiting suppressive cells. Conversely, mutated *KRAS* has been demonstrated pro-inflammatory effects by upregulating inflammation-related transcription factors and effector cytokines in cancers. Additionally, murine models suggest *KRAS* involvement in endometriosis development, yet mechanisms are unclear. We aimed to explore *KRAS*-mediated inflammation in endometriosis using murine models due to its chronic inflammatory nature.
Methods: We established murine models of endometriosis by surgically transplanting uterine tissues from $Kras^{G12D}$ mice into wild-type recipients, or by injecting these tissues into the peritoneal cavities. The growth of endometriotic lesions was monitored using *in vivo* fluorescence imaging. Upon reaching the endpoint, we recorded the survival rates of lesions, lesion weights, and conducted pathological characterizations. Furthermore, we analyzed the immune cell composition using multi-parametric flow cytometry.
Results: The cystic lesions induced by both methods exhibited typical pathological characteristics, including squamous metaplasia in endometrial glands and heightened inflammation. Mutant Kras protein was expressed prominently in the epithelial region of the lesions. Notably, in our surgically-induced model, the endometriotic lesions showed increased weight and greater infiltration of immune cells in the *Kras*-mutated group. Specifically, we observed a higher number of plasmacytoid dendritic cells (pDCs) within the endometriotic lesions in the *Kras*-mutated group, while the counts of conventional dendritic cells (cDCs) remained unchanged, indicating a shift in dendritic cell subsets in the *Kras*-mutated endometriotic lesions.
Conclusion: Our findings indicate that the expression of mutant *Kras* in uterine epithelial cells likely contributes to the promotion of endometriosis lesion growth. The *Kras*-mediated microenvironment appears to influence the distribution of immune cells, favoring a condition rich in pDCs within the endometriotic lesions. However, the precise mechanism underlying this phenomenon warrants further investigation.

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1342 – P3.09.32

The Role of Extracellular Vesicles In Renal Cell Carcinoma Progression

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Purpose: With approximately 430,000 cases, kidney cancer represents 4.6% of the total number of cancer diagnoses in 2020, according to WHO. Nighty percent of these are Renal Cell Carcinoma (RCC), a neoplasm originating in the nephrons. The 5-year relative survival is related to staging and a drastic rate drop is observed when the tumor is metastasized. Extracellular vesicles (EVs) have emerged as a new paradigm of cell-cell communication supporting cancer progression. As little is known about EVs in RCC, we aim to obtain an EV profiling and address its role in RCC progression.
Methods: Given RCC heterogeneity, the study involves the evaluation of two RCC lines, 786-O and 769-P. HEK293 cells are used as control. It was implemented the 3D culture to mimic the tumor architecture. The lineages used in the 3D model were characterized by molecular and functional assays like western blot (WB), live-dead, wound-healing and transwell assay. Spheroid derived-EVs were isolated by affinity column and characterized by nanoparticle track analysis (NTA), WB, flow cytometry and transmission electron microscopy (TEM). In upcoming experiment, it will be performed co-incubation of EVs with spheroids and WB evaluation of hypoxia and metastasis pathways.
Results: RCC spheroids mimic the architecture of kidney tumor, mainly by consolidating a hypoxic core demonstrated by confocal microscopy images. We demonstrated that 786-O has active HIF-1α and higher levels of vimentin and MMP-2, -3, -8 and -9, pro-tumoral markers. Functionally, we show that 786-O cells migrate faster than 769-P cells. Data from NTA, flow cytometry and TEM from spheroid derived-EVs converged, showing that 786-O-derived EVs have a larger mean size than those derived from 769-P (229nm versus 144nm, respectively) and that tetraspanins CD9 and CD63 are present on the surface of these spheroid derived-EVs.
Conclusion: In summary, the *in vitro* data shows a spectrum of RCC aggressiveness that is related to cellular phenotype. As next steps, we aim to evaluate whether cell phenotype is reflected in its derived EVs via their unique RNA and protein content, which could functionally affect hypoxic-metastatic processes. This could open door for further investigation of therapeutic targets.

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LYVE-1+ perivascular macrophages represent a therapeutic target in the tumour microenvironment

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Background: Tumour associated macrophages (TAMs) are a plastic population of immune cells that have been implicated in promoting a variety of pro-tumoral pathways. In a spontaneous murine model of mammary adenocarcinoma (*MMTV-PyMT*), a subset of TAMs co-expressing the lymphatic vessel endothelial hyaluronan receptor-1 (LYVE-1) and the immune-suppressive enzyme heme oxygenase-1 (HO-1) reside proximal to vasculature within the tumour microenvironment (TME). In this current study we aimed to investigate the role of these cells in tumour progression.
Methods: To assess the transcriptomic profile of the perivascular niche, spontaneous *MMTV-PyMT* tumours were analysed using image-guided spatial transcriptomics. Subcutaneous tumours were generated via injection of *PyMT*-derived Py8119 tumour cells into the mammary fat pad of *Csf1r*^{Dtr} *x Lyve1*^{Cre} mice to study the effects of specific LYVE-1$^+$ TAM ablation upon diphtheria toxin (DTx) administration. Spontaneous tumour models were generated when *MMTV-PyMT* mice were crossed with *Csf1r*^{Dtr} *x Lyve1*^{Cre} mice. The role of HO-1 was assessed using *Hmox1*$^{fl/fl}$ x *Lyve1*^{Cre} mice (to genetically inactivate HO-1). Tumour growth was assessed using callipers and immune profiles were analysed via flow cytometry of enzyme-digested tumours.
Results: Transcriptomic analyses of 15 perivascular regions from three tumours reveals regions of high HO-1 expression which coincide with the presence of LYVE-1$^+$ TAMs. These HO-1$^+$ niches have a unique transcriptomic profile enriched for genes associated with a pro-tumoral microenvironment (*Aurka*, *Angplt4*, *Krt14*) along with immune suppression (*Hmox1*, *Ptges*). Hypothesising that areas enriched in LYVE-1$^+$HO-1$^+$ TAMs are pivotal for tumour progression, we proceeded with specific ablation of the LYVE-1$^+$ TAM subset in the TME using DTx in tumours grown in *Csf1r*^{Dtr} *x Lyve1*^{Cre} animals. Ablation of LYVE-1$^+$ TAMs resulted in significant control of tumour growth in spontaneous (*MMTV-PyMT*) and subcutaneously (Py8119) implanted mammary tumours. Interestingly, genetic knockout of HO-1 expression in LYVE-1$^+$ TAMs did not control tumour growth but did improve CD8$^+$ T-cell infiltration to the TME.
Conclusion: This study, and the novel transgenic models generated in it, highlight an unappreciated role for LYVE-1$^+$ macrophages in driving tumour progression, that is independent of their HO-1 expression. Thus, LYVE-1$^+$ TAMs represent a therapeutic target within the TME to control tumour progression.

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1441 – P3.09.34

Immunophenotyping in peripheral blood and bone marrow niche of multiple myeloma: focus on innate CD8+ T lymphocytes

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Purpose: Multiple myeloma (MM) is an incurable malignant hematological disorder characterized by the monoclonal proliferation of transformed plasma cells (PC) in the bone marrow (BM). In addition to the conventional immune system components, cancer immune surveillance involves innate T-cells (ITC), including the newly described innate panKIR(+) NKG2A(+) CD8 T-cells (CD8 ITC). We sought to identify the phenotypic signature of ITC (iNKT, MAIT, γδ-T cells, and CD8 ITC) in the tumoral microenvironment and peripheral blood (PB) of MM.
Methods: Using spectral flow cytometry, we analyzed ITC subsets in PB and BM samples from newly diagnosed MM (NDMM) patients and healthy donors (HD). In BM, we focused our analysis on CD8 ITC, separating them into two subsets based on NKG2A and KIR expression. We also analyzed activation (CD122, CD137) and exhaustion (PD-1, TIM-3) markers, IFN-γ production in response to innate-like stimulation, and their cytotoxic content (Granzyme B).
Results: First, we showed that NDMM patients had a higher proportion of PB CD8 ITC among CD8 T-cells (and not iNKT or γδ-T cells among T-cells), as compared to HD. Moreover, PB CD8 ITC from NDMM patients were functionally impaired, as attested by their reduced IFN-γ production in response to IL-12/IL-18 stimulation, as compared to HD. Secondly, taken as a whole, ITC accounted for 10 to 20% of resident (CD69+) T-cells in BM from HD, a proportion that tended to increase in BM from NDMM patients. Remarkably, this resident (CD69+) T-cell population of NDMM patients co-expressed CD122 and CD137 and was mainly composed of KIR+ CD8 ITC. Moreover, there was no difference in the expression level of exhaustion markers (PD-1 and TIM-3) by CD8 ITC between HD and NDMM. Finally, Granzyme B frequency in KIR+ CD8 ITC patients was increased in NDMM, as compared to HD.
Conclusion: These data show that ITC represent a significant proportion of BM resident cells. Specifically, the co-expression of CD69, CD122 and CD137 in NDMM KIR+ CD8 ITC suggests specific activation by tumor antigens and bystander activity of this cell subset contributing to immune control of the BM MM niche.

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1511 – P3.09.35

Next generation of spatial biology: high-throughput multiplexed Imaging Mass Cytometry™ with whole slide modes

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Purpose: Gaining spatial insights into tumor tissue cellular composition holds great promise for informing clinical and translational researchers about immunotherapy success predictors, disease progression, and etiology. Imaging Mass Cytometry™ (IMC™) facilitates deep characterization of the tumor microenvironment (TME) diversity and complexity. With the ability to simultaneously assess cell phenotype and function using 40-plus metal-tagged antibodies on a single slide, IMC eliminates issues related to fluorescence-based spectral overlap, tissue autofluorescence, and multiple washing and acquisition cycles.
Method and Material: Currently, IMC allows user-defined regions of interest (ROI) in tissues for evaluating cellular and structural composition. To improve the IMC user experience, we introduced two new whole slide imaging (WSI) modes: ultrafast preview mode (PM) and high-throughput tissue mode (TM). PM swiftly samples the entire tissue at predefined intervals to capture a low-resolution image of all markers in the antibody panel, providing quick insights for ROI placement while preserving tissue integrity for higher-resolution imaging. PM and TM seamlessly enable acquisitions on the same slide without extra processing steps. TM rapidly captures whole tissue images at a lower resolution (7 μm pixel size) suitable for quantitative spatial analysis of tissue biology. Tailored for high-throughput applications, TM, along with a new 40-slide loader for the Hyperion XTi™ Imaging System, allows automated and continuous imaging of over 40 large tissue samples (400 mm2 per tissue) per week.
Results and Discussion: We demonstrate the use of WSI modes with the newly developed Maxpar® Human Immuno-Oncology IMC Panel Kit. This 31-marker panel, combined with catalog antibodies, creates a 40-plus-marker panel, enabling comprehensive high-plex tumor and immune cell profiling. Tumor tissue microarrays (TMA) and whole tumor tissue sections were stained with the expanded panel. Single-cell analysis of selected ROIs, guided by PM data, provided quantitative analyses of spatial biology at single-cell resolution. Additionally, TM on whole tumor sections, followed by pixel-based analysis, yielded a spatially resolved quantitative assessment of specific tumor and immune components of the TME.
Conclusion: This work demonstrates the expanded capabilities of IMC and establishes it as a reliable high-plex spatial biology imaging platform with high-throughput imaging capabilities ideally suited for translational and clinical applications.

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1512 – P3.09.36

Novel Whole Slide Imaging Modes for Imaging Mass Cytometry Reveal Cellular and Structural Composition of Mouse Glioblastoma

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Introduction: Mouse tumors are valuable models for brain malignancy research, overcoming challenges in studying the human brain. The mouse brain serves as a miniature version of the human brain, facilitating whole tissue visualization for spatial context. Imaging Mass Cytometry™ (IMC™) offers a powerful means to quantitatively analyze protein composition in the brain tumor microenvironment (TME) without autofluorescence or spectral overlap issues. The Hyperion XTi™ Imaging System by Standard BioTools™ employs IMC technology to assess over 40 structural and functional markers simultaneously, shedding light on TME organization and function.
Method and Material: We utilized whole slide imaging (WSI) with a 40-marker panel, combining the Maxpar OnDemand™ Mouse Immuno-Oncology IMC Panel Kit and the Maxpar® Neuro Phenotyping IMC Panel Kit on mouse normal and glioblastoma (GBM) brain tissue. The panel included mouse-specific antibodies highlighting tumor and immune components of the mouse tumor microenvironment (TME). Employing Hyperion™ XTi, we utilized two new features: Ultrafast preview mode (PM) for rapid screening of entire brain sections, guiding selection for region of interest-based IMC analysis and single-cell analysis (SCA); and high-throughput tissue mode (TM) for detailed whole-slide scans, quantified using pixel-based analysis (PBA) to elucidate TME composition.
Results and Discussion: Using TM, we successfully visualized both normal mouse brain and mouse GBM tissue in coronal and sagittal sections. PBA analysis revealed quantitative spatial expression patterns of structural and immune markers throughout the tissue. In normal tissue, we observed well-organized structures including neurons, oligodendrocytes, vascular-adjacent astrocytes, and axonal tracks. In GBM tissue, we detected necrotic cores, areas with high immune infiltration, extracellular matrix deposits, and activated tumor cells. SCA of GBM tissue revealed extensive vascularization, replication of Olig2+ cells, activation of Ras signaling, and abundant infiltrating immune cells.
Conclusion: Overall, we demonstrate the successful application of two novel WSI modes and highlight the power of IMC technology to simultaneously explore dozens of relevant biological outputs to better understand the TME of GBM and other tumors.

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Novel whole slide imaging modes for imaging mass cytometry unveil extensive cellular heterogeneity in human gliomas

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Introduction: Gliomas, particularly glioblastoma (GBM), pose significant diagnostic and therapeutic challenges, with a median survival of just over one year post-diagnosis. GBM can manifest as multi-lesion, remote, or diffuse tumors, often lacking peripheral immune cells in the tumor microenvironment (TME). Notable features include necrosis, hemorrhage, and pseudo palisades, reflecting its high heterogeneity and necessitating deeper exploration. Understanding the cellular and spatial composition of the TME is crucial for interpreting GBM's origin, progression, and informing treatment strategies.
Method and Material: We utilized a 40-plus-marker neuro-oncology Imaging Mass Cytometry™ (IMC™) antibody panel to examine the cellular and structural landscape of the brain tumor microenvironment (TME). This panel was applied to a tissue microarray (TMA) containing numerous human glioma cores, revealing the spatial distribution of over 40 distinct molecular markers. Leveraging the Hyperion XTi™ Imaging System, we employed two new features for whole slide scanning. Ultrafast preview mode enabled swift screening of tumor cores for specific expression signatures related to immuno-oncology processes, guiding targeted imaging and subsequent single-cell analysis. Simultaneously, high-throughput tissue mode conducted a detailed scan of the brain tumor TMA, followed by pixel-based analysis, providing insights into the spatial composition of the TME.
Results and Discussion: Using tissue mode imaging, we effectively mapped cell populations in human gliomas, including neurons, astrocytes, microglia, and oligodendrocytes, across the entire TMA. Various tumor cell phenotypes and immune cell types were detected across all TMA cores. Single-cell analysis provided quantitative assessment of the brain TME's cellular composition. We classified neuronal states and quantified immune cell infiltration across normal, astrocytoma, and GBM tissues. Significant cellular and protein heterogeneity was observed between cores, highlighting IMC's capability for spatial evaluation of high-plex protein composition in the brain TME, without complications like autofluorescence or spectral overlap.
Conclusion: Empowered by the neuro-oncology panel and new whole slide imaging modes, IMC accelerates neurological research and provides insights into the spatial complexity of gliomas and other tumors.

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Neutrophils-like monocytes increase in patients with colon cancer and induce dysfunctional TIGIT+ NK cells

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Myeloid-derived suppressor cells (MDSCs) are a heterogeneous family of immune cells including a granulocytic (CD14neg/CD15+/HLA-DRneg) and a monocytic subtypes (CD14+/CD15neg/HLA-DRneg). In the present study, we found a population of CD14+ monocytes expressing the granulocyte marker CD15 that significantly expand in both peripheral blood (PB) and tumoral tissues of patients with colorectal cancer (CRC). Further phenotypical analysis confirmed the granulocytic-like features of this monocyte subpopulation that is associated with an expansion of granulocyte-monocytes precursors (GMPs) in the PB of these patients. Functionally, they secreted large amount of IL-10 and suppressed NK cell activity, crucial for the establishment of antitumor immune response. Mechanistically, this granulocytes-like monocyte population could promote the acquisition of the immune checkpoint inhibitor TIGIT in NK cells and accordingly, increased frequency of dysfunctional TIGIT+ NK cells were found in both peripheral blood and tumoral tissue of CRC patients. Collectively, we provided new mechanistic explanation for tumor immune escape occurring in CRC by showing the expansion of this new class of myeloid suppressor cells in both PB and CRC tissue that significantly impaired the effector function of NK cells thereby representing a potential therapeutic target for cancer immunotherapy.

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1589 – P3.09.39

The impact of ER stress on γδ T cells in the tumor microenvironment

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Gamma delta T cells (γδ T cells) are unconventional T lymphocytes with a role in the immune surveillance of cellular stress. γδ T cells respond to a wide range of tumors and tumor infiltrating γδ are the most significant favorable prognostic immune population among 39 cancer types. However, a suppressive tumor microenvironment (TME) can impair γδ function, and the mechanisms are not fully understood. Thus, we aim to elucidate cellular communication between tumor cells and tissue resident γδ T cells to provide a basis for the development of effective anti-tumor therapies. The TME is often characterized by increased endoplasmic reticulum (ER) stress, a cellular response to an accumulation of un-/misfolded proteins in the ER lumen that can be promoted by several environmental insults (e.g. hypoxia, glucose deprivation). To restore cell homeostasis, the ER activates pathways collectively known as unfolded protein response (UPR), and an enhanced UPR correlates with poor clinical outcomes in cancer patients. Crucially, the UPR can support tumor growth by regulating immune cell function. We hypothesize that ER stress is a mechanism used by tumor cells to dysregulate the activation and function of tumor-infiltrating cutaneous γδ T cells. To this end we induced ER stress pharmacologically in primary human cutaneous squamous cell carcinoma cell lines using thapsigargin and treated cutaneous gd T cells with the conditioned medium of these cells. This induced the upregulation of activation and cytotoxic markers and concomitantly reduced the expression of NK cell receptors (NKRs) and the production of pro-inflammatory cytokines by gd T cells. These findings support the hypothesis that cutaneous γδ T cells respond to ER stress within the TME and this modulates their activation and function. Our findings will provide important new insights into the immune surveillance and anti-tumor function of cutaneous γδ T cells under ER stress and help optimize their future clinical applications for cancer therapies.

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1643 – P3.09.40

Stereo-imaging mass cytometry: expansion to 3D of multiparametric single-cell analysis in tissue

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Biological investigations require collection of 3D Imaging data for a comprehensive understanding of cellular and molecular spatial distribution. Imaging Mass Cytometry (IMC) reveals cell landscape, abundance and phenotype in tissue. Relevant results were obtained with IMC applied to studies on infiltrating immune cells in tumor. Initial attempts direct at 3D IMC with the aim of expanding measurable parameters, such as assessment of tissue architecture and vasculature, as well as to refine analyses related to cell phenotyping, positioning and their functional interactions. Here, we propose Stereo-IMC, an unprecedented method to advance IMC from conventional 2D to 3D of larger tissue analysis. Alteration in tissue volume resulting from the transition from hydrated to dry, the latter essential in IMC analysis, was addressed through use of antibody-permeant hydrogels. An antibody labelling strategy using both metal and fluorescence, confocal microscopy, IMC and computing corrected the mismatch in cell positioning due to transition of tissue in the different the states (hydrated vs. dry), thus reallocating single cell in volume. Organs of different structure and cell density were used for the purpose, at last challenged in a pancreatic cancer model. Comparative evidence obtained using Stereo-IMC versus conventional 2D IMC raises the method as an expansion towards a 3D multiparametric analysis of single-cell in relationship with tissue microenvironment and for tissue architecture. Results are instrumental in the development of an optimized 3D IMC system to improve investigation of tumor microenvironment.

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Colorectal Cancer microenvironment controls Mast Cell phenotypical plasticity

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Purpose: Mast cells (MCs) are tissue-resident immune cells characterized by their cytoplasmic granules containing different proteases and by the surface expression of the high-affinity receptor for IgE (FcεRI) and c-kit (CD117), the receptor for stem cell factor (SCF). Once activated, MCs released several mediators and can orchestrate different immune responses in both physiological and pathological conditions including cancer. In particular, MCs can infiltrate Colorectal Cancer (CRC) but the precise role of mucosal versus connective-like intestinal MC subsets during CRC development is still unclear. The aim of our study is to investigate whether a particular MC subset is involved in CRC progression and to analyse how tumor microenvironment shapes MC plasticity.
Methods: We employed a mouse model of chemical-induced inflammatory colorectal cancer (AOM/DSS) as well as tumor biopsies of CRC patients. We initially performed microscopic and flow cytometric analyses in order to study MC frequency, localization and phenotype. We further investigated by *in vitro* and *in vivo* assays the contribution of soluble factor enriched in tumor microenvironment in shaping murine MC plasticity.
Results: By evaluating the expression of selective proteases within the granules of tumor infiltrating MCs, we demonstrated the prevalence of a connective tissue-like phenotype in both murine and human samples.
Focusing on the tumor microenvironment of AOM/DSS-treated mice, we found a higher concentration of SCF and IL-33 in tumor lesions compared with tumor-free tissue. Moreover, we demonstrated that a sustained *in vitro* stimulation of MC primary cultures with SCF and IL-33 promotes the expansion of a connective-like MC subset. Consequently, trough *in vivo* experiments we observed that SCF-neutralization induce a decrease of the connective tissue-like MC subset accompanied by inhibition of tumor burden.
Conclusion: Our results demonstrate that connective tissue-like MCs accumulate in both murine and human colorectal cancer lesions supporting a role for this subset in the control of tumor progression. Moreover, we underscored the ability of SCF in combination with IL-33 to shape MC phenotype.

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Siglec ligand-receptor axis: novel insight into extracellular vesicle mediated immunomodulation in colorectal cancer

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Colorectal cancer is one of the most prevalent forms of cancer worldwide. A major change in tumour signature is the up-regulation of sialic acids, supporting an immunosuppressive milieu. Sialic acids form Siglec ligands, which interact with immunomodulatory Siglec receptors. Data from our group suggest that cancer associated fibroblast (CAFs) within the colorectal tumour express high levels of Siglec ligands.
Extracellular vesicles (EVs) are key mediators of communication within the tumour microenvironment. This study aims to compare the Siglec ligand profiles of EVs derived from CAFs and CRC cells and investigate their respective effects on NK cell function.
We have optimised a method to analyse Siglec ligands on EVs via single-particle flow cytometry with the Cytek NL2000. Using this method, we will assess the abundance of CD24 (Siglec 10 ligand) on HCT116 cancer cell line and cancer cell secretome conditioned hTERT transduced MSCs (n=3). We will also examine α2,3- & α2,6-sialic acids on EVs from both sources via western blot and compare them with the respective cell membranes.
We examined uptake of CFSE stained HCT116/MSC EV in primary human NK cells via flow cytometry (n=2). Additionally, expression of Siglec 10 post treatment was investigated (n=2). Next, we will look at HCT116 and secretome treated MSC EV ± sialidase treatment on NK Siglec 10 expression (n=3). The ability of NK cells to kill cancer cells post treatment will also be examined.
We anticipate EVs derived from secretome treated MSCs will display unique profiles of Siglec ligands compared HCT116 EVs, in line with our findings revealing heightened Siglec ligand expression in CAFs. Our preliminary results suggest a preference for hTERT MSC EV uptake and a pattern towards downregulation of Siglec 10 expression with treatment with both EV groups. Building upon this observation, our future investigations aim to highlight how the anticipated differences in Siglec ligand composition between CAF-derived and CRC cell-derived EVs could further elucidate their distinct immunomodulatory effects on NK cells.
Investigating the impact of tumour microenvironment-derived EVs on immune cells is crucial for advancing our understanding of tumour immunology and developing EV and sialic acid targeting therapies.

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Exercise-induced extracellular vesicles delay tumor growth by igniting inflammation in immunologically 'cold' triple-negative murine breast carcinomas

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Purpose: Preclinical meta-analyses on breast cancer demonstrate that physical activity reduces tumor incidence and growth. Independent studies have also reported exercise effects on the murine breast cancer microenvironment. However, there is a gap in understanding the molecular mechanisms behind the beneficial effects of exercise. Exercise-induced biomolecules can be secreted into the circulation either in soluble form or packaged into carriers such as extracellular vesicles (EVs). This study aims to investigate the effect of exercise-induced plasma EVs, a potential exercise mimetic, on tumor growth and immune microenvironment in two murine models representing triple-negative breast (TNBC) cancer
Methods: Size exclusion chromatography was used to isolate exercise-induced EVs from plasma of healthy Balb/c (n=30) and C56BL/6 (n=30) female mice that underwent 10 sessions of 30-minute treadmill running. The EVs were characterized using electron microscopy, nanoparticle tracking analysis, and dynamic light scattering. Two murine TNBC models were generated by subcutaneously implanting 4T1 cancer cells in Balb/c mice and EO771 cancer cells in C56BL/6 mice (n=60 per strain). Tumor-bearing mice received five doses of 1×10^6 exercise-induced EV particles either prophylactically or therapeutically setting. Extensive immune profiling of the tumor microenvironment was conducted using flow cytometry.
Results: The amount of EVs in the plasma of active mice was higher than in the sedentary group. Both prophylactic and therapeutic administration of exercise-induced EVs delayed tumor growth by 35% in the 4T1 model and by 45% in the EO771 model ($p<0.05$) compared to respective untreated controls. Noticeable differences in the proportions of tumor-infiltrating lymphoid, but not myeloid, cell subpopulations suggested that exercise-induced EVs have immunomodulatory effects. We observed a significant influx of CD8+ lymphocytes in tumors, along with a decrease in the exhaustion marker PD-1, in all groups treated with EVs in both tumor models. Moreover, the level of PD-L1 on tumor cells was reduced, suggesting perturbations in the PD-1/PD-L1 axis upon treatment with EVs.
Conclusion: Our study demonstrates that treatment with exercise-induced EVs triggers a pro-inflammatory antitumor immune response, and converts immune ‘cold' tumors to 'hot', associated with better outcomes. These findings provide rationale for furhter investigations of EVs as potential immunomodulatory exercise mimetics.

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Xenografting mouse models to study human cutaneous γδ T cells in health and disease

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Gamma delta (γδ) T cells play important roles in the surveillance of cellular stress, tumors, and infection, that help maintain tissue integrity and modulate adaptive responses to these stimuli. γδ T cells can recognize malignant cells via surface molecules and display killing activity upon activation. γδ T cells respond to a variety of solid and hematological tumors *in vitro* and in *in vivo* xenograft models, and the presence of tumor infiltrating γδ T cells was the most significant favorable prognostic immune population among 39 human cancer types. In clinical trials, adoptive transfer of e*x vivo* expanded γδ T cells lead to temporary tumor regression and increased survival of leukemia patients. Hence, γδ T cells are promising candidates for anti-tumor immune therapeutic approaches. Due to technical difficulties to isolate enough γδ T cells from human skin, most studies on cutaneous γδ T cell biology were focused on murine skin resident γδ T cells. Here we are using novel methodologies to expand functional cutaneous γδ T cells *ex vivo*. Upon adoptive transfer of these cells into mice that have received xenografted engineered human skin or skin tumors, we can study their migration, maintenance, and phenotypic adaptation *in vivo*. Specifically, we have established a squamous cell carcinoma (SCC) xenograft mouse model in which the grafted SCC tissue resembles tumors of patients macroscopically and microscopically. In this model, γδ T cells engrafted in the spleen, healthy skin and the tumor tissue. Crucially, these cells displayed an activated phenotype and function after isolation from the tumor mass. This model enables in depth and mechanistic studies of the biology of cutaneous γδ T cells in the tumor microenvironment. Additionally, the *in vivo* tumor model can be utilized to study the therapeutic potential of γδ T cells in cutaneous carcinomas, paving the way to novel anti-tumor treatments.

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1894 – P3.09.45

Heterotypic 3D Cancer Spheroids as a Controlled Modeling Platform for Studying Tumour Microenvironment

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Purpose: The adoption of 3D cell culture models has greatly improved our understanding of tumour biology as these models closely represent characteristics of *in vivo* tumours, such as cell-cell interactions, hypoxia and pH rate, exposure to nutrients and metabolites, and gene expression profiles. However, 3D models are usually composed of only one cell type and, more often than not, an immortalized cell line. However, they usually lack crucial tumour components like macrophages and fibroblasts. Innovative strategies, including co-culture systems and organoids, address this limitation by integrating diverse cell types, thereby offering a better representations. However, so far there is a lack of 3D controlled *in vitro* models to study tumour and immune cell interactions.
Methods: In our study, we optimized 3D culture conditions for six epithelial ovarian cancer (EOC) cell lines and their co-cultures with fibroblasts or THP1 monocytes. 3D spheroids were measured and observed in different growth conditions and at a different starting point seeding densities. Using qPCR, we compared gene expression profiles focusing on stemness, epithelial-mesenchymal transition, and immune interaction genes. Additionally, flow cytometry analysis was employed for cancer cell, fibroblast and monocyte marker expression evaluation in diferent culture conditions.
Results: We observed that cell lines that were unable to form 3D cell cultures in standard culturing conditions, were able to form spheroids when cultured with fibroblasts. Upon analyzing the gene expression in the 3D cultures and 3D co-cultures with fibroblasts, we found significant differences in the expression of genes such as *SNAI1*, *POU5F1*, *VEGF*, and *FAP*. In addition, flow cytometry analysis showed that the number of monocytes infiltrating the spheroids varied depending on the cancer cell line used in 3D culture model.
Conclusion: Our findings provide input for creating and developing better 3D models that reflect immune-tumor interactions and new *in vitro* models to study and modulate the tumor microenvironment.

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1934 – P3.09.46

Role play by endogenous galectin-1 in an experimental model of melanoma

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Galectin-1 is an endogenous lectin ubiquitously expressed in different tissues and immune cells, as well as by tumor cells. Numerous studies have identified a pivotal tumor promoting role for galectin-1 expressed by the cancer cells. However, the role play by endogenous expression of galectin-1 within the tumor microenvironment remains to be fully dissected. In the present work, the experimental murine model of melanoma using *B16F10* cell line has been used to study the function of endogenous galectin-1 during tumor development. Endogenous galectin-1 expression accelerated tumor burden upon subcutaneous implantation of *B16F10* cells. The endogenous expression of galectin-1 was associated with increased expression of proteins involved in immune checkpoints such as PD-L1 by myeloid cells and CCR8 by intratumoral Treg cells. Taken together, these results underscore the importance of the immunomodulatory role play by the endogenous expression of galectin-1 in the context of melanoma. Our findings identifies galectin-1 as a key regulator of the tumor microenvironment and that strategies to specifically target galectin-1 expression may enhance host's anti-tumor immune responses that could behold great promise for cancer patients, especially when combined with immunotherapy.

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Boosting systemic anti-tumour immunity: the effects of electroporation on proteomic output and immune cell polarization in human ex vivo gastrointestinal cancer explant models

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Purpose: Electroporation-based antitumor therapies calcium electroporation (CaEP) and irreversible electroporation (IRE), have been shown to be effective on directly treated tumours by altering the tumour microenvironment (TME) and triggering a systemic immune response. We investigated the proteomic secretome following reversible electroporation (rEP), CaEP and IRE in upper and lower gastrointestinal (GI) cancer explants. We analysed the proteomic secretome to further understand the immunological response to EP and investigated the effect of electroporated tumour conditioned media (TCM) on immune cell polarization.
Methods: Following patient consent, *ex-vivo* tumour and matched normal tissue from GI cancer patients were exposed to reversible electroporation with/without 5.0mM $CaCl_2$ and irreversible electroporation using the ePORE electroporator (Mirai Medical, Galway). Treated explants were cultured at 37°C for 24 hours. The resulting supernatants, termed tumour or normal conditioned media, TCM and NCM were analysed via MSD 54plex ELISA to assess secreted factors. The effects of TCM and NCM on dendritic cell and macrophage polarisation was assessed by flow cytometry.
Results: MSD profiling of TCM and NCM from EP treated explants, showed a varied proteome that depends on the treatment delivered and tumour origin. In gastric cancer myeloid cell attractants such as IL-8 and MCP-4 were elevated on EP treatment and not in colorectal cancer (CRC). Normal tissue was less responsive to all treatments than tumour tissue. Inflammatory markers such as IL-1α ($p=0.03$), TNF-α ($p=0.022$) and IL-10 ($p=0.048$) and macrophage released chemokines MIP-1α ($p=0.048$) MIP1-β ($p=0.018$) and MIP-3α ($p=0.0043$) were altered with CaEP. DCs and macrophages were differentially affected by EP treatment at different GI sites. TCM lowers M1 macrophage marker expression in CRC but differentially affects upper GI cancers. TCM from CRC tumour explants alter DC marker expression.
Conclusion: Alterations in the immunomodulatory secretome of tumour tissue after EP affects immune cell function, alters the tumour microenvironment and could illicit an abscopal response. Further interrogation is required to fully elucidate the effects of EP at different sites and could facilitate tailored regimens based on tumour site whilst enhancing a systemic immune response to clear distal metastatic tumours.

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2139 – P3.09.49

Stromal cell-mediated immunosuppression: sialylated ligands as orchestrators of immune evasion in colorectal cancer

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Background: Cancer-associated fibroblasts (CAFs) play a critical role in the tumour microenvironment, often inhibiting the efficacy of cancer immunotherapy. Targeting CAFs directly holds significant potential in improving cancer treatment outcomes. However, the mechanisms underlying CAF-mediated immunosuppression remain poorly understood. Recently, the Siglec-sialic acid axis has emerged as novel mechanism contributing to immune evasion in cancer. This study aims to investigate the involvement of sialic acid in CAF-mediated immunosuppression specifically in colorectal cancer (CRC).
Methods: CAFs and matched normal-associated fibroblasts (NAFs) were isolated from tumour and adjacent non-cancerous tissue, respectively. Expression of fibroblast markers (α-SMA and FAP), sialic acid (SNA-I and MAL-II) and Siglec-7/9 and 10 ligands on NAFs/CAFs were analysed using flow cytometry. Immunosuppressive effects of CAFs on T cells and NK cells were assessed using CAFs-immune cells co-culture and the expression of immune cells immunosuppressive/activation markers, as well as Siglec-7/9 receptor expression were analysed using flow cytometry.
Results: CAFs expressed higher levels of sialic acid and Siglec-7/9 and 10 ligands compared to NAFs and tumour cells. CAFs also induced exhausted immunomodulatory CD8+PD1+ and CD8+Siglec-7/9+ T cell phenotypes. Furthermore, CAFs induced Siglec-7/9 on NK cells and reduced expression of the activating receptor NKG2D, leading to decreased NK cell cytotoxicity. Strikingly, de-sialylation of CAFs reversed these effects.
Conclusion: We show for the first time that CAFs can induce immunosuppressive Siglec receptor expression on T and NK cells. Our study underscores the pivotal role of sialylated ligands on CAFs in immunosuppression. Targeting the Siglec-sialic acid axis on CAFs is a promising strategy to enhance anti-tumour immunity in CRC.
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2225 – P3.09.50

The impact of the STING pathway on the antitumor response of short-term fasting

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Cutaneous melanoma is the leading cause of death from skin cancer in the world and has relatively high resistance to treatment with radiotherapy and chemotherapy. The partial success achieved with the use of immunotherapy, especially checkpoint inhibitors, therefore, it suggests that immunological interventions are an interesting strategy to be used. Short-term fasting (STF) aims to induce the effect of differential stress resistance, where tumor cells are more compromised than normal cells due to low energy levels during cancer treatment. This occurs because normal cells can better adapt to stress by redirecting energy expenditure towards maintenance and repair, while tumor cells remain focused on proliferation due to mutations in oncogenes and tumor suppressor genes. Considering the importance of the STING signaling pathway in the recognition of DNA released by apoptotic cells and in the subsequent immune response generated, our aim was to investigate the role of STING in the antitumor effect of STF. Mice WT or STING KO were given a subcutaneous injection of B16-F10 cells. When the tumor became palpable, the animals underwent 48 hours of fasting, with free access to water. Our results demonstrated that 48-hour fasting proved to be efficient in controlling tumor growth without negatively affecting the frequency of infiltrated myeloid and lymphoid cells in the tumor. Weight loss during the fasting period was quickly regained when the animals resumed feeding. However, control over tumor growth was maintained until the day of euthanasia. In STING KO animals, there was no statistical difference in tumor weight, suggesting that the STING signaling pathway participates in the antitumor response generated by short-term fasting.

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2251 – P3.09.51

Associations between soluble PD-L1/PD-1 and circulating immune cells for prostate cancer progression

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Purpose: This study aims to investigate the role of soluble forms of PD-L1 and PD-1 (sPD-L1 and sPD-1) and its potential interaction with circulating immune cells, in influencing prostate cancer (PCa) development and progression, considering specific immunologically cold profile of PCa.
Methods: The study involved 75 patients diagnosed with pT2 and pT3 prostate cancer, along with 41 healthy individuals. The levels of sPD-L1 and sPD-1, as well as specific immune cells: T lymphocytes (CD4+, CD8+), B lymphocytes, regulatory T cells (Tregs), Natural Killer cells (NK), myeloid derived suppressor cells(MDSC), in the peripheral blood of all patients before and three months after radical tumor removal were evaluated. sPD-1 and sPD-L1 were measured in plasma using ELISA method, while immunophenotyping was conducted using flow cytometry technique. The correlations were assessed against the clinicopathological findings of PCa.
Results: Elevated levels of sPD-L1/sPD-1 in the plasma of prostate cancer patients were found compared to healthy controls. Associations between elevated levels of sPD-L1 and sPD-1 and prostate cancer advancement were found. The results indicate the source of low preoperative sPD-L1 might be associated with the secretion of immune cells. High concentrations of sPD-L1 in the blood of prostate cancer patients correlate with Tregs and MDSC as well as with recurrence of cancer. A correlation between sPD-1 and NK cells was observed.
Conclusions: sPD-L1 emerges as a promising prognostic marker in prostate cancer (PCa), potentially influencing disease progression through interactions with Tregs. NK cells could be one of potential sources of sPD-1in PCa.

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P3.10 VACCINES

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62 – P3.10.03

Specific cellular and humoral response after the third dose of anti-SARS-CoV-2 RNA vaccine in patients with Immune-Mediated Rheumatic Diseases on immunosuppressive therapy

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Purpose: Data on cellular and humoral immunogenicity after the third dose of anti-SARS-CoV-2 vaccines in patients with immune-mediated rheumatic diseases (IMRDs) are scarce. Herein, we evaluated the adaptive immune response in IMRD patients treated with different immunosuppressive therapies (conventional synthetic disease-modifying antirheumatic drugs [csDMARDs], biological disease-modifying antirheumatic drugs [bDMARDs], and targeted synthetic disease-modifying antirheumatic drugs [tsDMARDs]) after the booster of the anti-SARS-CoV-2 vaccine to determine whether any drug reduced the vaccine's response.
Methods: A single-center prospective study was conducted, including patients presenting with IMRD and healthy controls (HC). Specific anti-SARS-CoV-2 interferon-gamma (IFN-γ) production was evaluated between 8-12 weeks after the third dose of the SARS-CoV-2 vaccine. In addition, anti-Spike IgG antibody titers were also measured.
Results: Samples were obtained from 79 IMRD patients (51 women, 28 men; mean age 57 ± 11.3 years old): 43 rheumatoid arthritis, 10 psoriatic arthritis, 14 ankylosing spondylitis, 10 undifferentiated spondyloarthritis, and 2 inflammatory bowel disease-associated spondyloarthritis (IBD-SpA). In total, 31 HC (mean age 50.9 ± 13.1 years old, 67.7% women) were included in the study. Post-vaccine results displayed positive T-cell immune responses in 68 out of 79 (86.1%) IMRD patients (82.3% of those without prior COVID-19). All HC and IMRDs patients had an antibody response against the SARS-CoV-2 receptor-binding domain; however, the HC response was significantly higher (median of 18,048 AU/mL) than in IMRDs patients (median of 6590.3 AU/mL, $p < 0.001$). MTX and leflunomide were associated with lower titers of IgG and IFN-γ responses. Among bDMARDs, adalimumab, etanercept, and guselkumab are associated with reduced cellular responses.
Conclusion: Our preliminary data show that the majority of our IMRD patients develop cellular and humoral responses after the SARS-CoV-2 booster vaccination, emphasizing the relevance of vaccination in this group. However, the magnitude of specific responses was dependent on the immunosuppressive therapy administered. Specific vaccination protocols and personalized decisions about boosters are essential for these patients.

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126 – P3.10.04

Assessment of lipid nanoparticle (LNP) uptake and its effects on immune cells in hepatocellular carcinoma (HCC)-burdened versus healthy mice

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Purpose: Initial *in vivo* testing of novel lipid nanoparticle (LNP)-based nano-vaccines e.g. for tumor treatment is usually performed in healthy wild type mice. However, considering their intended later application in tumor patients, the biodistribution of the LNPs and the biological activity of their cargo may differ on organ and single-cell level in tumor bearing- versus healthy individuals. For example, tumors establish an immunosuppressive tumor-microenvironment (TME) and induce immunoregulatory cell types, which imprint a tolerogenic status in immune cells. Here, we aimed to identify differences in LNP biodistribution and drug efficacy on organ and single-cell level in healthy versus hepatocellular carcinoma (HCC)-burdened mice.
Methods: HCC cells (Dt81Hepa1-6) were spleen-injected into C57BL/6JRj mice to induce liver tumors. Liver-targeting LNPs containing luciferase-encoding mRNA for *in vivo/ex vivo* detection of transfected cells or Cy5-labeled eGFP mRNA to monitor LNP uptake (Cy5) and eGFP expression on organ and single-cell level were generated using the NanoAssemblr platform. After intravenous LNP injection luciferase activity was monitored *in vivo* (IVIS) and luciferase activities and Cy5 intensities were assessed on organ level. Liver- and spleen- derived single-cell suspensions were subjected to flow cytometry to assess their state of activation. The responsiveness of liver non-parenchyma cells (NPCs) and spleen cells towards immunostimulatory agents was assessed after overnight incubationon by flow cytometry.
Results: Stable LNPs formulations of 100 nm in size with an mRNA encapsulation efficiency >90% were successfully developed. Intraveneously applied LNPs enriched in healthy and HCC-burdened mice in the liver. However, in healthy mice transgene expression occurred in various liver NPC types but in HCC-burdened mice predominantly in tumor-associated macrophages. Furthermore, liver NPC isolated from HCC-burdened mice were refractory to adjuvant stimulation. In a rescue experiment, addition of IL-10 neutralizing antibody overcame this unresponsive state.
Conclusion: LNP biodistribution differs on single-cell level in healthy and tumor-burdened mice, which needs to be considered for nano-vaccine design. Liver NPC from HCC-burdened mice are refractory to adjuvant treatment, yet co-applying agents that inhibit anti-inflammatory mediators and suitable adjuvants may restore responsiveness. Altogether, our study suggests that the efficacy of nano-formulations for therapeutic purposes needs to be tested in representative preclinical models.

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199 – P3.10.05

Mass cytometry unveils baseline and vaccine-induced cellular correlates of immune response variability to SARS-CoV-2 vaccination in the elderly

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Purpose: Vaccine response varies widely among individuals, particularly in vulnerable groups like immunocompromised and elderly, posing a significant concern. In recent years, the utilization of mass cytometry for immune profiling has unveiled inter-individual disparities in immune cellular composition as an important predictor of systemic response to diseases. However, there has been limited exploration into cellular immunity post-SARS-CoV-2 vaccination in the elderly. Therefore, in this study, we performed deep immune cell profiling of phenotype and function to identify cellular factors associated with vaccine responsiveness in low and high responders from a cohort of Norwegian older adults.
Methods: PBMCs obtained before and after second dose of a SARS-CoV-2 vaccine, were stimulated with either spike peptide, Cytostim, or left untreated for 22 hours. Post-stimulation, cells were stained with a cocktail of 40 antibodies targeting phenotypic markers and intracellular cytokines, then analyzed via mass cytometry. Cell type identification was accomplished through unsupervised clustering using FlowSOM, with resulting clusters visualized on UMAP-embedded space. To compare cluster abundances between groups, univariate negative binomial regression models were employed for cell counts of each cluster.
Results: We demonstrate a significant correlation between elevated baseline frequencies of $CD27^{+}IgD^{-}$ class-switched memory B cells and robust vaccine response. Additionally, our study identifies heightened levels of $CD27^{-}CD24^{+}CD38^{+}$ transitional B cells at baseline in individuals with strong vaccine responses. Post-vaccination, high responders had increased frequencies of $IFN\gamma^{+}CD4^{+}$ T cells following activation with spike peptide, coupled with a concurrent reduction in $CCR6^{+}$ Th17 cell subset frequencies. Furthermore, our findings suggest that enhanced vaccine response among the elderly is linked to the presence of a γδ T cell subset characterized by elevated expression of the inhibitory NKG2A receptor.
Conclusions: This study aimed to identify baseline differences in immune cell frequencies and function, alongside qualitative and quantitative changes after two vaccine doses. Utilizing unsupervised clustering and statistical analysis, we identified disparities in immune cell profiles before and after vaccination, linked to vaccine response. These insights provide a valuable guide for refining vaccination strategies for the elderly.

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397 – P3.10.06

The safety, early lung transcriptional responses and efficacy of differently adjuvanted subunit tuberculosis vaccines after intrapulmonary delivery

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Tuberculosis causes approximately 1.3 million deaths annually, and the only vaccine available provides incomplete protection. Mucosal vaccination elicits memory immune responses at the site of infection that respond rapidly to pathogen encounter, making it a promising strategy to achieve optimal tuberculosis vaccine efficacy. Currently, a lack of understanding of adjuvant activity in the respiratory tract hinders the development of mucosal subunit vaccines. We have previously shown that intratracheal (IT) administration of Advax™, a polysaccharide adjuvant, combined with the subunit protein CysVac2, is protective against *Mycobacterium tuberculosis*. Here, we compared CysVac2 with Advax or alum and MPLA to model the clinically approved adjuvant AS04™. IT immunisation of CysVac2 with either adjuvant generated lung Th17 cells and was protective against *M. tuberculosis* challenge. Using lung function measurement techniques, we found there was no significant change in lung function at 7 days or 6 weeks after a full schedule of IT vaccinations, demonstrating the safety of these vaccines.
Whole-lung transcriptional responses to IT immunisation with CysVac2 vaccines were also analysed 4 hours and 7 days after delivery using the Nanostring™ system. At 4 hours post-immunisation, both adjuvants generated a shared immune signature of both pro-inflammatory and immunoregulatory genes, and at 7 days both upregulated genes associated with adaptive immune responses. Interestingly, IT immunisation with CysVac2 and alum/MPLA stimulated inflammasome and type I IFN signalling-associated genes. Contrastingly, IT CysVac2/Advax promoted local upregulation of genes associated with TREM-2 receptor signalling and pulmonary opsonins. Using mice deficient for the T-cell homing receptor CXCR3 we showed that IT CysVac2/AlumMPLA, but not CysVac2/Advax, was partially dependent on CXCR3 for vaccine-specific T cell and total Th17 cell recruitment to the lungs.
In conclusion, our findings confirmed that IT-delivered protein/adjuvant vaccines are safe using novel lung function testing techniques. While alum/MPLA stimulated upregulation of the type I IFN pathway, Advax generated comparable immune responses via non-conventional immune pathways. Ultimately however, both adjuvants generated lung Th17 cells that correlated with protection against *M. tuberculosis* infection.

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540 – P3.10.07

Boosting Cell-Mediated Immunity to Burkholderia pseudomallei Outer Membrane Protein Antigen with Cationic Nanoparticles

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Meliodosis, caused by the bacterium *Burkholderia pseudomallei*, is a severe and often fatal infectious disease that is prevalent in tropical regions but is expanding globally due to the rise of anti-microbial resistance. Given its high mortality rate and the challenges associated with diagnosis and treatment, effective vaccines are urgently required. Studies in mice have shown that IFNγ secreting CD4 T cells in mice are critical for protection. We have previously demonstrated the ability of small (50-60nm) biodegradable polymer nanoparticles to enhance antigen-specific cell-mediated immunity. In this study, we evaluated the potential of cationic (DOTAP stabilised) or anionic (no surfactant) poly(lactic-co-glycolic acid) (PLGA) and poly (lactic acid) (PLA) nanoparticles with varying lactide:glycolide ratios as adjuvants when combined with the antigen BpOmpW, a *B.pseudomallei* protein identified by McClean & colleagues as a potential adhesin. Following intramuscular vaccination, BpOmpW-specific T cell frequencies and cytokine secretion was assessed by intracellular staining, while IFNγ, and IL-17 secretion responses were evaluated through ex vivo Fluorospot and ELISA on splenocyte supernatants. Intramuscular vaccination with BpOmpW and cationic PLGA or cationic PLA nanoparticles significantly enhanced the induction of antigen specific IFNγ+ CD4+ T cell response and IgG titres compared to vaccination with antigen alone. In contrast, vaccination with antigen and DOTAP-free nanoparticles induced weaker T cell and antibody responses. These data highlight the potential of cationic PLGA NPs as adjuvants to boost BpOmpW specific cellular immunity and antibody responses and support further evaluation of cationic NP as adjuvants in subunit vaccines against *Burkholderia pseudomallei.*

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565 – P3.10.08

SphB1 is a potential antigen for inclusion in a pertussis mRNA vaccine to prevent nasal infection with Bordetella pertussis and overcome the challenge of emerging PRN-negative strains

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Current acellular pertussis (aP) vaccines protect against whooping cough but fail to prevent nasal carriage and transmission of *B. pertussis* (*Bp*). Pertactin (PRN)-deficient *Bp* strains have emerged in countries using aP vaccines. Here we examined the protective efficacy of mRNA-based pertussis vaccines in a mouse model and assessed the role of PRN and the antigen autotransporter subtilisin-like protease (SphB1).
Mice were immunized (i.m.) twice, aerosol-challenged with *Bp*, and CFUs were assessed in lung and nasal tissue. Preliminary data suggest monovalent mRNA pertussis vaccines encoding SphB1 or PRN protected against lung infection and significantly reduced CFUs in the nose. In contrast, a commercial aP vaccine (Adacel) or an mRNA vaccine encoding filamentous hemagglutinin (FHA), pertussis toxin (PT) and fimbrae (FIM) failed to prevent nasal infection. Addition of PRN or SphB1 enhanced protection in the nose and addition of both PRN and SphB1 conferred the highest level of protection against nasal infection.
Assessment of immunogenicity of the pertussis mRNA vaccines demonstrated that they induced potent IgG2c in nasal homogenates and IFN-γ-secreting T cells (Th1 cells) in the spleen. While SphB1 was not a major target for T cells, addition of SphB1 to the vaccine significantly increased FHA- and PRN-specific IFN-γ production assessed by ELISA and flow cytometry.
These studies demonstrate the potential of mRNA-based vaccines in protecting against *Bp* infection of the lung and nose and identified a promising novel autotransporter for inclusion in a pertussis mRNA vaccine to prevent nasal colonization and to address the emergence of mutant strains of *Bp*.

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593 – P3.10.09

A third Mumps-Measles-Rubella immunization elicits mumps-specific cellular responses in young adults with distinct responses in immune subgroups

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Background: Despite vaccination, mumps outbreaks occur worldwide in young adults indicating waning of vaccine-induced responses. A third Mumps-Measles-Rubella (MMR3) booster immunization may be a solution to increase the level of protection for this specific risk group. We previously demonstrated that such a third MMR immunization could indeed significantly boost mumps neutralizing antibodies but cellular responses remained unexplored.
Objectives: In this study, mumps-specific T and B cell responses were unraveled four weeks and 1 year following a MMR3 immunization in thirty young adults (18-25 years). These responses were compared to Mumps infection-induced responses 1-2 and 7-10 months post infection. Secondarily, correlations were investigated among antigen-specific responses and to *ex vivo* frequencies of cell subsets of the innate, T and B cell compartments.
Methods: *Ex vivo* phenotyping on peripheral blood mononuclear cells (PBMCs) was done using high-dimensional flow cytometry. Mumps-specific T and B cell responses were investigated by measuring activation-induced marker expression (AIM) and by ELISpot assay, respectively.
Results: *Ex vivo* immune cell phenotyping established insight in kinetics of innate, T and B cell responses. MMR3 immunization significantly induced activated mumps-specific $CD4^+$, but not $CD8^+$, T cell responses characterized by expression of one or more activation markers (CD137, CD154, OX40, CD69). Additionally, enhanced mump-specific B cells were observed 1 month post-immunization. Mumps infection, as compared to immunization, induced significantly higher antibody, $CD4^+$ T cell and B cell responses and in addition provided $CD8^+$ T cell responses. While B cell responses correlated positively to IgG and virus neutralization levels, these responses did not correlate to $CD4^+$ T cell responses. Finally, based on pre-existing immunity and kinetics of mumps-specific responses following MMR3 vaccination, participants could be divided in distinct immune subgroups. These subgroups were characterized by different responses to MMR3 immunization with distinct correlations among mumps-specific responses and *ex vivo* immune cell subsets.
Conclusion: A third MMR immunization potentially increases protection of young adults against mumps infections as it provides strong antibody responses and significant mumps-specific T and B cell responses albeit with clear distinct responses in subgroups.

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774 – P3.10.10

Age differentially impacts SARS-CoV-2 spike-specific adaptive immune responses induced by adenoviral versus mRNA vaccines

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Purpose: Despite older subjects are more at risk of severe COVID-19, especially few months after vaccination, at which extent age and the type of vaccine affect the maintenance of antibody and cellular responses has not been addressed. By this study, we observed spike-specific immune responses elicited by vaccination with two doses of BNT162b2 or ChAdOx1-S and subsequently boosted with a single dose of BNT162b2 or mRNA-1273.
Materials and Method: We enrolled healthy donors with no known history of SARS-CoV-2 infection (n=230) at 6 months on average after the second dose of homologous immunization: 128 subjects were vaccinated with two doses of BNT162b2 vaccine, and 92 subjects were vaccinated with two doses of ChAdOx1-S vaccine. Ninety-nine participants were followed up, and 43 newly enrolled volunteers were recruited, to provide a blood sample at 6 months on average after a mRNA booster dose. The donors were stratified by age as young (*Y*, 18- 40 years), middle-aged (*M*, 41- 65 years), or old (*O*, >65 years).
We evaluated the titers of SARS-CoV-2-spike RBD-specific IgG, and we analyzed the *ex vivo* response with the Human IFNγ ELISpot, the production of cytokines (IFNγ, TNF, CD107, and CD154) in Intracellular Cytokines Staining and the spike-specific expansion and functionality in Tetramer Staining after expanded upon stimulation for 10 days.
Results: Primary vaccination with BNT162b2 ensures better maintenance of spike-specific antibodies than ChAdOx1 vaccination, while both vaccines induce similar memory T cell responses. The booster dose markedly improves the maintenance of both humoral and cellular responses, especially in subjects previously vaccinated with ChAdOx1-S. Aging profoundly affects long-term humoral responses, while the effects on cellular responses are less pronounced, mainly affecting the $CD4^+$ compartment in BNT162b2-recipients. Overall, higher T cell responses are observed in elderly subjects after primary vaccination with ChAdOx1-S and a booster mRNA dose, compared to those vaccinated with 3 mRNA vaccine doses.
Conclusion: In summary, our findings indicate that spike-specific cellular responses elicited by various vaccine formulations persist for up to 6 months post-final vaccination. However, we showed pronounced decline of adaptive responses in older individuals who received mRNA, but not vector vaccines.

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802 – P3.10.11

Using Human Lymph Node Culture Models to Characterize T cell Activation Dynamics and Early Immune Responses to Vaccines

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Purpose: Vaccination has long been an effective strategy in reducing morbidity and mortality by safely inducing the formation of immunological memory. T cell activation in memory-forming sites such as the lymph node (LN) is critical in initiating adaptive immunity which facilitates vaccination and ultimately generates a protective immune response. However, early immune responses induced by vaccines are not well characterized in human tissue. We previously utilized our collaborator's well established human tissue resource and demonstrated that T cells from blood, lymphoid, and mucosal sites exhibit conserved transcriptional states during activation in CD4+ cells. However, these T cells were isolated from dissociated tissue, depleting the spatial and signaling contributions of the tissue microenvironment.
Methods: To preserve these effects, we developed a method for culturing intact human LN slices. This culture system overcomes the limitations of liquid culture by preserving tissue architecture, local signaling, and maintaining physiological cell ratios. We utilized α-CD3/28 antibody tetramers, superantigen mimicking antibodies, and vaccines to stimulate both conventional liquid and slice cultures of human LNs up to 24 hours and utilized CITE-seq and flow cytometry to generate datasets.
Results/Conclusions: Stimulating slice and corresponding liquid culture with α-CD3/28 antibody tetramers yield distinct activation patterns and resting cell states with CITE-seq analysis. Antigen independent stimulation in liquid culture produces more robust activation compared to slice culture. However, α-CD3/28 perturbation bypasses physiological cell-cell interactions, while antigen dependent stimulation like superantigens facilitate MHCII/TCR binding and may benefit from the intact microenvironment in slice culture. With superantigen mimicking antibody we observed a discrete T cell metabolic intermediate state and a diverging activation trajectory in slice and liquid culture compared to α-CD3/28 stimulation. The establishment of this robust human LN platform enabled us to assess early immune responses induced by vaccines with single-cell genomics. By profiling these initial cell states, we hope to elucidate mechanisms or signatures which produce long-lasting, durable memory responses to vaccination. Using the methods described above, we have compared LN cultures perturbed with the Measles-Mumps-Rubella (MMR) and COVID-19 mRNA vaccines, revealing dose-responsive and vaccine-specific gene signatures across immune cell subsets.

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867 – P3.10.12

Effectiveness, immunogenicity and safety of the novel DS-5670 vaccine platform against SARS-CoV-2: evidence from two randomized clinical trials

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Importance: DS-5670 is a vaccine platform comprising the mRNA antigen derived from the spike protein receptor-binding domain (RBD) from SARS-CoV-2 encapsulated in lipid nanoparticles.
Objective To examine the immunogenicity and safety of monovalent DS-5670a (original strain RBD; Study 146) or bivalent DS-5670a/b (original strain and omicron BA.4-5 RBDs; Study 212) as boosters and comparator (BNT162b2 or mRNA-1273)
Design: Randomized, observer-blinded, active-comparator, non-inferiority studies. Enrollment began on January 31, 2022 (Study 146) and May 19, 2023 (Study 212) with 52-week observation and follow-up periods for both studies.
Participants: Study 146: adults (≥18 years) who had completed primary vaccination with monovalent BNT162b2 or mRNA-1273 against the original strain. Study 212: individuals (≥12 years) who had received primary and booster vaccinations with BNT162b2, with the last dose being the bivalent original/BA.4-5 composition.
Main Outcomes and Measures: Study 146: the primary endpoint was geometric mean fold-rise (GMFR) in serum neutralization titers against SARS-CoV-2 (original strain) at day 29; secondary endpoints were geometric mean titer (GMT) of anti-SARS-CoV-2 antibody, and COVID-19 incidence over time. Study 212: co-primary endpoints were GMT and seroresponse rate of anti-SARS-CoV-2 (omicron BA.5 strain) serum neutralization titers at day 29.
Results: Both studies exceeded non-inferiority margins. Study 146 (n=4518): adjusted GMFR ratios for DS-5670a were 1.464 (97.5% CI, 1.112 to 1.927) vs BNT162b2 and 1.772 (97.5% CI, 1.335 to 2.353) vs mRNA-1273. DS-5670a provided high serum neutralization titers and effectively prevented symptomatic COVID-19. Study 212 (n=701): the adjusted GMT ratio of DS-5670a/b to bivalent BNT162b2 was 1.712 (95% CI, 1.509 to 1.944), and the between-group difference in seroresponse was 21.4% (95% CI, 13.8 to 28.6). There were no serious AEs associated with DS-5670 in either study.
Conclusions and Relevance: Monovalent and bivalent compositions of DS-5670 were effective against symptomatic COVID-19 and well-tolerated, with broad neutralization activity across omicron sub-lineages. This platform can be utilized to produce new vaccines against future SARS-CoV-2 variants.

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868 – P3.10.13

Effectiveness, Immunogenicity and Safety of the Novel DS-5670 Vaccine Platform Against SARS-CoV-2: Evidence From Two Randomized Clinical Trials

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Importance: DS-5670 is a vaccine platform comprising the mRNA antigen derived from the spike protein receptor-binding domain (RBD) from SARS-CoV-2 encapsulated in lipid nanoparticles.
Objective: To examine the immunogenicity and safety of monovalent DS-5670a (original strain RBD; Study 146) or bivalent DS-5670a/b (original strain and omicron BA.4-5 RBDs; Study 212) as boosters.
Design: Randomized, observer-blinded, active-comparator, non-inferiority studies. Enrollment began on January 31, 2022 (Study 146) and May 19, 2023 (Study 212) with 52-week observation and follow-up periods for both studies.
Setting: Hospitals and clinics across Japan.
Participants: Study 146: adults (≥18 years) who had completed primary vaccination with monovalent BNT162b2 or mRNA-1273 against the original strain. Study 212: individuals (≥12 years) who had received primary and booster vaccinations with BNT162b2, with the last dose being the bivalent original/BA.4-5 composition.
Interventions: Study 146: monovalent booster dose of DS-5670a or comparator (BNT162b2 or mRNA-1273). Study 212: bivalent booster dose of DS-5670a/b or BNT162b2.
Main Outcomes and Measures: Study 146: the primary endpoint was geometric mean fold-rise (GMFR) in serum neutralization titers against SARS-CoV-2 (original strain) at day 29; secondary endpoints were geometric mean titer (GMT) of anti-SARS-CoV-2 antibody, and COVID-19 incidence over time. Study 212: co-primary endpoints were GMT and seroresponse rate of anti-SARS-CoV-2 (omicron BA.5 strain) serum neutralization titers at day 29.
Results: Both studies exceeded non-inferiority margins. Study 146 (n=4518): adjusted GMFR ratios for DS-5670a were 1.464 (97.5% CI, 1.112 to 1.927) vs BNT162b2 and 1.772 (97.5% CI, 1.335 to 2.353) vs mRNA-1273. DS-5670a provided high serum neutralization titers and effectively prevented symptomatic COVID-19. Study 212 (n=701): the adjusted GMT ratio of DS-5670a/b to bivalent BNT162b2 was 1.712 (95% CI, 1.509 to 1.944), and the between-group difference in seroresponse was 21.4% (95% CI, 13.8 to 28.6). There were no serious AEs associated with DS-5670 in either study.
Conclusions and Relevance: Monovalent and bivalent compositions of DS-5670 were effective against symptomatic COVID-19 and well-tolerated, with broad neutralization activity across omicron sub-lineages. This platform can be utilized to produce new vaccines against future SARS-CoV-2 variants.

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958 – P3.10.14

Clonotypic analysis of T cell responses reveals long-term vaccine efficacy

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As a fundamental component of the immune system, adaptive immunity is crucial in defending against viral infections. Cellular immunity works in controlling the infection as well as promoting humoral immunity; however, antigen-specific T cells were less investigated and less considered in the designing of SARS-CoV-2 vaccines. To characterize the T cell responses in SARS-CoV-2 vaccinees on a clonotype level, we traced the dynamics of spike (S)-specific T cells before and after the vaccination, and determined their epitopes and restricting human leukocyte antigen (HLA) alleles. We observed that the dominant follicular helper T (Tfh) clonotypes induced by vaccination were associated with the diverse humoral immune response, and can be used as an indicator of antigen-specific antibody longevity. In contrast, pre-existing S-responsive T cells were contracted post-vaccination and thus were unlikely to contribute to the protection established by vaccination. These findings suggest the pivotal role of T cell immunity in defending against SARS-CoV-2 infection, and could be helpful for the vaccine design in the future.

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1071 – P3.10.15

Non-waning immunity and protection upon a single immunization with a self-boosting vectored vaccine

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Background: Cytomegaloviruses (CMV) elicit a lasting immune response with remarkably large populations of antigen-specific $CD8^+$ T cells for life. Therefore, CMV has been proposed and investigated as a novel vaccine vector. Strict species specificity of CMV has compelled vaccine developers to use HCMV in clinical trials. We propose that non-human CMVs used in cross-species settings may overcome several limitations of HCMV vectors and be superior in terms of safety and immunogenicity. On one hand, such constructs are naturally unable to replicate in human cells, omitting the need to generate recombinant single-cycle CMV vectors grown on complementing cell lines. On the other, immune evasion genes are host-specific and do not affect responses in species discordant settings. A recombinant murine CMV (MCMV) vaccine vector expressing the spike protein of SARS-CoV-2 ($MCMV^S$) showed a robust and long-lasting protection against distinct SARS-CoV-2 variants in the mouse model. We show in this study the immunogenicity and efficacy of our MCMV-based vector vaccine in the non-cognate hamster host.
Methods: We immunized hamsters with $MCMV^S$ and longitudinally quantified humoral and cellular immunogenicity following different immunization routes. At ten months post immunization, immunized hamsters were challenged with SARS-CoV-2 including the Omicron BA.5 variant.
Results: Hamsters tolerated $MCMV^S$ well. They developed neutralizing antibodies and antigen-specific T cell responses which expanded in size and breadth over time. Comparing intramuscular, subcutaneous, intranasal and intraperitoneal application routes, we found that robust humoral and cellular immune responses to spike antigens were elicited after all infection routes, but in particular after intramuscular injection. All immunized animals fully controlled productive SARS-CoV-2 infection upon challenge with the vaccine seed strain or with the antigenically distinct BA.5 variant at ten months post-immunization.
Conclusion: Our data suggest that a single-dose vaccination with an MCMV-based vector vaccine elicits exceptionally long-lasting and broad protection in a non-cognate host species. Hence, this technology might be a promising platform for the development of broadly reactive and long-lasting immune responses and has the potential for use in clinical settings.

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1177 – P3.10.16

Micronutrient supplementation supports immune response to seasonal influenza vaccine in mice

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Purpose: Although nutritional gaps are prevalent in several micronutrients reported to support immune function, the significance of their deficits/supplementation for efficacy of vaccines, particularly those with low efficacy, as it is seasonal influenza vaccine, has not been fully investigated, yet. The present study examined influence of supplementation combining vitamins C and D, oligoelements zinc, selenium and manganese, and N-acetyl-cysteine on germinal centre (GC) and serum IgG responses to seasonal quadrivalent influenza vaccine (QIV) in mice.

Methods: Study encompas female BALB/c mice that were given QIV in two doses (28 days apart). The supplementation started five days before the first injection. Phenotypic and functional characteristics of cells from secondary lymphoid organs (SLOs) (draining lymph nodes and spleens) were analyzed by flow cytometry, whereas serum IgG response to QIV and levels of cytokines from SLO cell cultures were determined by ELISA. Redox status parameters were evaluated in spleens by spectrophotometric methods.

Results: The supplementation increased the magnitude of serum IgG response 28 days post the first QIV dose, through stimulation of GC reaction in SLOs, as indicated by increase in the frequency of GC B cells and follicular CD4+ T helper (Th) cells, and Th cell IL-21 production. Additionally, 14 days following the second QIV dose the supplementation supported more favorable (viz. more effective in the context of virus infection clearance) IgG2a response through favoring Th1 response. This could be ascribed not only to its indirect action related to antioxidant properties (confirmed by redox status analyses), but also to direct action on Th cell differentiation, as indicating by the ratio in production levels of Th1 (INF-γ) /Th2 (IL-4) signature cytokine ratio upon QIV restimulation in SLO cell cultures.
Conclusion: Thus, the study forms solid base for further studies aimed at repurposing use this safe and inexpensive micronutrient preparation as an adjuvant for virus influenza vaccine and possible some other virus vaccines.

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1180 – P3.10.17

BpOmpW antigen administered with CAF01 adjuvant stimulates comparable T cell responses to Sigma adjuvant system

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Purpose: There are no licensed vaccines to protect vulnerable populations from the potentially fatal tropical infection, melioidosis, despite its causative agent, Burkholderia pseudomallei, being endemic in tropical and subtropical regions. A promising vaccine candidate, BpOmpW protected mice from melioidosis infection for up to 81 days and stimulated robust interferon-gamma responses in $CD4^+$, $CD8^+$, NK and NKT cells. In order to progress to human studies, the selection of an adjuvant as CAF01 with an acceptable human safety profile that stimulates appropriate correlates of protection is essential.
Methods: The T-cell responses were characterised by flow cytometry using a 15-multicolour antibody panel after re-stimulating splenocytes from BpOmpW-immunized mice. Cytexpert and Cytobank software were used for the analysis of the cytometry data.
Results: Here we demonstrate that the CAF01 vaccine adjuvant elicits optimal immune correlates of protection when administered with our BpOmpW vaccine. Specifically, we demonstrate that CAF01 administered with BpOmpW elicits robust Th1 responses, with potent IFN-γ responses in $CD4^+$ and $CD8^+$ T cells and NKT cells, in addition to Th17 and Th2 responses comparable to Sigma Adjuvant System
Conclusion: This formulation BpOmpW/CAF01 will be particularly effective in protecting susceptible populations including people with type 2 diabetes from melioidosis.

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1248 – P3.10.18

Development of a new selection model for selection vaccine candidates against multidrug resistant *Staphylococcus aureus*

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Staphylococcus aureus is a bacterium of great interest being responsible for high levels of hospital and community acquired infections, it is responsible for the rapid increasing of multidrug resistant bacteria. The infections are initiated by the host's asymptomatic colonization, where this bacterium forms biofilms, a complex structure composed by bacteria and extracellular matrix, from biofilms, the bacterium can spread to other tissues causing several diseases. A recent report from WHO pointed that *S. aureus* is one of the most important bacteria for the development of prophylactic and therapeutics strategies. Even tough several vaccine candidates have been proposed, until now there is no vaccine approved against these pathogen. Therefore, we propose the development of a new selection and validation model for vaccine candidates against *S. aureus*, our model combines bioinformatic, transcriptomic and proteomic analysis of the bacteria in two stages: from colonizing biofilms and biofilm-dispersed, virulent bacteria. For that, we used a cell substrate biofilm formation protocol, which allow the bacterium to form biofilm in a physiological model using lung epithelial cells for 48 h at 34ºC and 5% CO2 (simulating the nasopharynx condition), after this time, the biofilm was treated for 3 h at 38.5ºC (simulating fever), as control a 34ºC treated plate was also prepared. After the 3 h, the bacterium was plated on blood agar plates for CFU count, a scanning electron microscopy (SEM) was also performed to evaluate biofilm formation.: The results showed that fever simulation works triggering the bacterial dispersion form biofilms, two S. aureus strains were tested and for both the heat treatment provided 4 times more dispersion in relation to the control, SEM showed that the cells formed viable, well-structured biofilms, the samples were prepared for transcriptomic and proteomic analysis. As conclusion, we were able to develop a protocol that simulates a real curse of infection by S. aureus trigged by fever, this result will be used to analyze the bacterium profile during colonization and infection phases.

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1258 – P3.10.19

Evaluation of fimbrial subunits as vaccine candidates against Klebsiella pneumoniae infection in a mouse model of pneumonia

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Klebsiella pneumoniae infections pose a great burden worldwide, causing high morbidity and mortality, which is worsened by the increase in multidrug resistant strains. New therapeutic/prophylactic strategies are urgently needed to overcome antibiotic resistance and reduce the health and economic impacts of diseases caused by this pathogen. Fimbriae are important virulence factors involved in biofilm formation and adhesion to host cells. Their exposed location and conservation among clinical isolates make them interesting candidates for inclusion in protein-based vaccines. Therefore, the present work investigated the immunological potential type 1 and 3 fimbriae subunits in a murine model of *K. pneumoniae* lung infection. The genes encoding fimbriae subunits FimA and MrkA were cloned in procaryotic vector systems, expressed in *Escherichia coli* and purified by affinity chromatography. Subcutaneous immunization with the recombinant proteins using Alum as adjuvant induced specific IgG production, mainly of the IgG1 subclass; the antibodies efficiently recognized the native proteins at the bacterial surface and reduced biofilm formation *in vitro*. Furthermore, mice vaccinated with the co administered proteins, but not the individual antigens, showed reduced bacterial loads in the lungs after intranasal challenge with a virulent *K. pneumoniae* clinical strain. Overall, the results suggest that both type I and type III fimbriae subunits contribute to protection against *K. pneumoniae* lung infection, possibly by inducing antibodies that can bind to the bacteria and favor clearance by the host.

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European Journal of Immunology Clinical-Basic-Translational

1376 – P3.10.20

Can Lacticaseibacillus rhamnosus modulate the immune response to inactivated Influenza vaccine in young and old rats?

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Purpose: The senescence of the immune system bears the burden of poor response to vaccination. The Influenza virus is a common etiological factor that humans encounter every year. The elderly are at greater risk of undesirable outcomes, even when vaccinated, since the vaccine response rate is 45-65%. To address the poor immunological response to vaccination in the elderly, we sought a non-invasive and comfortable "adjuvant" in the form of probiotic Lacticaseibacillus rhamnosus that could boost the immune response and affect the vaccination outcomes.
Methods: Young (3) and old (24 months) Dark Agouti female rats were set into four experimental groups (n=6). One from each group of young and old rats received L.rhamnosus supplemented to the water (~$1x10^9$ CFU per day) 7 days before the vaccination and during the next 3 weeks after it (L.rhamnosus+vaccinated). The control groups drank tap water *ad libitum* (control+vaccinated). All 4 groups were vaccinated with the seasonal Influenza vaccine (inactivated, fragmented virus). Three weeks after the vaccination, the experiment was ended and the thymuses and blood were retrieved for flow cytometry, qPCR, and ELISA analysis.
Results: Our results indicated that supplementation with L.rhamnosus induced a significant increase ($p<0.05$) in the frequency of Foxp3+CD25+CD4+TCRαβ+ cells in the blood of young, but not old vaccinated rats. Also, their thymuses were significantly ($p<0.05$) larger compared to the control vaccinated group of the same age. Following this was the significantly lower ($p<0.05$) frequency of memory CD90-CD45RC-CD4+ T cells in the blood of the young L.rhamnosus+vaccinated group and CD28nullCD8+ T cells ($p<0.05$) in both young and old L.rhamnosus+vaccinated groups compared to the control group. Interestingly, the concentration of proinflammatory IFNγ was significantly lower ($p<0.01$) in sera retrieved from both young and old vaccinated rats that received L.rhamnosus supplementation.
Conclusion: The immunomodulatory effect of L.rhamnosus is complex and affects both primary and secondary lymphoid systems. Our preliminary results suggest that L.rhamnosus supplementation: can be beneficial to vaccination outcomes for both young and old but is guided by different mechanisms; it could potentially affect slower thymus involution and take part in postponing the immunosenescence. Future experiments should provide us with additional answers.

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1387 – P3.10.21

The IL33/ST2 axis negatively regulates vaccine adjuvant induced cell-mediated immunity in a sex-dependent manner

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Introduction and Purpose: Cell mediated immune responses, particularly antigen specific $CD8^+$ T cells and Th1 cells are vital in prophylactic and therapeutic interventions for cancer and intracellular pathogens. It has been demonstrated that IL-33 is a negative regulator of vaccine-induced antigen-specific cellular immunity in mice, however, the role of biological sex in those immune responses has not been addressed. Therefore, the main purpose of this work is to elucidate the impact of biological sex on IL33/ST2 axis regulation of vaccine induced cell-mediated immune responses.**Methods:** Female and male C57BL/6 mice were vaccinated on days 1 and 14 in both leg flanks with ovalbumin and 50nm polymeric nanoparticles as adjuvant. Wild type mice (WT) and mice lacking the cytokine IL-33 (RD8) or its receptor ST2 (ST2) were employed. Antigen-specific $CD8^+$ and $CD4^+$ T cell responses were determined by tetramer staining and intracellular cytokine staining and secretion of IFN-γ by restimulated splenocytes was assessed by ELISA. Antigen-specific serum antibody titres were determined by ELISA. **Results:** Vaccination with antigen (ovalbumin) and adjuvant nanoparticles enhanced antigen-specific $CD8^+$ and $CD4^+$ T cell responses compared to vaccination with antigen alone. These responses were higher in female than male mice. Vaccine induced T cell responses were negatively regulated by IL-33 and ST2 but this effect was most marked in female mice. Specifically, the number of antigen specific $CD8^+$ T cells assessed by tetramer staining and percentage of antigen specific IFN-γ producing CD4 and CD8 cells was elevated in female IL-33 and ST2 deficient female mice. Vaccine induced antigen-specific antibody responses were also higher in female than in male mice, but this effect was independent of the IL-33/ST2 axis.**Conclusion:** IL33 and ST2 negatively regulated the ability of adjuvant nanoparticles to promote antigen specific T cell responses. The adjuvant more effectively induced T cell and antibody responses in females than in males and the immunomodulatory effect of IL-33 and ST2 was most marked in females. This indicates that biological sex should be considered when pursuing immunomodulatory strategies concerning the IL-33/ST2 axis.

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1451 – P3.10.22

Heterologous Bacillus Calmette-Guérin infection reverts the monocytic myeloid-derived suppressor cell phenotype induced by prior Mycobacterium tuberculosis immunization into innate and adaptive effector cell responses.

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Tuberculosis remains one of the deadliest infectious diseases worldwide. Despite the heterologous BCG vaccine, which shows a limited protection, a *Mycobacterium tuberculosis* (Mtb)-based homologous vaccine is not available to date. We found earlier that double immunizations with heat-killed Mtb massively induced monocytic myeloid-derived suppressor cells (M-MDSC) in mice. Our new results indicated that induction of M-MDSC is not an exclusive feature of Mtb, but Mtb appears quantitatively superior over other bacteria, including *Mycobacterium bovis, Mycobacterium smegmatis, Listeria monocytogenes*. In contrast, BCG vaccination did not generate M-MDSC or other myeloid cell types under any condition tested. We also investigated how Mtb and BCG vaccines affect subsequent live BCG infection in mice. Infection of Mtb-immunized mice with heterologous BCG resulted in increased effector neutrophil, macrophage, and DC generation specially in the lung, despite elevated M-MDSC frequencies. Thus, the balance between suppressive and effector immune responses tipped towards increased DC maturation and T cell activation, leading to a protective phenotype with lower bacterial loads. Remarkably, BCG-immunized mice infected with homologous BCG showed higher overall bacterial loads, with no induction of effector cell frequency and function. These results offer new insights into the relationship between M-MDSC induction, T cell response, and protection from disease following homologous versus heterologous challenges. Our findings may impact Mtb-based TB vaccine development to achieve a robust protection against Mtb.

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1506 – P3.10.23

Immune stimulating properties of lipid nanoparticle formulations containing a polyethylene-glycol alternative

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Purpose: Lipid nanoparticles (LNPs) are an established platform for mRNA delivery. Currently approved LNPs comprise four components: an ionizable lipid, cholesterol, a phospholipid, and a polyethylene-glycol (PEG)-conjugated lipid. PEG is a key component since it prevents aggregation and ensures particle stability but also influences LNP behaviors in biological systems. There is evidence that PEG is immunogenic and contributes to rare adverse reactions to mRNA-LNP vaccines. PEG-specific antibodies can accelerate blood clearance of nanomaterials. Thus, we explored the hydrophilic polymer Poly(2-methyl-2-oxazoline) (PMOZ) as an alternative to PEG lipids in mRNA therapeutics.
Methods: SM-102-based LNPs containing different percentages of PMOZ were prepared using the microfluidics mixing technique, and physicochemical characteristics were evaluated by dynamic light scattering. Transfection efficiency was evaluated in different cell lines and primary human PBMCs by quantifying GFP expression by flow cytometry. The immunostimulatory profile of the LNPs was determined by evaluating expression of co-stimulation and activation markers by means of flow cytometry and by quantification of cytokines and chemokines in cell culture supernatants of PBMCs using a cytokine bead array. Toxicity was evaluated by staining cells with a viability dye. Immune stimulatory properties *in vivo* were addressed in mice after intramuscular injection.
Results: Cells exposed to LNPs with higher amounts of PMOZ responded with reduced percentage of GFP expressing cells and reduced upregulation of surface CD80, CD83 or CD86 on innate immune cells than cells exposed to LNPs with lower amounts of PMOZ. Furthermore, there was an inverse correlation of percentages of PMOZ in LNPs and induced cytokine and chemokine responses in supernatants of transfected PBMCs. The stronger immunostimulatory properties of LNPs containing lower percentages of PMOZ was also observed *in vivo* in LNP-injected mice.
Conclusion: PMOZ shows the potential to be used in LNPs for mRNA vaccine delivery as an alternative to PEG. Transfection efficiency and immunostimulatory potential of LNPs can be modulated by altering the amount of PMOZ.

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1522 – P3.10.24

Delivering SARS-CoV-2 mutated RBD antigens to the CD40 receptor: A strategy for inducing long-term antibody responses

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Purpose: The rapid availability of SARS-CoV-2 vaccines was pivotal during the COVID-19 pandemic. However, the emergence of variants (VOCs/VOIs) evading antibody (Ab) neutralization has reduced vaccine efficacy. Urgent needs are to develop new vaccines/strategies: i) targeting VOCs/VOIs; ii) inducing long-lasting Ab responses.
Methods: CD40.Pan.CoV is a Dendritic Cell (DC) targeting vaccine consisting of a mAb specific to human (h)CD40 fused with RBD harbouring K417N, L452R, T478K, E484Q, N501Y mutations common to VOCs and a Nucleocapsid sequence, (>95% conserved across Sarbecoviruses). Immunogenicity and viral protection were tested in hCD40 and hCD40/K18-hACE2 transgenic mice, respectively. Animals were immunized at days 0/21 with the CD40.Pan.CoV vaccine (10µg) plus poly-ICLC (50µg, i.p.) or the mRNA BNT162b2 vaccine (1µg, i.m.) and either sacrificed (n=5-10/Group) 14 days post-boost or infected (Wuhan strain; n=6-11/Group) 7 days post-boost. Control mice received poly-ICLC or PBS. In another set of experiments, mice primed with two doses of BNT162b2 were boosted at month (M) 8 with either BNT162b2 or CD40.Pan.CoV+ poly-ICLC (n=4-5/Group) and serum was collected monthly till M16. The levels of binding and neutralizing RBD-IgG were assessed with MSD tests. Splenic B cells were analysed by flow cytometry.
Results: The rate of protection reached 100% in CD40.Pan.CoV and BNT162b2 vaccine groups with no animals exhibiting clinical symptoms or viral replication in the lungs unlike mock animals. CD40.Pan.CoV induced IgG-specific RBD responses as potent as the BNT162b2 vaccine but with a broader range of cross-reactivity and neutralization against δ/ε/γ/κ VOC and higher frequency of germinal centre B cells. In BNT162b2-pre-immunized animals, BNT162b2 or CD40.Pan.CoV boost (M8) increased IgG-specific RBD responses. However, post-boost dynamics revealed different decreasing slopes of binding and neutralizing Ab responses with CD40.Pan.CoV maintaining long-lasting responses.
Conclusion: Our study presents the proof of concept of the efficacy and immunogenicity of a vaccine targeting a mutated-RBD and a conserved nucleocapsid region (Npep) to the CD40 receptor. The CD40.Pan.CoV vaccine induced humoral and B cell responses against VOC/VOIs, complete protection against SARS-CoV-2 with potentially superior immune outcomes than BNT162b2, and specifically antibody longevity when used as a boost. CD40.Pan.Cov vaccine will be moved to a phase I/II clinical trial in 2024.

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1533 – P3.10.25

Functional in vitro B cell assay for Tetanus and Diphtheria containing vaccine batch release using memory B cells from healthy donors

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Objectives: Vaccines containing inactivated toxins (toxoids) provide protection by eliciting a neutralizing antibody response against bacterial toxins such as tetanus and diphtheria. Release testing of vaccines ensures that efficacy and safety of the vaccine product are maintained in all batches. Currently, release of tetanus toxoid (TT) and diphtheria toxoid (DT)-containing vaccines relies on *in vivo* experiments mimicking the vaccine response.
Results: Here, we developed an *in vitro* assay based on the stimulation of switched memory B cells containing TT/DT antigen-specific B cells isolated from buffy coats. Exposure of TT/DT-specific memory B cells within the memory B cell fraction to vaccine antigen induced differentiation into IgG TT- and DT-specific antibody-secreting cells detected by ELISpot. The results further demonstrate that the protocol elicits a specific response to the target TT/DT antigens. Importantly, the assay provides robust and reproducible data on the immunogenicity of TT and DT vaccine components regardless of the presence of adjuvants, other antigens or excipients contained in the final vaccine formulation. Additionally, use of vaccine samples containing heat-treated TT displayed a decreased TT-specific response, indicating that the B cell assay is sensitive to alteration of vaccine antigen and its epitopes, an important prerequisite for batch release testing.
Conclusions: Our cell-based test system can serve to prove the functional integrity of the vaccine antigens and it might offer a potential alternative to animal experiments currently used for batch testing of vaccines containing TT and DT components by complementing biochemical assays.

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1656 – P3.10.26

Staphylococcus aureus-specific TIGIT+ Treg are present in the blood of healthy subjects – a hurdle for vaccination?

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Staphylococcus aureus poses an enormous burden of morbidity and mortality worldwide. Making an efficacious vaccine has however proven extremely challenging. Due to colonizing interactions, pre-existing *S. aureus*-specific $CD4^+$ T cells are often found in the human population and yet a detailed characterization of their phenotypes and how they might in turn impact vaccine efficacy are thus far unknown. Using an activation induced marker assay to select for *S. aureus*-specific $CD4^+$ T cells in an effector function-independent manner, phenotypical analysis was carried out via single-cell RNA sequencing. *S. aureus*-specific $CD4^+$ T cells consisted not only of effector (Teff) but also of regulatory (Treg) phenotypes. As compared to polyclonally activated $CD4^+$ T cells, Teff showed an enrichment for the expression of Th17-associated cytokine genes, which was further elevated in skin-tropic (cutaneous lymphocyte antigen, CLA, positive) cells. The vast majority of *S. aureus*-specific Treg was found to highly express the co-inhibitory receptor TIGIT. Remarkably, an antagonistic anti-TIGIT monoclonal antibody was shown to increase IL-1b production in response to *S. aureus*. Therefore, our results uncover the presence of *S. aureus*-specific $TIGIT^+$ Treg in the blood of healthy subjects suggesting that these cells could be responsible for blunting the response to re-infection and vaccination and indicate TIGIT as a potential targetable biomarker to overcome pre-exposure-induced immunosuppression.

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1679 – P3.10.27

Vaccination with BrpA, Biofilm Regulator Protein A, induce antibodies that affect biofilm formation in Streptococcus mutans

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Streptococcus mutans plays an important role in caries development due to its ability to produce and tolerate an acidic environment, as well as its capacity to form biofilms on the tooth surface. Many proteins have been investigated as vaccine candidates against *S. mutans*, particularly those involved in bacterial adhesion and biofilm formation. Biofilm regulator protein A (BrpA) is a virulence factor necessary for biofilm accumulation and maintenance of envelope integrity. Bioinformatics analysis suggests that BrpA is a transmembrane protein; however, the cellular location of BrpA has not been experimentally investigated. The present study aimed to determine the cellular location of BrpA, along with other traits associated with its potential inclusion in a vaccine against *S. mutans*, such as immunogenicity, prevalence among clinical isolates, accessibility to interact with antibodies, and the ability of these antibodies to block biofilm formation *in vitro*.The results indicate that mouse immunization with BrpA can produce a high amount of IgG antibodies. Western blot analysis showed that BrpA is present in 75% of the analyzed strains. Flow cytometry analysis revealed that BrpA is a membrane-exposed protein, making it accessible to interact with antibodies generated against the recombinant protein. Lastly, we tested the ability of these antibodies to block native BrpA, impairing biofilm formation by the bacterium. The strains pre-incubated with the antibodies formed less biofilm than the control. Together, these results suggest that BrpA stands out as a promising vaccine candidate against *S. mutans*.

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1733 – P3.10.28

A T cell inducing prime-boost vaccine for elephant endotheliotropic herpesvirus (EEHV)

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Purpose: Elephant endotheliotropic herpesviruses are a major threat to juvenile Asian elephants where losses due to EEHV induced haemorrhagic disease (EEHV-HD) remain high. There are no efficacious antiviral therapies or vaccines yet. Notably, the target cells for EEHV *in vivo* are unclear and monocytes/macrophages have been proposed as such.
Since immunity against herpesviruses particularly relies on cellular responses, we aimed to devise a T cell-inducing vaccine. Accordingly, we applied reverse vaccinology techniques aiming to design an EEHV vaccine that prevents death and severe disease not using external (glycoprotein) antigens.
Methods: We used a modified vaccinia Ankara (MVA) viral vector as priming vaccine and an adjuvanted protein formulation each containing two antigens as boost, creating a heterologous prime-boost vaccine against EEHV-HD.
In a first-in-elephant proof-of-concept trial, this prototype vaccine was tested for safety (and immunogenicity) in three adult elephants. We used a modified IFN-g release point-of-care, vaccine-specific whole blood stimulation assay as a way to determine immune responses with RT-qPCR as readout first as described for HCMV not least. This was necessary to overcome limitations of sample transport times from the zoo to the laboratory. RNA from preserved stimulated whole blood was used to determine the immune reaction via RNA Sequencing and assessment of differential gene expression pre- vs. post-vaccination using systems immunology approaches.
Results: A complete lack of adverse reactions post-vaccination strongly suggests that this heterologous prime-boost vaccine can be safely used in elephants. Recall IFN-g responses to our candidate antigens were observed against a background of reactions caused by the latent infection that exists in all elephants. Of diagnostic value for the immune reaction were Abs that could be detected against the candidate antigens using Western blot.
Over representation gene analysis (ORA) demonstrated that the vaccine induced a $CD8^+$ and $CD4^+$ T cell-based immune cell composition. Further ORA and GSEA as well a review of DEG demonstrated the Th1 bias of the antigen-specific immune response.
Conclusion: These results support a further evaluation of this prototype vaccine in young elephants as the target population for vaccination against EEHV-HD.

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1760 – P3.10.29

The M2SR intranasal H3N2 single replication live influenza virus vaccine induces more potent and broad mucosal secretory IgA response in older adults than the high-dose inactivated FluzoneHD vaccine

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The M2-deficient single replication (M2SR) H3N2 intranasal influenza vaccine was shown to protect from a drifted H3N2 virus in a phase 2a challenge human trial, inducing cross-reactive neutralizing antibodies, as well as IgA and secretory IgA (sIgA) antibodies. Here we compared the vaccine induced responses of M2SR to the licensed quadrivalent inactivated Fluzone High Dose (FluzoneHD) vaccine in a phase 1b trial of 305 older adults (65-85 years). Participants were randomized to receive placebo, FluzoneHD, M2SR or MS2R+FluzoneHD co-administration. Antigen microarrays spotted with 90 recombinant hemagglutinin (HA) and neuraminidase (NA) proteins of 58 influenza strains were used to profile the magnitude and breadth of serum IgG and IgA, and mucosal IgG and sIgA antibody responses at enrollment and 28 days post-vaccination.

While both FluzoneHD and M2SR induced significant serum IgG and IgA responses to influenza HA proteins ($p<0.001$), only M2SR induced nasal sIgA responses. Significant antibody responses to NA proteins were not detectable with any vaccine, except for FluzoneHD induced serum IgG response to B NA. Interestingly, although M2SR included only an H3N2 strain, it induced broad serum sIgA responses to H1 and B HA proteins as well, while in the nasal swabs the sIgA response was specific to H3 proteins only. The combination of M2SR and FluzoneHD was synergistic in inducing serum IgG to the conserved HA2 subunit and swab sIgA to H3N2 HA proteins. We compared the effects of both vaccines on individuals in the lowest quartile when ranked based on baseline H3 sIgA magnitude. M2SR induced significantly higher mucosal IgG and sIgA H3N2 responses in this group compared with FluzoneHD.

Overall, the H3N2 M2SR vaccine induced a significant mucosal sIgA response to H3 proteins in older adults, while FluzoneHD did not affect their sIgA levels. This effect was particularly significant for individuals with pre-vaccination low H3N2 mucosal antibody responses. M2SR also induced a heterologous serum IgG responses to influenza subtypes that were not included in the vaccine. These results suggest that M2SR vaccines may be used in populations with a limited response to the traditional influenza vaccines and may induce more broader protection from multiple influenza subtypes.

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Leishmania extracellular vesicles mediate protection against cutaneous leishmaniasis

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Purpose: Leishmaniasis is a group of vector-borne diseases caused by several species of *Leishmania* protozoan parasites. The disease manifests as cutaneous, mucocutaneous, or visceral leishmaniasis depending on the infecting *Leishmania* species and the nature of the host response. As a neglected tropical disease, leishmaniasis is a serious global health problem that necessitates the development of new disease prevention measures to limit its spread. In this study, we sought to evaluate the immunogenicity of *L. major*-derived extracellular vesicles in the BALB/c mouse model of cutaneous leishmaniasis.
Materials and methods: *L. major*-derived extracellular vesicles (EVs) were purified under GMP-adaptable conditions from axenic cultures of late-log phase metacyclic promastigotes via multimodal chromatography and tangential flow filtration. EVs were characterized by proteomics, tunable-resistive pulse sensing, and scanning electron and atomic force microscopy. 6-8-week-old naive female BALB/c mice were immunized twice (2 weeks apart) with 3 or 10 µg of EVs as such or combined with different vaccine adjuvants. Cellular and humoral immune responses were evaluated 2 weeks after booster injection and immune protection after live parasite challenge was followed longitudinally.
Results and conclusions: GMP-adaptable EV purification protocol allowed consistent and reproducible isolation of extracellular vesicles enriched in several *Leishmania*-specific antigens (proteomics) with the expected vesicular structure and size (microscopy). EVs generated humoral and cellular immunity and provided protection against live parasite challenge. In conclusion, our data suggest the feasibility of utilizing parasite-derived vesicles as candidate vaccines against *Leishmania major*-induced cutaneous leishmaniasis.

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1901 – P3.10.31

Saponin-based adjuvants: deciphering the molecular mechanism of QS-21

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Purpose: Adjuvants are key immune activators added to vaccine formulations that prolong the immunogenicity of the antigen and stimulate a strong long-lasting immune response. However, toxicity problems, among others, restrict the use of many of them in humans. Traditionally, aluminum-based salts have been used as adjuvants in the clinic, but more recently the saponin-based family of adjuvant formulations have gained relevance due to their efficiency. Nonetheless, their molecular mechanism is yet unknown and scant mechanistic studies have been performed to date. Therefore, our work seeks to decipher the molecular target responsible for their activity.
Methods: Synthetic saponin variants were chemically developed deriving from QS-21's structure, in order to obtain a simplified and less toxic molecule, but with the same effector capacity. Moreover, a photoaffinity probe consisting of a light-activatable group and a biotin tag was added to the structure of our saponin probe, enabling subsequent photo-crosslinking and detection with streptavidin. Western blotting and proteomics assays were performed to detect the molecular target of our saponin. Once some hits were discovered, validation assays were carried out, both microscopy and in vitro inhibition of the target, to ensure the results' accuracy. Last, an in vivo experiment was performed to check the antibody responses against our saponin in presence and absence of an inhibitor.
Results: Western blot results showed some lysosomal proteins as potential targets, which were also upregulated compared to the results obtained by the proteomics. Furthermore, binding assays were performed and checked by confocal microscopy showing the co-localization of the target with our saponin. Moreover, a commercial inhibitor was used to assess the veracity of the target, which validated our previous results. Last, in vivo results show less antibody titers in the neutralized mice.
Conclusion: Qs-21 appears to have a lysosomal protein as its intracellular target, provoking the lysosomal disruption and the subsequent cross-presentation to the MHC-I molecules of the immune system.

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1969 – P3.10.32

Distinct cellular and serological cross-reactive responses upon COVID-19 Original/BA.1 bivalent booster vaccination

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Purpose: COVID-19 booster vaccination is currently advised to subpopulations at high-risk of severe COVID-19. In autumn 2022, bivalent vaccines encoding both Spike protein from SARS-CoV-2 Original and Omicron BA.1 variants (Original/BA.1) were rolled out to enhance protection against infection with emerging Omicron variants. Hereby, we study the vaccine-induced variant-specific anti-Spike T cell and serological response across the general Dutch population (including non-risk groups), with special focus on the booster´s benefit in cross-reactivity across different adult age groups.
Methods: In a long-term follow-up, PBMCs and serum from 24-89 years old (y.o.) participants (n=86) of three COVID-19 vaccination longitudinal studies were collected before and after receiving the Original/BA.1 booster (Comirnaty or Spikevax). An IFNy-ELISpot served to study the Spike-specific T cell response towards the S1 and S2 subunits of the Original, Omicron-BA.1, and -BA.4/-BA.5 strains; the latter to study vaccine-induced cross-reactivity towards the then most prominent circulating variant. Additionally, we measured anti-S1 IgG concentrations in serum for the Original and Omicron-BA.1 variants. Nucleocapsid serology determined SARS-CoV-2 infection.
Results: Booster vaccination with Original/BA.1 increased the Original- and Omicron-BA.1 Spike-specific IFNy-T cell response only among older adults (≥70 y.o.), while IgG concentrations were increased across all age groups. Notably, the pre- and post-booster Spike-specific IFNy-T cell response towards both vaccine strains was similar; and showed additional cross-reactivity towards Omicron-BA.4/-BA.5. By contrast, at both timepoints, anti-S1 IgG concentrations were higher for the Original strain compared to Omicron-BA.1; and interestingly, the fold-change ratio between IgG concentrations at both timepoints was higher for Omicron-BA.1 compared to the Original strain. These findings were similar among participants with or without previous SARS-CoV-2 infection, which showed comparable fold-change ratios of both the IFNy-T cell response and IgG concentrations.
Conclusion: Firstly, our results demonstrate that the Original and Omicron-BA.1 cellular response in ≥70 y.o. adults can be increased with booster vaccination. Secondly, boosting with the bivalent Original/BA.1 vaccine induces IgG concentrations across all adult age groups, with a more significant change of Omicron-BA.1 specific antibodies. Overall, the higher cross-reactive capacity of T cells compared to antibodies supports the importance of vaccine-induced cellular immunity for protection against emerging variants.

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2076 – P3.10.34

Monitoring of the immune response against SARS-CoV-2 in patients with common variable immunodeficiency vaccinated with mRNA vaccines

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Common variable immunodeficiency (CVID) is a disorder characterized by B cell dysfunction and impaired immunoglobulin production. Individuals with CVID typically exhibit reduced levels of immunoglobulin G (IgG) and IgA, often accompanied by low IgM levels. Additionally, they may display diminished switched memory B cells and a poor response to vaccinations. Therefore, it is crucial to closely monitor the vaccination status of these patients to assess their vaccine response. Following the SARS-CoV-2 pandemic, the emergence and deployment of mRNA vaccines have become a critical issue for the general population, with even greater significance for individuals with immunodeficiencies such as CVID. Consequently, it is imperative to evaluate and track both the cellular and humoral responses to mRNA vaccines in CVID patients. To address this, we enrolled 26 CVID patients alongside a healthy control group and monitored their response to five doses of mRNA vaccines, comprising four doses of mRNA-1273 and one dose of the BNT162b2 bivalent vaccine. The humoral response was assessed by measuring serum IgG anti-spike protein (anti-S) antibodies and their neutralizing activity against prevalent variants. Meanwhile, the cellular response was quantified by IFN-γ production specific to SARS-CoV-2. Our findings revealed that CVID patients initially exhibited lower anti-S IgG levels compared to controls, although subsequent vaccine doses led to an increase in anti-S IgG levels, albeit with individual variability. Some patients required up to three doses to seroconvert. Moreover, cellular response levels increased with each vaccine dose, and patients demonstrating a cellular response also exhibited higher anti-S IgG levels. Furthermore, successive vaccine doses enhanced neutralization against the wild-type strain but did not improve neutralization against emerging variants, even with the use of the bivalent vaccine. In conclusion, monitoring vaccine response in CVID patients enables the identification of non-responders, facilitating appropriate precautionary measures. Our results underscore the heterogeneous response among CVID patients, with some requiring multiple doses to achieve seroconversion. While successive doses bolster humoral and cellular responses, their efficacy is limited to strains included in the vaccine formulation, emphasizing the importance of tailoring vaccinations to prevalent strains, akin to strategies employed for influenza virus vaccination.

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T cell responses to the replication-complex of SARS-CoV-2 remain subdominant in the current landscape of SARS-CoV-2 immunity

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Purpose: During the first wave of the COVID-19 pandemic, a subset of highly exposed healthcare workers remained seronegative despite blood biomarker evidence of exposure and longitudinal expansion of SARS-CoV-2-specific T cells, indicating 'abortive' infections. In this cohort, T cells preferentially targeted the replication transcription complex (RTC) of SARS-CoV-2, a non-structural protein complex essential for the replication of the virus. In contrast, in individuals with detectable infections (seropositive, PCR+) with Wuhan-Hu-1 virus, RTC-specific responses were subdominant to structural-specific T cell responses. However, it is unclear what impact repeated exposure to SARS-CoV-2 variants and vaccine breakthrough infections would have on the RTC-specific T cell compartment. Here, we investigate the prevalence of RTC-specific T cell responses in a 'contemporary' (Sept2023-May2024) London-based cohort of healthy donors, after vaccination and likely exposure to SARS-CoV-2 Omicron variants.
Methods: PBMC from healthy donors were collected and T cell assays (IFN**γ** ELISpot, Intracellular cytokine staining, Activation induced marker assay) were performed to quantify the prevalence of T cell responses to structural and RTC proteins of SARS-CoV-2. These data were compared to existing data on pre-pandemic responses, responses induced by detectable Wuhan-Hu-1 infection and responses induced by abortive infection.
Results: RTC-specific responses remain subdominant in the 'contemporary' cohort despite potential repeated exposure to SARS-CoV-2, with comparable magnitude to the RTC-specific responses observed pre-pandemic. Further, these responses are lower in magnitude than RTC-specific responses induced by abortive infection. Structural-specific T cell responses, including those to antigens not included in vaccines, are expanded above levels seen pre-pandemic.
Conclusions: Repeated exposure to Omicron variants has not boosted RTC-specific T cell responses. Further, the preferential targeting of structural proteins beyond spike in the 'contemporary' cohort suggests repeated exposure to SARS-CoV-2 variants has boosted this immunity. Together, these data suggest boosting RTC-specific T cell responses, shown to correlate with abortive infection, with an RTC-based vaccine may offer a further layer of protection against infection with current and future SARS-CoV-2 variants than is currently seen in exposed vaccinated cohorts.

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2212 – P3.10.36

An investigation of immune interference following sequential platform vaccinations in a guinea pig filovirus infection model

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Filoviridae are a family of negative-strand RNA viruses that include several important human pathogens, including Ebola virus (EBOV), Sudan virus (SUDV) and Marburg virus (MARV). Recent deadly EBOV outbreaks in Western Africa highlight the potential for these emerging viruses to have a devastating global impact. Several EBOV vaccine platforms are currently progressing through clinical trials in the US and abroad, including a live-attenuated, replication-competent recombinant vesicular stomatitis virus (rVSV)-vectored vaccine (ERVEBO®) and a live-attenuated, replication-incompetent chimpanzee adenovirus type 3 (ChAd3)-vectored vaccine, both expressing the immunogenic EBOV glycoprotein (GP). While the plug-and-play nature of vectored vaccines have many advantages (ease of production, path to licensure, etc), concerns remain around immune interference obstructing the use of platform technology for subsequent vaccinations. Here, we investigated whether pre-existing antigen- or vector-specific vaccine-induced responses impact the immunogenicity or efficacy of anti-filovirus vaccines. Groups of n=12 female Hartley guinea pigs (gpigs) were vaccinated intramuscularly in a prime-boost regimen four weeks apart, with one vaccine containing homologous rVSV-SUDV-GP, and the other a heterologous EBOV or MARV GP expressed by either an rVSV or ChAd3 vector. Sera was collected pre-vaccination and 14 days post-prime and post-boost for evaluation by ELISA and microneutralization assay. Four weeks post-boost, gpigs were challenged with 10,000 plaque forming units of guinea-pig adapted SUDV (GPA-SUDV) via intraperitoneal injection. Sera was collected at 6 days post-infection to evaluate viremia, and any gpigs surviving to 28 days post-infection were considered protected and euthanized. Sera ELISAs (using recombinant SUDV GP) and microneutralization assays against authentic SUDV demonstrated significant immunogenicity post-vaccination compared to unvaccinated controls. All vaccinated gpig groups, regardless of timing of homologous antigen, or combination of antigens and vectors, demonstrated 100% survival following GPA-SUDV infection, a significant difference from unvaccinated gpigs (83% lethality). Further, vaccination prevented significant weight loss, temperature elevation, clinical signs of disease and viremia post-infection in comparison to controls. This experiment suggests that there is little to no significant vaccine-associated immune interference as a result of heterologous, sequential vaccinations with vector platforms currently in clinical development.

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Complete cross strain protection against congenital cytomegalovirus by vaccine targeting key components of the virus life cycle that induces antibody and T cell immunity

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Purpose: Congenital cytomegalovirus (cCMV) is a leading cause of hearing loss/cognitive impairment in newborns. A vaccine is complicated by existence of multiple CMV strains enabling reinfection. Consequently, a vaccine must exceed convalescent natural immunity. The guinea pig is the only small animal model for cCMV but requires guinea pig CMV (GPCMV). Viral glycoprotein complexes (gB, gM/gN, gH/gL/gO and pentamer complex/PC) are important for cellular virus tropism. The fusogenic gB glycoprotein is essential for entry into all cell types by all routes. A GPCMV non-replication competent DISC vaccine strain demonstrated the importance of neutralizing antibodies to viral glycoproteins for protection in this model but failed to provide cross strain protection against a new clinical strain of GPCMV evading antibodies by being closely cell associated rather than cell free virus. Consequently, the T-cell response against CMV is equally important to target cell associated virus.
Methods: GPCMV antibody (viral glycoproteins) and T-cell (IE1/pp65) target antigens were evaluated as recombinant defective adenovirus (Ad5/35) based vaccines against horizontal and vertical challenge models with multiple strains of wild type GPCMV.
Results: A trimeric gB complex based Ad vaccine (AdgB) induced potent neutralizing antibodies, unlike gB monomer. However, cell associated virus was able to disseminate and similar results were obtained for gH/gL vaccines. GPCMV encodes functional homologs of IE1 and pp65 (GP83) pathogenicity factors targeting PML bodies and IFI16/cGAS-STING pathway respectively. Ad vaccines (AdIE1/AdGP83) induced T-cell responses in animals and reduced viral load in target organs against horizontal challenege but were not fully protective with AdIE1 exceeding AdGP83. A combination strategy of AdgB and AdIE1 (targeting humoral and cell-mediated responses) against mixed wild type strains of GPCMV in a congenital protection study was evaluated. 4 groups of female animals (N=10/group) were vaccinated with either AdgB, AdIE1, AdgB+AdIE1, or unvaccinated before mating. During 2nd trimester, animals were challenged with GPCMV. Pup cCMV transmission in unvaccinated was 91% but the AdgB+AdIE1 group had no detectable virus in pup tissues unlike single antigen vaccines.
Conclusion: Results demonstrate the importance of combined immune responses to antibody (gB) and T-cell (IE1) antigens for potent cross strain protection against cCMV.

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2224 – P3.10.38

Evaluation of the protective efficacy of novel vaccine candidates against *Acinetobacter baumannii* and *Pseudomonas aeruginosa* pathogens

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Acinetobacter baumannii and *Pseudomonas aeruginosa* are highly multidrug-resistant bacteria and major contributors to nosocomial infections. They are classified by the World Health Organization as critical priority pathogens that require immediate action to develop novel antibiotics. However, no approved vaccines for these pathogens are currently available. We have identified several proteins involved in cell attachment of *P. aeruginosa* and *A. baumannii* to lung epithelial cells, some of which are homologous between *P. aeruginosa* and *A. baumannii.* Therefore, the aim of this project is to compare the protective efficacy and cross-reactivity of these homologous antigens (AgN) against *A. baumannii* and *P. aeruginosa. A. baumannii* AgN possesses seven, and *P. aeruginosa* AgN possesses nine predicted CD4 T cell epitopes, suggesting the potential of these antigens to induce cellular immune responses, and are of interest because Th1 and Th17 responses may contribute to protection against these pathogens. Mice immunised with recombinant *A. baumannii* AgN or *P. aeruginosa* AgN (with Sigma Adjuvant System (SAS) as adjuvant) expressed significantly more IFN-γ compared to the SAS adjuvant-only group (4-fold increase, p=0.0035 for *A. baumannii* AgN and 6.4-fold increase, p=0.007 for *P. aeruginosa* AgN), indicating strong T cell stimulation. However, IL17 expression was only significantly increased in the *P. aeruginosa* AgN-immunised mice (9.2-fold increase, p< 0.0001)
Mice immunised with SAS-adjuvanted *A. baumannii* AgN and subsequently challenged in an acute pneumonia model showed reduced bacterial lung colonisation (0.74-$\log_{10}$, p<0.0102) and spleen dissemination (0.9-$\log_{10}$, p<0.0426), highlighting its potential as a vaccine. Mice also showed robust IgG titres prior to the challenge. Although challenge studies on *P. aeruginosa* AgN are ongoing, its ability to stimulate more robust IFN-y and IL-17 responses, suggests it is a promising vaccine candidate.
Overall, these novel vaccine candidates against these two pathogens contribute to the urgent need for effective vaccines against antibiotic-resistant infections.

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2236 – P3.10.39

Cellular immunity against SARS-CoV-2 after different vaccination regimens

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We longitudinally explored cellular immune response to SARS-CoV-2 in four COVID-19 vaccines regimens.
Venous blood was obtained from healthy volunteers at three time-points: before the third dose of the vaccine, 21 days and 6 months after. COVId-19 infection during the follow up was an exclusion criterion. A total of 21 persons completed the follow-up. BNT162b2 was administered as a booster in 7 persons after BNT162b2 primary series, in 7 after BBIBP-CorV, and 3 after ChAdOx1 nCoV-19. In 4 persons BBIBP-CorV was used as a booster after BBIBP-CorV primary series. Cellular immune response to SARS-CoV-2 was assessed by interferon (IFN)-γ release assay and flow cytometric in vitro proliferative response to S-protein, along with unstimulated and pokeweed-stimulated blood as negative and positive control, respectfully. Total lymphocytes, T, B and NK cell were counted in a population of lymphocyte blasts after 7 days of antigen stimulation as absolute numbers, using absolute counting tubes. In addition to antigen specific analyses, samples were assessed for the T, B and NK cell counts before incubation.
There were not any statistically significant differences in total lymphocytes, T, B and NK cell counts between the groups and time-wise. Statistically significant increase in IFN-γ levels for all stimulations (Ag1, Ag2, Ag3) was observed 21 days after the third dose in regimens where BNT162b2 was administered as a booster after the same vaccine or BBIBP-CorV primary series. Higher IFN-γ levels after booster were measures in samples in other groups without statistically significant difference. After 6 months, IFN-γ release upon Ag1, Ag2 and Ag3 stimulations was comparable to the level before the booster. In vitro incubation with S-protein did not reveal any differences in T, B and NK cell blast numbers among vaccination regimens.
Small number of participants is a major limitation of the study. However, longitudinal alterations after vaccination warrant further assessment.

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P3.11 VACCINES FOR IMMUNOTHERAPY

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36 – P3.11.01

Lynch syndrome-related neoantigens prediction and validation for a dendritic-cell based cancer prevention vaccine

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Purpose: Lynch syndrome (LS), caused by germline mutations on DNA mismatch-repair genes (MMR) predisposes to colorectal and endometrial cancer (CRC, EC) amongst other tumors. Although CRC prevention is effective, no strategies exist for most LS-related tumors. Ex-vivo generated and tumor-antigen-loaded dendritic cell (DC) vaccines have been used as cancer immunotherapy; however, their full potential would likely be as a preventive approach in high-risk cancer patients. LS is a paradigmatic model for its limited and predictive mutational spectrum in repetitive DNA sequences (microsatellites, MS). We aimed to Identify and validate LS-related frameshift-derived neopeptides (FSDN) to develop a cancer preventive DC-based vaccine.
Methods: Search of LS-related coding MS (cMS) mutations and prediction of neoantigens with high coverage on common HLA-I and II alleles (pVACbind; pVACtools v2.0.1). Sequencing and analysis of FSDN-mutations presence on colorectal adenomas (CrAD), EC and CRC samples from LS patients, non-LS tumor sequences and RNA and DNA sequences from tumoral cell-lines. In-vitro FSDN immunogenicity analysis on tissue infiltrating lymphocytes (TILs) from LS CrADs, CRCs and normal mucosa by IFN-y ELISPOTs, flow cytometry. Detection, expansion, and characterization of neoantigen-specific CD8+ T-cells (Dextramers, IFNy+ magnetic selection, flow cytometry).
Results: 98 neoepitopes from 53 coding-MS-containing genes were prioritized. In silico analysis showed that ≥1 neoantigen-related mutations are found in all analyzed CrADs (31), EC (8) and CRC (52) LS samples and in 18-84% non-LS MSI tumors. FSDN mutations were found in DNA (66% cMS) and cDNA (69.8%) from MSI tumoral cell lines. In-vitro analysis showed that 71% FSDN gave a positive IFNy response in ≥1 LS patient (n=9). FSDN-specific TILs were detected and isolated from CrAD and normal colonic mucosa from 7 LS samples (n=12, 58%). Based on the results we prioritized a set of 24 FSDN for the vaccine.
Conclusion: Our predicted neoepitope set has optimal coverage among LS patients (HLA alleles, associated cancers and prevalence) and is capable of inducing IFNy inflammatory responses in LS-derived TILs. A phase Ib clinical trial will start on 2024 to determine the safety and efficacy of the autologous DC-based vaccine in LS-individuals.

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756 – P3.11.02

Chronic infection induces a neutralizing B cell response to Elastase B of Pseudomonas aeruginosa

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Purpose: *Pseudomonas aeruginosa* (PA), known for causing severe acute nosocomial infections and chronic lung infections, exhibits extensive intrinsic and extrinsic resistance mechanisms. Pathogenesis of PA involves various virulence factors, including secreted proteases. Among these, elastase B (LasB), a highly potent exoprotease with broad substrate specificity capable of dissolving connective tissue and inactivating host defence proteins, emerges as a pivotal virulence factor and a promising target for antivirulence therapies. Although the immunogenicity of LasB in humans has been recognized for decades, a comprehensive analysis of the humoral immune response is lacking.
Methods: In this study, we investigate the humoral immune response to LasB in humans by screening a cohort of individuals with cystic fibrosis (CF), mostly with detectable or recurrent PA infections, followed by single B cell analysis of selected donors. Monoclonal antibodies (mAbs) derived from patients were produced based on B cell receptor repertoire analysis of LasB-specific B cells and characterized *in vitro* and *in vivo*.
Results: Among 102 individuals with CF, six donors were selected for single cell analysis of LasB-specific B cells. B cell receptor repertoire analyses revealed a polyclonal antibody response with evidence of clonally related B cells within each donor. Produced mAbs, originating from various clonal groups and donors, exhibited varying binding epitopes and neutralizing potency against LasB, including evidence of mAbs with superior activity. These mAbs completely inhibited cytolytic LasB activity and protected against LasB-induced loss of cell integrity and cell death. Notably, broadly neutralizing mAbs were identified with activity against several LasB variants, without detection of mutations leading to a loss-of-function of neutralizing antibodies.
Conclusion: Our findings offer novel insights into the intricate host-pathogen interactions involved in chronic PA infections by elucidating the humoral immune response to LasB. Patient-derived anti-LasB mAbs represent an innovative antivirulence therapeutic approach aimed at disarming PA during invasive infections. Leveraging the long half-life of human IgG, this approach holds promise for extending to passive immunization strategies for individuals at high risk of PA infections.

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844 – P3.11.03

Epigenetic Inhibitors Together With TLR9 Agonist elevated IFN response and Th1 immunity

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Purpose: Cancer immunotherapy is a successful treatment that triggers person's own immune system during fight against cancer. However, some patients might show immunotherapy resistance, or worse, cancer hyper-progression. Therefore, immunotherapy is preferred as combinational therapy. Another novel cancer treatment is epigenetic therapy which solves dysregulations of cancer cells in terms of epigenetic modifications. They can prevent relapsing of cancer by repairing of abnormal gene expressions. Additionally, some have an influence on immune cells which can resolve suppressive environment of tumor. In this study, we aim to combine both immunotherapy and epigenetic inhibitors which FDA has been already approved to use against cancer to elevate the effectiveness of both therapies and limit their possible side effects. We used nucleic acid-based adjuvants and ovalbumin as a cancer vaccine and epigenetic inhibitors including DNA methylation and histone deacetylation inhibitors.
Methods: Splenocytes were stimulated with unmethylated CpG-containing oligonucleotides (CpG-ODNs) as a TLR9 agonist together with epigenetic inhibitors. We have checked production of cytokines using ELISA assay and reporter cell lines. After selecting the combination with highest Th-1 biased immune response, we have applied the combinational therapy to established melanoma tumor using B16-OVA cell line on C57BL/6 mice as a treatment. We have followed tumor growth, survival rate and antigen specific immune responses.
Results: We have shown that combination of K-type CpG-ODNs with Zebularine elevated Th1-biased immune responses by increasing type-I and II IFN production, in addition, up-regulation of co-stimulatory molecules (CD86 and CD40) and down-regulation inhibitory signals (PDL1) was observed *in vitro* splenocyte. *In vivo* experiments revealed that antigen specific production of type II IFN increased, however; IgG2c/IgG1 ratio remained unchanged after the novel combination.
Conclusion: We have found that DNMT inhibitor Zebularine showed coadjuvancy with K-type CpG-ODN against murine melanoma. Next, we will apply loading the combination into nanovesicles like liposomes to increase the delivery efficiency to tumor side.

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872 – P3.11.04

mRNA-Encoded SFTSV Neutralizing Antibodies: Evaluation of Therapeutic Potential

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Purpose: Severe fever with thrombocytopenia syndrome (SFTS) was first discovered in 2009 as the causative agent of SFTS virus (SFTSV), an infectious disease caused by the SFTSV that is primarily transmitted through tick bites, localized to Korea and China. Despite its potential threat to public health because of high fatality rate, no effective prophylactic vaccine or therapeutic antibody are yet available. Recently, mRNA has been in the spotlight as a preventive and therapeutic vaccine or antibody. The advantage of mRNA is that it can be produced quickly and easily for the desired antigen gene.
Methods: In this study, we developed a promising mRNA-based therapeutic antibody candidates against SFTSV Gn in vitro and in vivo.
Results: In this study, we developed a promising mRNA-based therapeutic antibody candidates against SFTSV Gn. Administration of varied lipid nanoparticle-encapsulated mRNA-based therapeutic antibody candidates successfully elicited high neutralizing antibody titers in vitro. The mRNA-based therapeutic antibody candidates expressed anti-Gn antibodies dose dependently. We found that mRNA -based therapeutic antibody treatment significantly improved survival compared to the control group and protected against a lethal SFTS virus challenge in both vaccinated wild-type mouse and IFNAR-1 KO mouse in vivo
Conclusion: This study suggests that this mRNA-based therapeutic antibody candidates may be potential as an effective SFTSV therapeutic antibody.

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963 – P3.11.05

Recombinant carrier-peptide fusion vaccine for allergen-specific immunotherapy of allergy to cat

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Purpose: Worldwide, over 200 million individuals suffer from allergy to cats. Allergen-specific immunotherapy (AIT) is a highly effective allergen-specific treatment, but current allergen extract-based vaccines can induce severe adverse reactions, are not effective against all cat allergen molecules and require multiple and cumbersome administrations.
We report the engineering and characterization of a cat allergy vaccine based on a recombinant fusion protein consisting of hypoallergenic peptides derived from the three most important cat allergens Fel d 1, Fel d 4 and Fel d 7 fused with the PreS-surface antigen from hepatitis B virus as an immunological carrier protein.
Methods: Five recombinant fusion protein variants containing differently arranged allergen-derived peptides, (designated SuperCat 1-5) were produced, characterized and investigated regarding their allergenic activity (i.e., IgE-reactivity and induction of basophil degranulation), T-cell reactivity and cytokine release in cat allergic patients' samples. Their ability of inducing protective IgG responses was evaluated by immunization of rabbits in comparison with registered extract-based vaccines.
Results: SuperCats 1-5 lacked relevant IgE-reactivity and did not induce basophil activation demonstrating lack of allergenic activity. Allergen-specific IgG antibodies induced with only two injections in rabbits with SuperCat 1 and 5, but not with registered extract-based vaccines, blocked cat allergic patients' IgE binding to Fel d 1, Fel d 4 and Fel d 7 and allergen-induced basophil activation. Even induction of cross-protective IgG antibodies to cross-reactive major dog and horse allergens was observed. Furthermore, SuperCats 1 and 5 induced the production of the tolerogenic cytokine IL-10 in cultured PBMCs from allergic patients.
Conclusion: The in vitro data suggest that SuperCats are safe AIT vaccines capable of inducing protective IgG antibodies preventing allergen-induced basophil activation in cat allergic patients. In particular Supercats 1 and 5 are highly promising antigens for AIT of cat allergy.

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1123 – P3.11.06

Study of Mycobacterium indicus pranii and human beta defensin-2 as a combinatorial therapy against Mycobacterium tuberculosis

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Purpose: To reduce the host damage and enhance the elimination of mycobacteria, host directed therapeutics (HDT) can modulate specific host responses related to inflammation. Some HDTs have the potential to complement the existing Tuberculosis treatment by targeting both the bacteria and the host immune system. Among them, *Mycobacterium indicus pranii* (MIP) and human beta defensin-2 (hBD-2) have emerged as promising candidates for the treatment of various infectious diseases, including tuberculosis (TB). They have the ability to directly kill *Mtb* bacilli and also induce a cell-mediated immune response, which is lacking in conventional antitubercular drugs. In this study, we used MIP and hBD-2 as novel adjuvants to evaluate their effects on bacterial clearance and Th1 immune response in THP-1 cell line infected with an avirulent strain of *Mtb*.
Methods: THP-1 cells were differentiated into macrophages by using PMA. PBMCs were isolated using Ficoll Hypaqe Density Gradient method from the whole blood and monocytes were differentiated into macrophages. Cultured macrophages were infected with avirulent strain of *Mycobacteria.* Then, infected cells were treated with MIP, hBD-2 or both. Pro-inflammatory response was measured using gene expression of pro-inflammatory cytokines by qPCR. Bacterial clearance was assessed using CFU assays and Pyroptosis was checked using flow cytometry.
Results: When cells were treated with MIP and hBD-2 together, a strong pro-inflammatory response was observed. This signifies a robust Th1 immune response, which further reduced the bacterial count in the CFU assays. Flow cytometry expression of Caspase-1 showed the enhanced pyroptosis of infected cells after treated with combined MIP and hBD-2.
Conclusion: The current findings support the use of these powerful immunomodulators as adjuvants against mycobacterial infection as an effective strategy of therapy. However, the current findings must be verified in an appropriate animal model.

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1261 – P3.11.07

Advancing rheumatoid arthritis therapy by tolerogenic peptide-based vaccines

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Purpose: Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disorder, characterized by peripheral joint inflammation and bone destruction. Interestingly, around the onset of the disease, autoantibodies against citrullinated proteins cross-react with joint proteins, such as type II collagen (COL2). Currently, the medical treatment of established RA is the neutralization of inflammatory mediators such as TNFα. However, these treatments remain problematic, largely because of their lack of immune specificity and side effects. Therefore, there is a need for the development of more specific, safer, and more effective therapeutic interventions, targeting the initiation immune mechanisms. The genetic association of RA with certain MHC class II (MHCII) alleles indicated T cells as central players and, consequently, an important target for therapeutic intervention in the disease. We propose a tolerogenic peptide-based vaccination as a new therapeutic option for the treatment of early/established disease.
Methods: This process involves the expression of MHCII-$COL2_{peptides}$ complexes in eukaryotic cells, utilizing HEK cells. Collagen-induced arthritis (CIA) model is used to test our vaccine candidates in new unique physiologically accurate mouse strains. These strains were made by knock-in technology replacing the extracellular domains of HLA-DRA, HLA-DRB1*04:01 and CD74 in C57BL/6N mice, surpassing the limitations observerd in transgenic approaches. Fluorescently labeled MHCII-peptide tetramers aid in detecting COL2-specific T cells, while flow cytometry assesses T cell phenotype post-vaccination. ELIspot, ELISA, qPCR, and proteomic analysis provide deeper insights into antigen-specific T cell phenotypes.
Results: Among various COL2 peptide modifications, the AL179 peptide emerges as the most promising vaccine candidate. Vaccination with MHCII-AL179 complexes demonstrates potent suppressive effects and protects against arthritis in mice. Notably, the therapeutic effect can be transferred using T cells from vaccinated mice into COL2-immunized mice. Furthermore, the immune checkpoint inhibitor molecule VISTA, appears to play a significant role in the vaccination's efficacy.
Conclusion: We describe a vaccine that directly targets COL2-specific T cells, mediating a suppresive and tissue-specific effect on inflammation and the development of arthritis in peripheral joints. Leverageing our unique humanized mouse strains, we present promising avenues for developing an effective RA vaccine for human use.

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P3.12 VIRAL IMMUNOLOGY

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61 – P3.12.02

Revealing the role of lamin A/C in dendritic cell-mediated antiviral immunity

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Dendritic cells (DCs) are pivotal orchestrators of immune responses, crucially involved in fostering IFN-γ-producing CD8+ cytotoxic T lymphocytes (CTLs) and CD4+ T helper 1 (Th1) cells, pivotal for combating viral infections and optimizing vaccination efficacy. Moreover, lamin A/C, a nuclear envelope protein, has emerged as a key player in T cell immunity. However, the intricate dynamics between innate and adaptive immunity during viral infections, particularly the impact of lamin A/C on DC function in this context, remain elusive.
Herein, we unveil that mice lacking lamin A/C in myeloid LysM promoter-expressing cells exhibit diminished capacity to elicit Th1 and CD8 CTL responses, culminating in impaired clearance of acute primary VACV infection. Intriguingly, in vitro-generated GM-CSF BMDCs display elevated levels of lamin A/C. Notably, lamin A/C deficiency in GM-CSF BMDCs does not alter the expression of surface costimulatory molecules but profoundly impairs their ability to form immunological synapses with naïve CD4 T cells. This impairment correlates with perturbations in NFκB nuclear translocation, thereby modulating NFκB-dependent transcription. Furthermore, lamin A/C ablation induces epigenetic alterations in BMDCs, predisposing these cells to mount a suboptimal antiviral response upon TLR stimulation.
Our findings underscore the indispensable role of DCs in orchestrating interactions with CD4 T cells during antiviral responses, elucidating the intricate molecular mechanisms through which lamin A/C governs DC function via epigenetic and transcriptional regulation.

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144 – P3.12.04

HIV control status is associated with functional changes in memory natural killer cells

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Purpose: HIV controllers (HIC) are a unique subset of people living with HIV (PLHIV) who spontaneously control HIV. We aimed to characterize functional alterations in Natural Killer (NK) cells prior and post HIV infections in HIC and non-controllers (non-HIC). 1st-degree family members (FAM) of HIC and non-HIC were used to infer NK-cell functionality prior HIV infection.
Methods: General NK-cell markers (CD56 and CD16) were assessed by flow cytometry on whole blood samples from 1315 PLHIV (105 HIC vs. 1213 non-HIC) and 43 HIV-negative FAM of PLHIV of the 2000HIV-study (NTC03994835). 10 activating and inhibitory NK-cell receptors were measured on MACS-sorted NK-cells of 27 PLHIV (15 HIC vs. 12 non-HIC) and 21 FAM of the 2000HIV-TRAINED-study (NCT04968717). Functional NK-cell markers were assessed using supervised and unsupervised analysis, integrating UMAP and FlowSOM metaclusters (MT). NK-cell functionality was evaluated by co-culturing PBMCs with K562 cells and measuring IFNγ, granzyme B, and perforin via flow cytometry.
Results: HIC exhibited increased $CD56^{bright}$ NK-cells compared to non-HIC and a higher expression of DNAM, NKp30, and NKp46 in HIC. Unsupervised analysis of CD56-sorted cells comparing PLHIV with FAM identified the expansion of an HIV-specific NK-cell MT. Increased NKG2C and ILT2 and decreased KIR2DL2/3 expression (MFI) within the HIV-specific MT was seen in HIC compared to non-HIC. Because NKG2C is a memory receptor in cytomegalovirus (CMV)-induced NK-cells, we stratified the FAM according to their CMV serostatus, who are 50% CMV seronegative. We identified KIR2DL2/3 expression as a differentiating receptor between HIV- and CMV-specific MT because it was expressed lower in HIV-induced NK-cells. NKG2C+ NK cells from HIC produced greater amounts of IFNγ than non-HIC, while no differences in granzyme B and perforin were observed.
Conclusion: An HIV-specific NK-cell population was identified in PLHIV that differed from CMV-induced NK-cells. NK-cells in HIC are more functionally competent and characterized by increased NKG2C, ILT2 and decreased KIR2DL2/3 expression. These HIC-specific NK-cells with a memory phenotype produce IFNγ, which may contribute to HIV control.

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172 – P3.12.06

High levels of S2-specific IgA antibodies with decreased Fc-effector activity correlate with severe COVID-19

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Purpose: It has been suggested that individuals experiencing severe COVID-19 develop a distinctive SARS-CoV-2-specific humoral response, which is associated with disease progression. Here, we evaluated whether SARS-CoV-2 antibodies contribute to disease exacerbation in unvaccinated individuals infected with SARS-CoV-2.
Methods: We quantified IgG, IgA, and IgM levels against the SARS-CoV-2 Spike, Nucleocapsid, and Envelope proteins, as well as S2 and RBD Spike domains, in plasma obtained from individuals with severe (Group S, n=74) and mild (Group M, n=25) COVID-19 up to 31 days post-symptoms onset. We measured SARS-CoV-2-specific IgG and IgA avidity, neutralizing activity, and antibody-dependent cellular phagocytosis (ADCP) and cytotoxicity (ADCC).
Results: Group S had higher levels of anti-Spike IgA (p=0.0109), and anti-S2 IgA and IgG than Group M (p=0.0019 and 0.0076, respectively). No differences in neutralizing activity or avidity were detected between groups. Additionally, Group S exhibited greater ADCP and ADCC activities against RBD and S2 than Group M (ADCP-RBD: p=0.0167, ADCP-S2: p=0.0096, ADCC-S2: p=0.0244). After adjusting for IgG levels, Group S exhibited a slight reduction in ADCC (ADCC-S2/IgG: p=0.0594) and a modest increase of ADCP activity compared to Group M (ADCP-RBD/IgG: p=0.0563). However, Group S exhibited significantly lower ADCP activity than Group M (ADCP-S2/IgA: p=0.0164), when corrected by IgA levels. These results indicate that S2-specific humoral responses are distinct between those SARS-CoV-2-infected individuals that progress to either severe or mild COVID-19.
Conclusion: Our results demonstrate that individuals experiencing severe COVID-19 develop higher anti-S2 IgG and IgA titers, which are functionally distinct to those with milder COVID-19. Further work should deepen into the role of these antibodies in triggering the proinflammatory cytokine storm linked to the severity of COVID-19.

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211 – P3.12.07

B cell expression of the complement receptor 2 (CR2/CD21) constitutes a novel biomarker for COVID-19 severity

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Purpose: We previously detected a decrease in immunoglobulin (Ig) and complement levels in severe COVID-19 patients, suggesting an unbalanced Ig and complement consumption (Marcos-Jiménez et al.*Eur J Immunol*,2021). As part of the B lymphocyte co-receptor, complement receptor 2 (CR2/CD21) plays an essential role in B-cells function, linking the innate and adaptive humoral immunity. We therefore aimed to study the role of CD21 on B cells as a bridge between complement activation and the early humoral B-cells response in COVID-19 pathogenesis and clinical course.
Methods: Clinical and laboratory parameters of 162 subjects (aged 18-80 years) were collected during the second and third waves of the pandemic in Madrid (Spain), along with complement activation products and the serum Igs profile. Among them, we also immunophenotyped B-cells and plasmablasts from healthy donors (n=17) and patients who achieved a mild (n=16), moderate (n=18) or severe/critical/fatal (n=15) outcome, according to the WHO ordinal clinical severity scale.
Results: B-cells immunophenotyping revealed that their CD21 expression is lower in patients than in healthy donors. By performing ROC analysis, we established a cut-off point that distinguishes mild from moderate/severe patients. Multivariate analysis confirmed that CD21 expression at admission predicts disease severity (OR 0.059, p=0.003) and length of hospitalization (OR 0.0520, p=0.007). The significant variables in each multivariate model were considered to calculate the predicted probabilities of final outcome and hospitalization length, which showed that patients in the $CD21^{high}$ group are more likely to have milder disease and a hospitalization period of ≤ 7 days. In addition, in comparison with mild patients, moderate and severe/critical patients failed to increase the proportion of $IgG1^{+}$-plasmablasts after 5 days of hospitalization. Accordingly, specific α-RBD Ig levels on day 10 were lower in severe/critical patients than in milder/discharged ones.
Conclusion: Our results support the determination of CD21 expression on B-cells as an early prognostic biomarker for worse clinical course and a longer hospitalization period. From a practical point of view, this could represent a new tool for hospital management optimization. We are currently validating the results in other respiratory infections and investigating whether CD21 low expression associates with less effective humoral responses.

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224 – P3.12.08

Interleukin-36γ is causative for liver damage upon infection with Rift Valley fever virus in type I interferon receptor-deficient mice

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Objectives: Type I interferons (IFN) are pro-inflammatory cytokines which can also exert anti-inflammatory effects via the regulation of interleukin (IL)-1 family members. Several studies showed that interferon receptor (IFNAR)-deficient mice develop severe liver damage upon treatment with artificial agonists such as acetaminophen or polyinosinic:polycytidylic acid.
In order to investigate if these mechanisms also play a role in an acute viral infection, experiments with the *Bunyaviridae* family member Rift Valley fever virus (RVFV) were performed.
Methods and Results: Upon RVFV clone (cl)13 infection, IFNAR-deficient mice develop a severe liver injury as indicated by high activity of serum alanine aminotransferase (ALT) and histological analyses. Infected IFNAR$^{-/-}$ mice expressed high amounts of IL-36γ within the liver, which was not observed in infected wildtype (WT) animals. In line with this, treatment of WT mice with recombinant IL-36γ induced ALT activity. Furthermore, administration of an IL-36 receptor antagonist prior to infection prevented the formation of liver injury in IFNAR$^{-/-}$ mice, indicating that IL-36γ is causative for the observed liver damage. Mice deficient for adaptor molecules of certain pattern recognition receptors indicated that IL-36γ induction was dependent on mitochondrial antiviral-signaling protein and the retinoic acid-inducible gene-I-like receptor. Consequently, cell type-specific IFNAR knockouts revealed that type I IFN signaling in myeloid cells is critical in order to prevent IL-36γ expression and liver injury upon viral infection.
Conclusions: Our data demonstrate an anti-inflammatory role of type I IFN in a model for virus-induced hepatitis by preventing the expression of the novel IL-1 family member IL-36γ.

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265 – P3.12.09

Anti SARS-CoV2 properties of quinacrine and its analogues identified from Treg regulators screening

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The COVID-19 pandemic caused by SARS-CoV2 infection resulted in disrupted immune responses in many patients, presenting as increased inflammation and acute respiratory distress syndrome. Regulatory T cells (Tregs) are a subset of CD4 T cells that maintain immune response homeostasis in the body by suppressing effector T cells, which could also inhibit anti-viral immune responses. However, conflicting observations were reported with respect to function and proportions of Tregs relative to severity of COVID-19 infection. In a high-throughput screen to identify small molecules targeting FoxP3$^+$ Tregs in primary T cells, we found that the anti-malaria and anti-inflammatory drug quinacrine could down-regulate FoxP3 and inhibit Treg suppressive function. With a structure related to, yet distinct from, chloroquine and hydroxychloroquine that were evaluated as re-purposing drug during pandemic, we observed that quinacrine also harbored an inhibitory effect on SARS-CoV2 replication in cell-based assays. By checking analogs of quinacrine that were identified by *in silico* prediction, we found that compounds with comparable effects on anti-SARS-CoV2 activity as quinacrine also showed down-regulation on FoxP3. This suggested a shared anti-viral function based on a quinacrine-like compound structure. In contrast, chloroquine and hydroxychloroquine did not affect FoxP3 levels. Next, we examined the effect of a potent quinacrine analog MP4 in Golden Syrian hamsters infected with SARS-CoV2 virus, which demonstrated lower total viral loads in throat swabs and significant lower overall symptoms than the control group. By analyzing the lung samples from SARS-CoV2 infected hamsters, we observed lower FoxP3 levels in the MP4-treated group after 6 days of infection, together with reduced production of IL-4, GM-CSF and IFN-γ. However, after 10 days of treatment, lower IL-10 and higher TNFα were observed, implying recovery of the immune response. Furthermore, we investigated effects of quinacrine and its analog on virus antigen specific T cell responses. Our preliminary data showed that CMV-antigen specific CD8 responses were enhanced by quinacrine and its analog, represented as higher IFN-γ and TNF-α productions. We are investigating effects of our compounds on anti-SARS-CoV2 antigen specific T cell responses in ongoing experiments.

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276 – P3.12.10

The impact of infection dose on CD8 T cell diversity and differentiation in acute viral infection

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Cytotoxic CD8 T cells are essential in fighting pathogens by differentiating into subsets with specialized functions: short-lived highly cytotoxic effectors, and memory cells with poor effector functions, but with high proliferation and differentiation potentials. While CD8 T cell responses upon infection are very robust at the population level, they are very variable at the clonal level, even for naïve cells expressing the same T cell receptor (TCR).
At the population level, T cell fate (response size and functional state) is influenced by factors such as infection dose, antigen affinity, and the amount of antigen and inflammation. Generally, larger T cell responses are associated with more extensive differentiation, leading to a more cytotoxic fate. Whether response size and T cell function are fully linked remains unknown. Likewise, it is unclear how population-level changes in T cell fate are regulated at the clonal level.
Here, we set out to investigate how different factors influence the relationship between response size and T cell function at the population and clonal level in acute infection in mice. We found that at the population level, whether and how response size and T cell functional state correlates depends on the priming conditions and response kinetics. To investigate how population-level changes in T cell fate are regulated at the clonal level, we made use of the cellular barcoding technology. Naïve OT-I cells carrying a unique heritable barcode were generated by retroviral transduction of thymocytes. We determined the fate of individual T cells by measuring the clone size and the functional states (using CD27 and CX3CR1 expression levels) generated by each clone upon acute viral infection.
Our results show that the infection dose has an impact on the diversity of the clonal responses: a higher virus inoculum induces a higher diversity at the clonal level, but lower clone size disparity. Additionally, the correlation between clonal size and differentiation is increased when a higher dose is used. Understanding how diversity of T cell responses is generated at the clonal level is crucial for improving T cell-based therapies, such as vaccines or chimeric antigen receptor (CAR) T cell therapies.

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304 – P3.12.11

Higher frequencies of Memory Natural Killer cells and altered specific response to SARS-CoV-2 antigens in the lung from critical COVID-19 patients

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Purpose: Natural Killer (NK) cells may be deregulated in the lung of critical COVID-19 patients and contribute to the progression and pathology of the disease. NKG2C+ cells represent a subset of NK cells displaying memory-like properties and may specifically respond to antigens. However, alterations in this subset have not been fully elucidated in COVID-19.

Methods: N=19 peripheral blood (PB) and N=9 lung infiltrates from Bronchoalveolar Lavages and Aspirates (BAL-BAS, respectively) from hospitalized COVID-19 patients were recruited. PB from Healthy Donors (HD) were included as controls. Expression of CD56, CD16, NKG2C, CD57, TRAIL, TNFa and CD107a were analyzed in CD45+Lineage (CD3, CD19, CD14) negative NK cells at baseline or after 16h stimulation with SARS-CoV-2 Spike-1 (S1) or nucleoprotein (NP) peptides or with polyI:C. Histological analysis of NKG2C+ in lung tissue from deceased COVID-19 patients was also performed.

Results: Mature NKG2C+CD57+ cells were enriched in CD56lowCD16+ NK cells from blood but not in the lung of COVID-19 patients compared to HD (p=0.006). In contrast, frequencies of NKG2C+CD57- NK memory precursors were higher in pulmonary samples from these individuals (p=0.0052). Consistently, NKG2C+ cells were detected within highly infiltrated areas in lung parenchyma from deceased COVID-19 patients. We observed an increase in these NK precursors in HD exposed to SARS-CoV-2 S1 (p=0.07), while in COVID-19 patients this effect was observed in response to NP (p=0.04). Additionally, such differences in memory NK responses were associated with significantly higher levels of TNFa (p=0.03) and an increased expression of CD107a in NK cells lung samples compared to circulating NK cells (p=0.0303). Furthermore, the killing receptor TRAIL was expressed at higher levels in memory NKG2C+CD57- lung precursors compared to COVID-19 patients' blood (p=0.01). Finally, induction of these markers in memory NK cells from the lung was associated with higher levels of inflammatory parameters such as increased C-Reactive Protein (p=0.0027), Ferritin (p=0.016) and Procalcitonin (p=0.016).

Conclusion: Increased proportions of highly activated TRAIL+TNFa+ memory NKG2C+ in the lungs from critical COVID-19 patients may play a pathological role and represent an attractive target for future therapeutic strategies.

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320 – P3.12.12

Dual blockade with α-PD-L1 and α-TIGIT antibodies restores immune activation on dendritic cells and T-cells during LCMV chronic infection

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Purpose: Chronic viral infections are characterized by sustained immune activation, which promotes the overexpression of inhibitory receptors in T-cells, including PD-1 and TIGIT. The interaction of PD-1 and TIGIT with PD-L1 and CD155 on APCs induces cellular-specific inhibitory signalling. Continuous inhibitory signalling of immune checkpoint pathways drives cellular immune exhaustion in chronic viral infection. Here, we aimed to overcome immune exhaustion by dual-blocking TIGIT and PD-L1 inhibitory pathways in the LCMV murine chronic infection model.
Methods: C57BL/6JOlaHsd mice were infected with $LCMV_{DOC}$ (n=20) or left uninfected (n=5). Once the chronic state is reached 20 days after infection, we administered 3 intraperitoneal doses of single (α-PD-L1; n=5 or α-TIGIT; n=5), combined treatment (α-PD-L1+α-TIGIT; n=5); or saline (n=5). The mice were euthanized 28 days post-infection. Splenocytes were harvested and immunophenotyped for activation markers (CD40, CD86 on DCs and CD11b on T-cells) by flow cytometry. Additionally, we tested a panel of pro- and anti-inflammatory cytokines in serum by ELISA Multiplex. Last, we evaluated viral infectious units by focus-forming assay (FFA) in the spleen.
Results: The dual blockade with α-PD-L1+α-TIGIT significantly increased activation by CD40 and CD86 on DCs (p=0.0077 and 0.0435, respectively). Meanwhile, by single α-PD-L1 blockade, we only observed a trend of CD40 increase on DCs (p=0.0597). Consistently, we found a specific signature of cytokines in animals treated with α-PD-L1+α-TIGIT with significantly higher levels of TNF, TNFRI, RANTES and IL-10. In CD4+ and CD8+ T-cells, α-PD-L1+α-TIGIT and α-PD-L1 blockade significantly increased activation by CD11b expression (p=0.0420, 0.0094 in CD4+; p= 0.0049, 0.0010 in CD8+, respectively). We observed a trend towards an FFU reduction by single α-PD-L1 blockade in the absence of changes for the rest of the conditions. Single blockade with α-TIGIT did not reveal any differences compared to untreated-infected animals.
Conclusion: Our work characterizes the immunoregulatory effect of α-PD-L1+α-TIGIT dual blockade in LCMV chronic viral infection. Our data support an enhanced immune activation of DCs, T-cells and specific cytokine profiles of dual α-PD-L1+α-TIGIT blockade and suggest the combinatorial targeting of both immune checkpoint pathways to reinvigorate immune function in chronic viral infection.

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360 – P3.12.13

ULBP2-expressing HCMV mutants modulate NKG2D expression and function of CD8+ T cells

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Purpose: Solid organ transplant recipients with insufficient HCMV memory T-cell responses are at high-risk of viral reactivation and life-threatening complications due to immunosuppression. Especially for the high-risk group of the HCMV donor$^+$/recipient$^-$ constellation, a protective HCMV vaccine, which is currently not available, would not only reduce the risk for HCMV reactivation and disease in those patients. Therefore, we aim for an effective live-attenuated HCMV that should be able to induce HCMV-specific protective T cells. We attempted to compare the activating and costimulatory NKG2D ligand ULBP2 expressed by HCMV mutants in the presence/absence of immune-evasins for HLA class I molecules (US2-6, US10, US11) that are expected to differ in their ability to activate HCMV-specific CD8$^+$ T cells, respectively.
Methods: In the parental strains TB40 (ΔUS2-US6) or the TB40R containing the immune-evasins US2-US6, the UL16 region was replaced by ULBP2 which is expressed under a weak or strong promotor. Fibroblasts infected with these virus mutants were phenotypic characterized for ULBP2 and HLA class I expression. Co-culture experiments between partially matched virus-infected HFF and human PBMCs, were used to study CD8$^+$ T cell activation and NKG2D receptor modulation was investigated. Further, HCMV-specific CD8$^+$ T cells were analyzed by IFN-γ ELISpot assays
Results: Owing to the presence of US11, downregulation of HLA class I expression occurred in all infected cells. However, the downregulation of HLA class I expression was significantly stronger in TB40R-infected cells due to the expression of US2-6. Moreover, CD8$^+$ T cells were activated by the HCMV variants as evidenced by upregulation of CD69. Upon interaction with ULPB2, a significant down-modulation of NKG2D was observed on CD8$^+$ T cells and the intensity correlated with weak or strong ULBP2 expression. In addition, HCMV-specific CD8$^+$ T cells were found in response to our HCMV mutants, demonstrating high potential of ULBP2-expressing HCMV variants to elicit a cellular immune response. Co-stimulation via the ULBP2-NKG2D axis will be analyzed using the JE6.1 T cell line with fluorescent reporters for NF-κB, AP-1 and NFAT.
Conclusion: A promising approach for HCMV vaccine development is the deletion of HLA class I immune-evasins while expressing the costimulatory ligand ULBP2.

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398 – P3.12.14

Latent cytomegalovirus infection and age do not affect de novo SARS-CoV-2 specific CD8+ T cell frequencies

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Immunosenescence, the age-related dysregulated immune response, substantially contributes to reduced vaccine responsiveness, increased susceptibility to viral infections and is associated with higher morbidity and mortality in the elderly. Latent cytomegalovirus (CMV) infections contribute to immunosenescence, characterized by lower CD4:CD8 ratios, increasing terminally differentiated T-cell populations (T_{emra}) and accumulation of CMV-specific memory $CD8^+$ T-cells. To what extent the inflating CMV-specific memory and decreasing naïve T-cell populations may impact the immune response against novel pathogens is not fully understood. The SARS-CoV-2 pandemic presented a unique opportunity to investigate whether CMV-status affects the formation of a *de novo* $CD8^+$ T-cell response against a novel pathogen following primary infection in young and aged individuals.
Here we analysed the impact of CMV status (CMV^+/CMV^-) on the ability to generate *de novo* SARS-CoV-2-specific $CD8^+$ T-cell responses in 41 younger and 39 older convalescent SARS-CoV-2 donors. $CD8^+$ T-cells were analysed for their ability to recognize 35 SARS-CoV-2, 7 CMV, 2 Epstein-Barr virus and 2 influenza virus derived epitopes, restricted by 10 common HLA class-I allotypes, using heterotetramer combinatorial coding combined with phenotypic markers allowing in-depth *ex vivo* profiling of antigen-specific $CD8^+$ T-cell populations.
The frequency of SARS-CoV-2-specific $CD8^+$ T-cells was comparable between all groups regardless of CMV status and was consistent across different SARS-CoV-2 epitopes. Age was the predominant factor associated with a reduction in total $CD8^+$ $T_{naive\text{-}like}$ cells, while both age and CMV infections contributed to an increase in $CD8^+$ T_{emra} cells. Interestingly, SARS-CoV-2-specific $CD8^+$ $T_{naive\text{-}like}$ cells were decreased in older convalescent individuals, with the lowest frequencies observed in older CMV^- individuals. Interestingly, the highest SARS-CoV-2-specific $CD8^+$ T_{emra} and $PD1^+$ populations were observed in older CMV^- individuals, while the SARS-CoV-2-specific $CD8^+$ T_{emra} population of CMV^+ individuals were comparable to those observed in younger individuals regardless of CMV status.
Together, our results demonstrate that age and latent CMV infection do not impact the frequency of *de novo* generated SARS-CoV-2-specific $CD8^+$ T-cells. However, the observed differences in memory phenotypes may indicate that age and CMV status may impact long-term immunity against SARS-CoV-2

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444 – P3.12.15

Eucalyptus essential oil inhibits cell infection by SARS-CoV-2 spikepseudotyped lentiviral particles

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Despite mass vaccination, there is an ongoing emergence of new SARS-CoV-2 variants, resulting in new infections and potential reinfections, so, the virus is still a public health concern. Therefore, research into cost-effective preventive methods is of great value. Essential oils are known for their antimicrobial activity and compared with synthetic drugs, have fewer side effects and are easily accessible to the entire population. Among them, eucalyptus essential oil (EEO) and its main component, eucalyptol, have been shown to have anti-inflammatory properties and antiviral activity against common viruses. In this work, we investigated whether EEO and eucalyptol can prevent infection in an *in vitro* spike-dependent system. First, we determined the cytotoxic effects of these extracts on HEK293T cells by the MTT colorimetric assay. Then, we used SARS-CoV-2 Spike-pseudotyped lentiviral particles to infect hACE2-transfected HEK293T cells, incubating the lentiviral particles with non-toxic concentrations of EEO and eucalyptol. Viral infection was evaluated with a luciferase-based assay. We demonstrate that EEO and eucalyptol incubation decreased the infection rate by approximately 72% and 99%, respectively. In addition, incubation of EEO and eucalyptol with VSV-G pseudotyped lentiviral particles also showed a decreased infection rate on HEK293T cells. Hence, in this study, we report that EEO and eucalyptol are great candidates for preventing viral infection by SARS-CoV-2 through a spike-independent pathway.

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536 – P3.12.16

Impact of SARS-CoV-2 vaccine and infection history on antiviral immunity post breakthrough infection

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Purpose: Hybrid immunity characterizing individuals infected with SARS-CoV-2 before their vaccination regimen was more robust than immunity deriving solely from vaccination. Yet, when the Omicron variant emerged, it infected all vaccinated patient groups due to mutations in the Spike protein. How immunity adapted after Omicron breakthrough infection depending on the hybrid status remains a poorly understood question.
Methods: Using a longitudinal prospective cohort of 714 healthcare workers, we selected individuals with hybrid immunity (n=15) or only vaccination immunity (n=15) before a BA.1 breakthrough infection. Control groups included individuals with hybrid (n=30) or vaccinal (n=15) immunity without breakthrough infection. We investigated antibody levels and functions and analyzed anti-RBD memory B cells phenotype.
Results: The levels of anti-RBD antibodies were increased upon BA.1 breakthrough infection, with a trend toward higher levels in the group with vaccination-induced immunity. Similarly, neutralizing antibodies against Omicron sub-variants were slightly higher in this group. This correlates with an increase in antibody avidity toward the spike protein. In this group, there was also a decrease in memory B cells against the ancestral strain coinciding with an increase in BA.1 specific and cross-reactive memory B cells. However, ADCC was lower in this group, probably as a consequence of class-switching towards IgG4 compared to individuals with hybrid immunity. As expected, the latter individuals also showed the strongest anti-N responses upon breakthrough infection.
Conclusion: Hybrid immunity prevents the dampening of ADCC responses by IgG4 class-switching but slightly limits the emergence of neutralizing antibodies against BA.1 following a breakthrough infection possibly through strong immune imprinting. The recall of the anti-N memory response post BA.1 breakthrough infection among individuals with hybrid immunity underlines the potential of utilizing cross-reactivity between diverse SARS-CoV-2 strains for vaccination purposes.

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582 – P3.12.17

Critical COVID-19 unveils the link between viral particle blood dissemination and prolonged Type I Interferon

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Purpose: A significant portion of patients suffering from hypoxemic SARS-CoV-2 pneumonia, insufficiently controlled by early type I interferon (IFN) immunity in respiratory epithelium, experienced a delayed and prolonged IFN response during hospitalization. Employing a comprehensive approach, we aimed to identify host and viral factors associated with severe COVID-19 in subjects with type I IFN response.
Methods: Prospective collection of blood and nasal samples from mild and critical COVID-19 patients allowed us to compare N-antigenemia, humoral immunity, pulmonary and endothelial damage, virological parameters via metagenomics, and innate immune responses through circulating proteins and transcriptomic markers. Simultaneously, we used a human primary respiratory epithelium model to evaluate the impact of the IFN response on the integrity of the epithelium.
Results: Over 89% of critically-ill patients showed plasma N-protein positivity at ICU admission, with the strongest correlation found between viral particle blood dissemination and a sustained type I IFN response. Additionally, we demonstrated that sustained type I IFN pathway activation can disrupt the respiratory epithelium, facilitating the virus's transition from the respiratory compartment to circulation, thereby involving the host in a detrimental cycle.
Conclusion: These results suggest that viral particles may trigger systemic type I IFN responses, potentially explaining the observed delayed responses in critically-ill patients. Moreover, our results suggest that IFN response is essential for the preservation of respiratory epithelium's integrity. Taking together, these findings underscore the critical importance of the timing and duration of type I IFN immunity in COVID-19.

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610 – P3.12.18

The immune landscape of antiviral responses in multiple sclerosis

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Multiple sclerosis (MS) is an immune-mediated chronic inflammatory disease of the central nervous system. There is a long-lasting association between MS and viral infections, which have profound influences on the immune system. The strong correlation between Epstein-Barr virus (EBV) and MS has recently gained more support. The allostatic perturbation resulting from viral infection is capable of triggering specific antiviral responses that can cause autoimmune reactions and support neuroinflammation through direct and indirect mechanisms. To better understand how the immune system can be modulated and activated by viruses, freshly isolated Peripheral Blood Mononuclear Cells (PBMCs) were drawn for the parallel study of both innate and adaptive immunity in untreated, treated people with MS (pwMS) and healthy donors (HD).

For the study of innate immunity, whole blood was stimulated with R848, mimicking a viral infection. The responses of the innate immune cells were studied by multiparametric flow cytometry analysis, that allowed to investigate 10 myeloid subpopulations and the production of 8 different pro-inflammatory cytokines and chemokines. For the study of adaptive immunity, PBMCs were isolated from whole blood and challenged over night with overlapping peptides pool spanning the entire sequences of several specific viral proteins. To fully capture the antigen-specific T cell response and to maximize sensitivity, two separate assays for the detection of the expression of surface activation-induced markers (AIM) and for intracellular cytokine staining (ICS) were set up to evaluate the immune antiviral responses against Epstein-Barr, Cytomegalovirus (CMV), Influenza and Sars-Cov-2 viruses. This study revealed that untreated MS patients exhibit a robust monocyte response to r848, which is accompanied by an anergic response from DCs. The adaptive immune reactivity to viruses implicated in MS pathogenesis displays varying patterns of responsiveness. In contrast, patients who received a high efficacy immune depleting therapy, showed a significant decrease of EBV virus-specific T cells responses. Collectively, these insights contribute to our understanding of the interplay between viral infections, immune dysregulation, and MS pathogenesis.

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617 – P3.12.19

Investigating non-structural proteins (NSPs) as novel targets for a SARS-CoV-2 and pan-coronaviridae vaccination

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Purpose: SARS-CoV-2 vaccine efforts have reduced infection rates and fatalities associated with COVID-19. However, SARS-CoV-2 still poses several challenges. Firstly, the high mutation rate of the major vaccination target, Spike, has led to the evolution of variants of concern able to evade vaccine induced immunity. Preparation for emerging human coronaviridae (HCoV) is also imperative. Therefore, this work aims to investigate targets for pan-coronaviridae vaccination, to provide protection against SARS-CoV-2 alongside current and emerging HCoVs. Specifically, we investigate non-structural proteins (NSPs), characterised by their indispensable roles early in the virus life cycle and high conservation across HCoVs.
Methods: Following in vitro expansion, we assessed the T-cell responses of recently infected individuals to 47 highly conserved peptides, derived from the NSP proteins. Cell lines were first screened for T-cell reactivity to pools of peptides and then screened against the individual peptides comprising the highlighted peptide mix. Intracellular cytokine staining for IFNg was used to determine whether T-cell responses were mediated by CD4+ or CD8+ T-cells. Subsequently, from successfully expanded NSP-specific T-cell lines, T-cell clones were generated by limited dilution cloning and characterised.
Results: Most donors displayed a response to at least one NSP peptide tested. This included responses to several NSP proteins from which peptides were derived, with both CD4+ and CD8+ T-cell responses identified. NSP-specific T-cell clones isolated were of high avidity and restricted through several common HLA alleles.
Conclusion: Responses to highly conserved NSP derived epitopes are common in recently infected individuals. The T-cell epitopes identified in this study are highly conserved across HCoVs and restricted through common HLA alleles, suggesting that T-cell cross-reactivity and therefore pan-coronaviridae reactivity could be likely. Further work is required to assess whether T-cells against these proteins can protect from infection.

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649 – P3.12.20

Localized Immunological Memory in Cynomolgus Macaques Following SARS-CoV-2 Infection

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection induces localized immunological memory, notably within the lungs and lymph nodes of humans. Our study aims to assess if similar observations can be recapitulated in cynomolgus macaques, a model for SARS-CoV-2 infection, and determine whether this model can be used to further investigate the potential of localized immune memory in preventing re-infection. Samples from the upper respiratory tract, blood, spleens, lymph nodes, and lungs of healthy and SARS-CoV-2-infected macaques were analyzed using qPCR, TCID50, flow cytometry, intracellular cytokine staining, and enzyme-linked immunosorbent spot assay. Advanced techniques such as single-cell RNA-sequencing and spatial transcriptomics (ST) were utilized to explore lung-resident immunological memory. Following the resolution of SARS-CoV-2 infection, significant $CD4^+$ and $CD8^+$ T cell responses and germinal center activities were observed in lymphoid and lung tissues, with gene ontology analysis revealing an enhancement in immune and inflammatory response pathways. ST analysis highlighted distinct immune responses within lung structures, notably in inducible bronchus-associated lymphoid tissue and alveoli, emphasizing their crucial role in localized defense against SARS-CoV-2. Our findings confirm that, akin to humans, cynomolgus macaques develop localized immunological memory following SARS-CoV-2 infection, with marked immune activation in lung-specific regions essential for countering reinfection. These findings highlight the critical role of local immune memory in SARS-CoV-2 prophylaxis, providing valuable perspectives for the formulation of targeted immunological interventions and vaccine development.

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760 – P3.12.21

Quicker and greater innate immune responses correlate with disease severity after controlled influenza infection of healthy adults

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Purpose: Influenza is a respiratory infection the severity of which varies considerably between individuals. Despite extensive research, morbidity and mortality remain high, while pathogenesis and immune determinants of influenza severity in humans are incompletely understood. Controlled human infection (CHI) studies involve the deliberate inoculation with pathogens and has the unique capacity to investigate host responses before, during and after infection while controlling for factors such as virus strain, dose and exposure. Here we report how early post-infection immune responses distinguish symptomatic and asymptomatic infection in volunteers challenged with influenza A(H3N2).
Methods: Healthy adults aged 18-55 years with serum neutralising antibody titres ≤1:20 were enrolled for inoculation with influenza A/Belgium/4217/2015 (H3N2) virus (SGS CPU, Belgium) intranasally, at a dose of $5x10^5$ TCID50 in 0.5ml PBS. Following inoculation, participants were quarantined until day 10 and returned for assessment and sampling at days 14, 28 and 180. Symptoms were monitored using self-reported symptom diaries. Viral load was quantified in nasal lavage fluid by qPCR. Soluble mediators were measured by multiplexed protein assays, cellular responses by flow cytometry and antibodies by ELISA, haemagglutination inhibition assay, and microneutralisation. Transcriptomic analysis was performed using RNA sequencing.
Results: Following inoculation of 27 individuals, 22 (81.5%) developed PCR-confirmed infection while 5 remained uninfected. Eighteen infected participants (81.8%) developed symptoms, with 4 remaining asymptomatic. A consistent pattern of greater and more rapid innate and adaptive antiviral responses was seen in symptomatic participants compared with asymptomatics. Early monocyte and dendritic cell activation correlated positively with symptom scores, soluble mediators, $CD8^+$ T and NK cell proliferation and later antibody responses.
Conclusion: The speed and magnitude of innate responses correlate with positive clinical and later immunological outcomes but also more severe symptoms after influenza infection. These findings suggest that early innate immune responses are a double-edged sword that could be fine-tuned by improved treatments and vaccines to mitigate disease, without preventing viral clearance.

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792 – P3.12.22

Autoantibodies neutralizing type III interferons are uncommon in patients with severe COVID-19 pneumonia

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Purpose: Autoantibodies neutralizing type I interferons (IFN) underlie about 15% of cases of critical COVID-19 pneumonia. The impact of autoimmunity towards type III IFNs remains unexplored

Methods: We included samples from 1002 patients with COVID-19 (50% with severe disease) and 1,489 SARS-CoV-2-naïve individuals. We studied the prevalence and neutralizing capacity of autoantibodies towards IFNλ and IFNα. Luciferase based immunoprecipitation method was applied using pooled IFNα (subtypes 1, 2, 8 and 21) or pooled IFNλ1-3 as antigens, followed by reporter cell-based neutralization assay.

Results: In the SARS-CoV-2-naïve cohort IFNλ autoantibodies were more common (8.5%) than those targeting IFNα2 (2.9%) and were less associated with older age. In the COVID-19 cohort the presence of autoreactivity to IFNλ did not associate with severe disease (OR 0.84; 95% CI 0.40-1.73), unlike to IFNα (OR 4.88; 95% CI 2.40-11.06; $p<0.001$). Most IFNλ autoantibody positive COVID-19 samples (67%) did not neutralize any of the three IFNλ subtypes. Pan-IFNλ neutralization occurred in five patients (0.50%), who all suffered from severe COVID-19 pneumonia, and four of them neutralized IFNα2 in addition to IFNλ.

Conclusion: Autoantibodies to type III IFNs are rarely neutralizing, and do not seem to predispose to severe COVID-19 pneumonia on their own.

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813 – P3.12.23

Screening and Application of SARS-CoV-2 Derived T cell Epitope Spectrum

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Purpose: Identifying a range of dominant SARS-CoV-2 derived T cell epitopes restricted by most prevalent HLA-A/DR allotypes for developing T cell-based vaccines and evaluating specific cellular immune responses to SARS-CoV-2 in most population.
Methods: We utilized multiple bioinformatics methods to predict candidate epitopes, and collected unexposed donor samples to perform dendritic cell-peptide-peripheral blood lymphocyte coculture experiment to assess the immunogenicity of candidate epitopes. Then, we collected several convalescents' PBMCs to culture with each validated epitope, and defined the HLA restriction and positive rate of epitope in population with prevalent HLA-A/DR allotypes. Several representational epitopes and inactivated virus were combined and generated a cocktail vaccine to immunize in HLA-A2/DR1 transgenic mice, and the humoral and cellular immunity elicited by this vaccine was systemically assessed. Based on ELISpot, we established SARS-CoV-2 specific $CD8^+$ T cell and $CD8^+$ T detection system.
Results: In this study, 93 $CD4^+$ T cell epitopes were screened by modified DC-peptide-PBL cell coculture system using healthy donor PBMCs. Subsequently, 117 $CD8^+$ T cell epitopes that were previously screened and 93 $CD4^+$ T cell epitopes were validated by convalescent individuals, covering most of the Chinese population in terms of HLA allotypes. For HLA-A restricted peptides, 117 of 117 (100%) could induce a specific $CD8^+$ T cell response in at least four samples. For HLA-DR restricted peptides, 91 of 93 (96.5%) could induce a specific $CD4^+$ T cell response in at least one sample. 27 epitopes restricted by HLA-A2 molecule, 19 epitopes restricted by HLA-DR1 molecule, and inactivated virus were combined and generated cocktail vaccine, and it elicited robust humoral and cellular immunity compared to single form vaccine in HLA-A2/DR1 transgenic mice. Moreover, SARS-CoV-2 specific T cell detection system based on ELISpot was established by validated T cell epitopes and exhibited great detection ability in unexposed and convalescent samples.
Conclusion: We screened and validated several SARS-CoV-2 derived T cell epitopes restricted by the most prevalent HLA-A/DR allotypes for facilitating the development of diagnostic, vaccine, and therapeutic measures for COVID-19.

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871 – P3.12.24

Clinical evaluation of HBV-specific T cell reactivity in CHB patients using a broad-spectrum T-cell epitope peptide library and ELISpot assay

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Purpose: The clinical routine test of HBV-specific T cell reactivity is still limited for random patients due to the lack of universal detection kit, thus the clinical implication remains disputed.
Methods: An universal ELISpot assay was set up using a peptide library which consists of 103 functionally validated CD8+ T-cell epitopes spanning overall HBsAg, HBeAg, HBx and HBpol proteins and fits to the human leukocyte antigen polymorphisms of China and Northeast Asia populations. 203 CHB patients were detected, and 33 CHB patients were longitudinally detected for 3 times with an interval of 3-5 months.
Results: Although the numbers of reactive HBV-specific T cells in PBMCs presented a significantly declined trend along with gradually increased serum viral DNA loads, HBsAg, HBeAg and ALT levels in CHB patients, but with a very low negative correlation coefficient (r = -0.21, -0.21, -0.27, -0.079, respectively). HBsAg-, HBpol-, HBx- or HBeAg-specific T cells maintained the tendencies similar to total HBV-specific T cells. NUCs/IFN-α combination led to much more reactive HBV-specific T cells than NUCs monotherapy. NUCs treatment more than 4 years presented higher reactivity of HBV-specific T cells than the NUCs treatment less than one year, especially TMF treatment, but different NUCs at the same treatment duration did not bring different reactivity of HBV-specific T cells. The dynamic numbers of reactive HBV-specific T cells were obviously increasing in CHB patients undergoing routine treatment, and came along with the gradual decline of serum viral DNA, HBsAg, HBeAg and ALT levels. The longitudinal trend of HBV-specific T cells possess an increased predictive power than the cross-sectional detection for liver function progression in CHB patients.
Conclusion: HBV-specific T cell reactivity mainly depends on host immune defense function rather than viral DNA load and antigens level in CHB patients undergoing routine treatment and their dynamic trend is a valued predictor for liver function progression.

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930 – P3.12.25

Recovery plasma from patients with COVID-19 and intravenous immunoglobulin as rescue passive immunization in SARS-COV-2 infection

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Purpose: To investigate the presence of specific anti-SARS-CoV-2 IgG and IgA antibodies in plasma from donors who recovered from COVID-19 and in IVIG batches produced from plasma collected during COVID-19 pandemic.
Methods and Materials: Donor convalescent plasma samples of 90 individuals collected in the period from 01.2021 to 06.2021 after SARS-CoV-2 infection and 13 batches IVIG derived from human plasma collected from 10.2021 to 03.2022. S1-binding SARS-CoV-2-specific IgG and IgA were determined by semiquantitative ELISA (Euroimmune, PerkinElmer Germany Diagnostics GmbH, Germany). According to the manufacturer's instructions for Etest/E calibrator > 1.1 is defined as a positive result). Neutralizing anti-SARS-CoV-2 antibodies were detected with ProcartaPlex Human SARS-CoV-2 Variants Neutralizing Antibody Panel 6plexl. The assay enables direct comparison of the neutralizing potential of antibodies towards the original wild type virus and five variants B.1.1.529 (o), B.1.617.2 (δ), P.1 (γ), B.1.351 (β), and B.1.1.7 (α). According to the manufacturer's instructions >20% of neutralization is positive result.
Results: Specific IgG was detected in 50/90 samples (55.5%), with levels ranging between 1.12 and 8.37, mean 5.19. Anti SARS-CoV-2 IgA was also detected in 50/90 samples (55.5%), mean (min-max) 5.84 (1.19 - 9.41). The levels of the two types of anti-SARS-CoV-2 specific immunoglobulins did not differ significantly (paired T-test p>0.05) The highest mean result of neutralization was detected against B.1.1.529 (o) (85%) and the lowest mean (54%) against B.1.617.2 (δ).
In IVIG specific IgG were detected in all 13 batches with levels ranging between 4.3 and 12.5, mean 9.3 compared to the control group of 3 batches with levels mean 0.4. The highest mean result (92%) of neutralization was detected against B.1.1.7 (α) and the lowest mean (75%) against P.1 (γ).
Conclusion: The effective use of CCP for passive immunization requires their preliminary examination for the presence and titter of SARS-CoV-2 specific IgG and IgA antibodies. The presence of specific Anti S1-IgG in Intravenous immunoglobulin enables their reliably effective use for passive immunization.

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1010 – P3.12.26

Characterization of a VSVΔG S (SARS-CoV-2) hybrid replicating virus as a possible model of mild COVID-19 disease

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SARS-CoV-2 has a broad spectrum of severity, ranging from mild to fatal. Several unknowns remain about the pathology of SARS-CoV-2, particularly about the dynamics of long COVID-19 (PASC). A limitation in the study of SARS-CoV-2 is the availability of facilities that support risk group 3 research. By using a VSVΔG S hybrid replicating virus, the study of spike-mediated viral migration and pathology can be done in a level 2 lab. This study aimed to characterize the immune effects of VSVΔG S (original) using molecular imaging and immune phenotyping.
This study tested two titres of VSVΔG S (SARS-CoV-2 original): $5x10^4$ PFU/mL and $1x10^5$PFU/mL, administered intranasally at a volume of 30µL per mouse. Naïve and VSVΔG (empty) controls were also used. The K-18 hACE2 mouse model was chosen as its hACE2 expression pattern matches that of humans. Weekly anatomical and fluorodeoxyglucose positron emission tomography (FDG-PET) imaging was used to track increased glucose metabolism as a marker of viral-associated inflammation in a murine model. Flow cytometry (FC) was performed on weekly blood samples and terminal organ cells to investigate immune cell population changes.
Early imaging results show a moderate increase in FDG concentration in lungs of infected mice compared to naïve and VSVΔG controls. Temporal increases in FDG uptake were observed in the heart, lungs, brain, spleen, and kidneys for both infected groups, indicating sustained inflammation up to 4 weeks post-infection. FC data revealed heightened percentages of neutrophils, monocytes, eosinophils, basophils, and NK cells in infected mice. Conversely, CD4+ T cells notably decreased in infected groups versus controls.
Immune cell data suggests a milder disease phenotype compared to literature reports of that produced by full SARS-CoV-2 infection in the same mouse model. Elevated myeloid cells, NK cells, and reduced CD4+ T cells match reports of mild to moderate disease and are associated with PASC. The absence of an animal model for PASC underscores the significance of the findings, as our hybrid replicating virus offers a promising option for *in vivo* study of this phenomenon.

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1016 – P3.12.27

Coinfection of VSVΔG S (SARS-CoV-2 omicron) and E0771.lmb breast cancer results in novel immune phenotypes and increased tumour volumes

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SARS-CoV-2 causes a systemic infection, with many patients experiencing a prolonged effect on the immune system, referred to as post-acute sequelae of SARS-CoV-2 infection (PASC). PASC is still not well understood, and due to the wide range of people affected, can lead to complications with a variety of diseases, including cancer. We have recently demonstrated that a hybrid replicating virus (HRV), VSVΔG, with the omicron spike protein, is a CL-2 pathogen demonstrating immunopathology like a mild SARS-CoV-2 infection/PASC. We wanted to characterize the co-occurrence of a chronic SARS-CoV-2 infection with cancer to understand systemic responses and evaluate disease and therapy responses impacted by infection.
This study used a K18-hACE2 mouse model. A subset of mice were implanted with E0771.lmb breast cancer cells, while others were infected with $1X10^6$PFU/mL of a VSVΔG S (SARS-CoV-2 omicron) HRV. Experimental groups included cancer only, virus only, and both, with infections occurring 3- or 14 days post-implant (representing different cancer stages). Fluorodeoxyglucose positron emission tomography (FDG-PET)/magnetic resonance imaging was performed weekly, and flow cytometry was performed on weekly blood draws and terminal organ harvests.
Co-infection of cancer and VSVΔG S showed increased FDG uptake in tested organs compared to cancer or VSVΔG S alone groups, particularly in the tumours, lymph nodes, and spleen. This corresponded to increased levels of NK cells and decreased CD4+ T cells and plasma cells in the same organs. Furthermore, D14-infected mice have increased macrophages in spleens and tumours. Tumour volumes were increased in combination groups compared to cancer alone, with the D14-infected group showing the largest tumours.
Intact VSV is known to be an oncolytic virus; therefore, the increase in tumour sizes in combination groups was unanticipated. This suggests that observed immune cell changes indicate a more immunosuppressive environment, with cells becoming pro-tumour or senescent. In D14-infected mice, it is possible that the addition of VSVΔG S when tumours were already established pushed the immune system into a state of exhaustion. This work represents valuable steps towards understanding these co-infections.

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1057 – P3.12.28

Mutations within the NS1 immunodominant T cell epitopes of the dengue virus serotype-2 cosmopolitan strain resulting in large outbreaks in Sri Lanka

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Purpose: Although the dengue virus (DENV) NS1 protein independently causes disease pathogenesis, there are limited data regarding NS1- specific T cell responses. Therefore, we sought to identify immunodominant epitopes within NS1, in individuals with past and acute DENV2 infection alongside mutational analysis of the DENV2 sequences in Sri Lanka to further characterize possible immune escape in these epitopes.
Methods: We utilized IFNγ ELISpot assays to map immune-dominant regions in those with past dengue (n=36) and characterized responding T cell subset by flow cytometry. To explore mutations within the immunodominant T cell epitopes within NS1, DENV2 strains from patients with acute dengue during 2017-2018 (n=59) were sequenced and analyzed. As key mutations were detected in the immunodominant T cell epitopes, we further evaluated the frequency of IFNγ ELISpot responses to the regions in the reference peptide (R22) in those with acute dengue fever (DF=35), dengue haemorrhagic fever (DHF=13) and healthy subjects (n=10). The probable HLA restriction was evaluated using SYFPEITHI (http://www.syfpeithi.de/) epitope prediction program.
Results: Individuals with past dengue (before 2016) showed the highest frequency of responses to a region within the wing domain of NS1(18/36). Out of the wing domain peptides, the highest responses were to the R22 peptide (9/20). This was found to be a CD4+ T cell epitope. However, 2017-2018 DENV2 isolates contained a T164M substitution in this epitope. Remarkably, T cell responses to the R22 bearing the T164M substitution (which we refer to as SL22) elicited a significantly higher frequency of T cell responses (p=0.04) in those with DHF (median10521, IQR 3030-20106 SFU/ million T cells) compared to those with DF (median 4900.6, IQR 615.69-8570.6 SFU/1 million T cells). The SYFPEITHI tool predicted that out the possible HLA restriction of this epitope to be through DRB1*1101(score=24) or HLA-DRB1*0701(score=22).
Conclusion: T cell responses to DENV2 NS1 epitopes were chiefly focused on the highly conserved wing domain region. As those with DHF had a higher frequency of responses to the peptide with the T164M mutation, it would be important to investigate if T cells specific to this region contribute to disease pathogenesis.

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1146 – P3.12.29

B cell populations in COVID-19 and post-acute impaired olfaction

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Background: There have been concerns about the effect of SARS-CoV-2 on the immune system, especially concerning "long COVID" or post-acute sequelae of COVID-19 (PASC). It has been reported that immunological dysfunction persists in PASC patients for at least 8 months post-COVID, including diminished naïve B cells. Previous studies have found that in acute COVID-19, plasmablasts and double negative B cells (DNs) are elevated and memory B cells are decreased. We studied the levels of different B cell subpopulations in the blood of COVID-19 patients in acute disease and convalescence and with and without PASC.
Methods: We collected a cohort of COVID-19 patients (n = 43) of varying disease severities in 2020-2021. Blood was collected at 1-3 different timepoints and PBMCs were isolated, stained for flow cytometry and analysed using the BD LSRFortessa cytometer. We also surveyed the patients for impaired olfaction as proxy for PASC (PASC+ n = 7, PASC- n = 16, no data n = 20). We divided the samples into acute (<30 d from symptom onset), convalescent (30-120 d) and recovered (>120 d) phases.
Results: We found that transitional, plasma, and plasmablast cells were proportionally higher and anergic, B1 and class switched memory cells lower in acute disease when compared with convalescent/recovered. Transitional cells were also significantly higher and anergic and class switched memory cells lower in the convalescent phase as compared with the recovered. We compared outpatients with inpatients: in acute disease non-switched and switched memory cells, B1 cells and CD21low CD38- cells were lower and plasmablasts higher in the inpatients. In recovered patients, higher proportions of anergic B cells could be found in the inpatients. In comparisons between PASC+ and PASC-, the only significant difference was found in the recovered phase, where naïve B cells were proportionally higher in PASC+ patients.
Conclusions: The B cell response to acute COVID is focused on rapidly acting APCs and maintaining homeostasis by producing new B cells. Memory cells are initially low, possibly due to the germinal center reaction not activating yet. Our finding of higher naive cells in PASC+ patients at 3-12 months post-COVID contrasts previous research.

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1171 – P3.12.30

Primary antibody response after influenza virus infection is first dominated by HA-stem antibodies followed by HA-head antibodies, which then remain stable over years

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Current influenza vaccines mainly induce antibodies against immunodominant, but highly variable, parts of the head domain of the hemagglutinin (HA) protein of influenza and therefore need to be updated yearly. New vaccine concepts are being developed that aim to generate broadly cross reactive antibodies against more conserved stem region of the HA protein. In humans, low numbers of stem-binding antibodies are present. However, as influenza exposure in humans is often complex and difficult to trace, it can be challenging to determine the origin of these stem-binding antibodies. Therefore, we investigated the antibody response against HA over time in non-human primates as an influenza naive animal model, that is physiologically and immunologically closely related the human. During initial infection with influenza A/Mexico/InDRE4487/2009 (Mex4487; $H1N1_{pdm2009}$) virus, we observed that the initial antibody response to HA stem in serum is relatively high, but decreases over time. Conversely, the response to the immunodominant HA head region starts low and plateaus at day 56 after challenge. Four years after the initial infection, both the anti-HA head and HA-stem antibody response were maintained at comparable levels to the day 56 values. In conclusion, primary influenza virus infection can result in long lived anti-stem antibody responses.

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1222 – P3.12.31

SARS-CoV-2 infection with the Alpha variant B.1.1.7 induced higher levels of IgG antibodies against spike, receptor-binding domain and nucleocapsid than infection with Wuhan-like variants in a Norwegian prospective, longitudinal COVID-19 household study

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Purpose: SARS-CoV-2 infection leads to a variety of different clinical outcomes. Higher degree of severity and specific symptoms of disease have shown to be associated with higher levels of SARS-CoV-2 binding antibodies. Here, we have studied antibody trajectories in adult participants with mostly mild SARS-CoV-2 infection included in a Norwegian household study between May 2020 and April 2021. We investigated whether the antibody responses were associated with virus variant (Wuhan-like or Alpha (B.1.1.7)), symptoms, severity of disease, or viral load.
Methods: SARS-CoV-2 infection was confirmed by PCR. Questionnaire data and symptom diaries were collected. Serum samples were collected during home visits on days (D) 0, 7, 14, 28, 42 and 180. IgG levels against spike (S), the receptor binding domain (RBD) and nucleocapsid (N) were measured using an in-house multiplex assay based on original Wuhan antigens. Viral load was measured using digital-droplet PCR, and the virus isolates were whole genome sequenced. Antibodies inhibiting RBD-ACE2 interaction were measured as a proxy for neutralizing antibodies. Multivariate longitudinal analyses are ongoing.
Results: In infected individuals (n=107), antibody levels increased over time until D42, and subsequently decreased until D180. Individuals infected with the Alpha variant had higher anti-S, anti-RBD, and anti-N IgG levels, showed greater inhibition of ACE2-RBD-interaction, and had higher viral loads than individuals infected with Wuhan-like variants. Fever and chills were associated with higher levels of anti-N antibodies, whereas high viral load was not. No specific symptoms were associated with higher levels of anti-S or anti-RBD IgG. Self-reporting of more severe disease seemed to be associated with higher anti-N IgG levels and increased inhibition of ACE2-RBD interaction, but these findings were not significant. Further analyses will be conducted.
Conclusion: This study indicates a higher immune activation by the more transmissible Alpha variant compared to earlier virus variants in non-hospitalized COVID-19 cases with mild disease. Moreover, we show that increased severity of disease is associated with increased anti-N-IgG responses, as previously shown for more severe SARS-CoV-2 infection.

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1223 – P3.12.32

Altered frequencies, phenotypes and functions of mucosa-associated invariant T cells and Vδ2+ γδ T cells in patients with severe COVID-19

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Purpose: COVID-19 exhibits a wide spectrum of clinical manifestations ranging from asymptomatic infection to severe respiratory insufficiency and extrapulmonary manifestations. Severe disease is associated with pulmonary inflammation and lymphopenia. We investigated the potential involvement of innate T cells, including invariant natural killer T (iNKT) cells, γδ T cells and mucosa-associated invariant T (MAIT) cells in patients with acute COVID-19.
Methods: Peripheral blood mononuclear cells (PBMC) from 16 patients with severe COVID-19, 22 with moderate disease, 3 with mild disease and 5 healthy donors were recruited from the STTAR biobank at St. James's Hospital, Dublin. The frequencies of MAIT cells, iNKT cells and the Vδ1, Vδ2 and Vδ3 subsets of γδ T cells, their expression of activation, differentiation, exhaustion and homing markers, and their production of interferon-γ (IFN-γ), tumour necrosis factor-α (TNF-α), interleukin-2 (IL-2), perforin, granzyme B and CD154 in response to stimulation with phorbol myristate acetate with ionomycin, anti-CD3 and anti-CD28 antibodies, IL-12 with IL-18 and the Vδ2 T cell ligand (E)-4-Hydroxy-3-methyl-but-2-enyl pyrophosphate (HMB-PP) was analysed by multicolour flow cytometry.
Results: The frequencies and phenotypes of Vδ1, Vδ3 and iNKT cells were similar in healthy donors and in patients with mild, moderate and severe disease. MAIT cell frequencies were significantly reduced in patients with moderate and severe disease and these cells more frequently expressed CD69, PD-1 and CCR6. The production of IFN-γ, TNF-α, IL-2, perforin, granzyme B and CD154 by ex vivo stimulated MAIT cells was similar in the 4 subject groups. The frequencies of Vδ2 T cells were similar in the 4 subject groups but compared to healthy donors, Vδ2 T cells from patients with moderate and severe disease had increased expression of PD-1, but not CTLA-4 or TIM-3. Vδ2 T cells from these patients were impaired in their ability to produce IFN-γ upon activation. However, no differences in the production of TNF-α, IL-2, perforin, granzyme B or CD40L were observed.
Conclusion: MAIT cells and Vδ2 T cells may promote antiviral immunity in patients with COVID-19 but appear to be functionally impaired in patients with severe disease.

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1262 – P3.12.33

Selective autophagy impedes KSHV entry after recruiting the membrane damage sensor galectin-8 to virus-containing endosomes

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When microorganisms invade host cells and access the cytoplasm, host defense mechanisms are deployed to direct pathogens to degradation by selective autophagy. Kaposi sarcoma-associated herpesvirus (KSHV) is an oncogenic ɣ-herpesvirus associated with several opportunistic malignancies, including the 4th most common cancer in Sub-Saharan Africa, Kaposi sarcoma. Numerous studies have investigated autophagy upon lytic reactivation of KSHV, however autophagy involvement in KSHV entry has remained unexplored. Using *in vitro* infection of human epithelial cell lines, we show that lipidation of the autophagy hallmark LC3 is induced shortly after KSHV entry. When components of the LC3 lipidation complex were depleted, infection increased. Accordingly, nuclear dot protein 52 kDa (NDP52), a receptor of selective autophagy, was recruited to endocytosed viral particles, and its reduction increased KSHV infection. Additionally, virus particles co-localized with both NDP52 and the endolysosome damage sensor galectin-8 upon KSHV entry and depletion of galectin-8 promoted KSHV infection. Using herpes simplex virus, listeriolysin, adenovirus and influenza virus as references, the KSHV data here reveal that, in contrast to what was previously thought about enveloped viruses, KSHV binding to EphA2 by its envelope protein gH could cause endolysosomal membrane damage, akin to non-enveloped viruses and bacteria. Taken together, our study identifies an important antiviral role for galectin-8 in the host cell-autonomous immune response to KSHV infection by recruitment of NDP52 and the autophagy machinery to virus-damaged endosomes. The resulting selective autophagy seems to restrict KSHV entry.

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1349 – P3.12.34

Reduced specific antibody response to SARS-CoV-2 vaccination associated with high clozapine dosages

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Purpose: Schizophrenia, affecting about 1% of the population, entails higher mortality rates and a lifespan reduction of 10 to 20 years. Around 30% of cases are resistant to conventional antipsychotic treatments, leading to the use of clozapine. While clozapine reduces overall mortality in severe schizophrenia cases, it's linked to various side effects, including a heightened risk of infections and pneumonia. Recent studies suggest that clozapine has immunomodulatory effects. Individuals on chronic clozapine treatment show patterns similar to primary immunodeficiencies, potentially reversible upon drug discontinuation, and inadequate vaccine responses. Given these findings and the COVID-19 pandemic, some studies have reported a higher risk of SARS-CoV-2 infection in individuals with psychotic spectrum disorders treated with clozapine.
This study aims to analyze the immune response to vaccination with the Spike (S) protein of SARS-CoV-2 in schizophrenia patients treated with clozapine, compared to those treated with other antipsychotics.
Methods: The study included 98 patients with schizophrenia and schizoaffective disorder, of whom 69 patients were taking clozapine. Demographic, clinical, and laboratory data were collected from all included patients. Antibodies against the S protein (Ab-S) were tested using the Elecsys® Anti-SARS-CoV-2 S test (Roche Diagnostics International Ltd., Rotkreuz, Switzerland).
Results: No significant differences were found in demographic, clinical, and laboratory data between patients with schizophrenia and schizoaffective disorders on clozapine, compared to those taking other antipsychotics. In the univariate analysis, Ab-S levels were increased in patients with smoking habit (Fold-Change, FC = 5.36, p=0.026) and with > 2 exposures to the virus (FC = 23.42, p < 0.001), and decreased with time since vaccination (Spearman Correlation, SC = -0.443, p<0.001) and with the clozapine dose (SC = -0.278, p=0.021). In the multivariate analysis, a significant decrease in Ab-S levels was observed in patients taking doses >350 mg/day of clozapine compared to those not taking clozapine (FC = 0.49, p=0.041), and to those on <200 mg/day of clozapine (FC = 0.39, p=0.024).
Conclusion: The use of high doses of clozapine in patients with schizophrenia and schizoaffective disorders is associated with a reduced response to vaccination against the S protein of SARS-CoV-2.

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Immune recovery biomarkers in PLWH on long-term cART and CD4AC above 500

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Purpose: Contemporary antiretroviral therapy (cART) has significantly increased the average life expectancy of people living with HIV (PLWH) while not resolving the issue of HIV reservoirs and low-level immune activation. We have previously described mitochondrial mass (MM) and membrane potential (MMP) as possible biomarkers of accelerated immune ageing in cART+ PLWH. The aim of this study was to investigate the applicability of superoxide dismutase activity in plasma (SOD3) and peripheral blood mononuclear cells (PBMC, SOD1) for monitoring of PWLH with complete response to cART.
Methods: Whole blood and freshly isolated PBMC from 20 PWLH (median age 46y, range 28-57y) on long-term cART (median 8.5, range 3-24y) with undetectable HIV VL, and CD4 absolute count (AC) >500 were analyzed. CD45RA, CD25, CD127, CD39, CCR7, CD27, CD28, CD57 and TIGIT expression, as MM and MMP in CD4 and CD8 T cells were analyzed by flow cytometry as previously described (FACSCanto II, BD Biosciences and Thermo Fisher). SOD3 and SOD1 activity were determined with Superoxide Dismutase assay kit (Sigma Aldrich). Statistical analyses were carried out with SPSS v. 23.
Results: In spite of restored CD4AC (median 748.0, range 576-1264), CD4/CD8 ratio largely varied among samples (median 1.12, range 0.5-2.12). Neither SOD1, nor SOD3 activity were associated with CD4/CD8 ratio, age or cART duration. SOD3 activity correlated directly with percentage of Treg (rho=.47, $p<0.05$), while high SOD1 activity was associated with a higher level of functional CD27+TIGIT-CD4 T (rho=0.48, $p<0.05$) and CD39 expression on Treg (rho=.511, $p<0.05$). Interestingly, in the settings of restored CD4/CD8 ratio (>0.9), a higher SOD1 activity correlated with higher CD4 T MM (rho=0.8, p=0.01) and higher MMP in both CD4 (rho=.75, $p<0.05$) and CD8 (rho=.77, $p<0.05$) T cells, while SOD3 was inversely correlated with CD57+ T cell subsets.
Conclusion: CD4/CD8 ratio is not sensitive enough for monitoring of PLWH on long term cART; SOD1, SOD3 and mitochondrial parameters may better describe accelerated immune ageing.

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Vδ2 T cells effector response in PLWH and PLWoH up to three months from mpox infection

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Purpose: The first evidence that *Orthopoxviruses* induced the *in vivo*-expansion and the recall of effector innate Vδ2 T-cells was described in a macaque model. Although, it was analysed an engagement of αβ T-cells specific response in patients infected with human monkeypox (mpox), little is known about the role of γδ T-cells during mpox infection. IFN-γ-producing γδ T-cells in the resistance to poxviruses may be a key role in inducing a protective type 1 memory immunity by influencing the effectiveness of vaccines. In this study, we analysed the kinetics of Vδ2 T-cells from symptoms onset (FSO) up to three months after mpox infection.
Methods: Nine MSM subjects (4 PLWH and 5 PLWoH) with confirmed mpox, were enrolled in a longitudinal study from May to July 2022. PLWH were all viro-immunologically suppressed. Blood samples were collected in the early phase of infection (T1) and at 3 months (T3) FSO. Vδ2 T-cells profile (CD45RA/CCR7), activation/exhaustion markers expression (CD38/HLA-DR/CD57/PD-1/TIM-3), cytokines production (IFN-γ/TNF-α) and CD107a expression after non-peptidic antigen stimulation, were assessed by multiparametric flow cytometry. Kinetics of Vδ2 T-cells response were compared with 13 healthy donors (HD) matched by sex and age. Mann-Whitney and Wilcoxon tests were used.
Results: At T1, Vδ2 T-cells frequency was lower than HD ($p<0.01$); in addition, an expansion of Effector Memory Vδ2 T-cells was observed ($p<0.002$), paralleled to a decrease of Central Memory Vδ2 T-cells ($p<0.0001$), reaching HD values after T3 FSO. Activation/exhaustion markers were significantly increased at T1 and resulted lower after T3. HLA-DR expression was higher in PLWH than PLWoH. No differences were observed for the other markers according to PLWH/PLWoH stratification. Vδ2 functionality decreased at T1 when compared to HD and it was associated to a CD38 higher expression ($p<0.02$). At T3, Vδ2 T-cells response was restored and seems linked to TIM-3 expression ($p<0.009$).

Conclusion: The presence of effector/activated Vδ2 T-cells in the early stages of infection and their capability to activate quickly, producing pro-inflammatory cytokines may be useful to enhance the early adaptive response to human mpox for the maintenance of the protective memory/effector T-cells response.

Founding: Ministry of Health, Programma Ricerca Corrente Linea 2.

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1578 – P3.12.38

Inflammatory milieu and specific T cells response after three months and one year from SARS-CoV-2 infection

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Purpose: Post-COVID-19 condition (PCC) is characterized by a plethora of symptoms, whose aetiology is not fully understood. The aim of this study was to analyse inflammatory/coagulative factors and the specific T-cells response to SARS-CoV-2 and investigate if they are associated with the presence of PCC.
Methods: Adult participants, diagnosed with SARS-CoV-2 during 2020-2021 were enrolled. Blood and plasma samples were collected after three (T3M) and 12 months (T12M) post-acute COVID-19. Demographic and clinical data were collected during each visit and structured in an electronic system. Plasma inflammatory (IL-6, IL-8, TNF-a, IL-1b) and coagulative factors (D-Dimer, E-sel, ICAM, VCAM) were tested by Elisa at T3M and T12M. Peripheral blood mononuclear cells (PBMC) were stimulated with Spike (S) and Nucleocapside (N) peptides to analyse SARS-CoV-2 specific T-cells response. IFN-γ production was quantified by Elispot assay. Mann-Whitney/Wilcoxon tests were used.
Results: We included 196 subjects between 2020-2021: 39% female, with a median age of 56.5 years. 78% of them were previously hospitalized for COVID-19. The median time between SARS-CoV-2 infection and the post-COVID evaluation was 86 days (78-91), 34% reported post-COVID symptoms, of which 68% with only 1 symptom. Most symptoms were respiratory (54%), followed by asthenia (30%) and neuropsychological (14%). At T3M and T12M, according with symptoms onset (presence or not), the inflammatory profile generally didn't change (IL-8, TNF-a, IL1b) except for IL-6 which decreased significantly from T3M to T12M (p=0.02), with no differences according to symptoms. All coagulation factors showed a general increase at T12M (D-Dimer: p=0.0002; E-Sel and VCAM: p=0.0001; ICAM-1 p=0.006), independently from symptoms. Spike response was higher than N at T3M (p=0.0009), by reaching the same level at T12M, and was not associated with symptoms. A significant expression of inflammatory/coagulative factors was measured a T12M post-infection.
Conclusions: In this cohort, inflammatory/coagulative milieu analysis showed altered coagulation factors one year after the acute COVID-19, by meaning that SARS-CoV-2-damage lasts for a long time after infection. Moreover, T-cells specific response was detected one year after COVID-19, even though lower than that observed 3 months post-infection, suggesting protective memory T-cells response maintenance overtime.

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Immune responses in HSV-2 genital herpes and HSV-2 meningitis: a comparative study

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Genital herpes, caused by herpes simplex virus type 2 (HSV-2), is a sexually transmitted infection with a prevalence of 13% worldwide. The main symptoms of HSV-2 are genital ulcers, though some carriers are asymptomatic. In rare cases however, individuals infected with HSV-2 can develop meningitis. It is poorly understood why some individuals develop meningitis, and to what extent the innate and acquired immune system play a role.
To address this question, we use the multivariate method OPLS-DA to identify immune response patterns that differentiate patients with meningitis (n=49) to those with genital herpes (n=38). The multiplex analyses include a panel of HSV-2-induced T cell cytokine responses, innate antiviral cytokine responses and chemokine responses as well as levels of HSV-2 type-specific serum antibodies and antibodies indicating co-infection with herpes simplex virus type 1 (HSV-1). OPLS-DA analysis shows that the immune profile differed significantly between the two different HSV-2 disease outcomes. Both baseline levels of cytokines as well as virus-induced immune responses vary significantly between patients with HSV-2 genital herpes and patients with HSV-2 meningitis and could to 67% explain the differences between these two disease outcomes.

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1655 – P3.12.40

TfR-1 as a potential therapeutic target in severe SARS-CoV-2 infection by mitigating macrophage activation syndrome

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Purpose: The COVID-19 pandemic has raised questions about the role of iron metabolism due to elevated ferretin levels in severe cases, linking it to hyperferritinemic syndrome and serious conditions like macrophage activation syndrome and septic shock. Thus, in this study we aimed to investigate changes in macrophage iron metabolism in a human primary system, avoiding animal-derived components where possible to mimic the *in vivo* situation. We analyzed key regulators of iron metabolism – Transferrin Receptor (TfR), Ferroprotein 1 (FPN1), Hepcidin, Ferritin, IRP-2, HIF-1, IL-6, IL-1β and IL-10 and examined their gene and protein expression as well as localization upon virus infection.
Methods: M1 macrophages from healthy donors were exposed to SARS-CoV-2 variants of concern (VoC). After 4 to 24 hours (with and without TfR-1-blocking antibody treatment), we performed virus neutralization assays and analyzed pro- and anti-inflammatory cytokine patterns and iron-regulating proteins. Moreover, viral copy numbers were determined by absolute quantification, and virus localization, TfR, and FPN-1 levels were illustrated by confocal microscopy.
Results: SARS-CoV-2 VoCs exhibited different cytokine and iron-regulating protein profiles. While Omicron sub-variants BA.5 and XBB1.5 showed low levels of inflammatory signals and iron regulation, the VoC Delta induced these to significantly higher levels. Thus, Delta exhibited an altered iron regulation. We could illustrate a correlation between pro-inflammatory signals mediated by Delta and altered iron regulation. If blocking TfR-1, IL-6 production was signifincantly reduced in Delta-exposed M1 macrophages, suggesting its potential as a therapeutic target during SARS-CoV-2 infection, dysregulated iron homeostasis, and associated cytokine storm.
Conclusion: Within a primary, human macrophage model, we demonstrated that SARS-CoV-2 variants differently shape pro- and anti-inflammatory signals, crucial for cellular iron balance, in M1 macrophages. In particular, Delta, which was associated with more severe COVID-19 compared to the more recent Omicron variants, exerted an altered inflammatory pattern and iron regulation with signs of TfR retention and macrophage activation syndrome, thus highlightening TfR-1 as potential therapeutic target.

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Interleukin-2 responses to whole spike antigen differentiate healthcare workers with frequent symptomatic SARS-CoV-2 infection and asymptomatic infection

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Purpose: Healthcare workers (HCWs) are at increased risk of SARS-CoV-2 infection. A proportion of HCWs develop frequent symptomatic COVID-19 despite vaccination, while others have no evidence of historic infection or symptomatic disease. We propose that differences in polyfunctional T cell responses contribute to different disease phenotypes, with highly functional cellular immune responses in asymptomatic individuals allowing rapid virus clearance and preventing the development of clinical disease.
Methods: The PRECISE Study, established in 2020, is a multi-centre longitudinal HCW cohort study investigating serological and clinical parameters of COVID-19 infection. Nucleocapsid antibody levels were measured in October 2020, April 2021, November 2021, and December 2022. HCWs reporting no prior SARS-CoV-2 infection with negative anti-N antibodies at all timepoints were considered never-infected/asymptomatic, while those with positive anti-N antibodies and more than one COVID-19 infection between December 2022 and September 2023 were considered frequent infections. Whole blood stimulation assays were used to measure the cellular response to spike antigens of the Wuhan, Delta (B.1.617) and Omicron (BA.2, BA.2.75, BA4/5, XBB.1.5, BQ.1.1) variants of SARS-CoV-2, with supernatant IFNγ and IL-2 levels measured after 24 hours. Univariate analysis with Chi-squared and Mann-Whitney U tests were used to assess differences in demographics and immunological outcomes.
Results: N=15 frequent infections and n=19 asymptomatic/never infected were recruited between November 2023 – January 2024. Repeat anti-N antibody testing demonstrated that n=10 of the never infected had developed detectable levels, suggesting asymptomatic infection since prior testing in December 2022. There were no differences in age (U=120.5, p=0.45), sex (χ^2=0.60, p=0.44) or COVID-19 vaccine status (χ^2=0.12, p=0.73) between groups. There are no differences in IFNγ responses between the groups to any variant. Frequent infections have a significantly lower IL-2 response to Wuhan (U=51, p=0.001) and XBB.1.5 (U=77, p=0.02), with no significant differences across other variants.
Conclusions: HCWs with frequent infections significantly abrogated IL-2 responses to spike antigen from the original Wuhan strain, and do not demonstrate increased IL-2 responses to Omicron variants, despite likely being infected by these variants. This may support functional T cell defects underling recurrent symptomatic COVID-19 infection in individuals at ongoing risk of SARS-CoV-2 exposure.

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1663 – P3.12.42

Significance of IgE antibodies against SARS-CoV-2 in different clinical conditions

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Purpose: COVID-19 appears to have a progression of three stages. The latter stages are characterized by a high level of cytokine release, which in turn triggers an uncontrolled reaction known as cytokine storm. Mast cells are involved in the cytokine storm. The presence of anti-IgE antibodies against SARS-CoV-2 in this phase has been previously reported, and appears to correlate with the severity of the disease. Our study aims to confirm the prognostic significance of IgE antibodies against SARS-CoV-2 across a spectrum of clinical presentations, including asymptomatic cases, individual with mild symptoms, hospitalized patients, and those requiring ICU admission.
Methods: The study included 87 patients distributed into the following groups: 22 critically ill hospitalized individuals (Critical); 21 non-critical hospitalized patients (Severe); 21 mild symptomatic non-hospitalized cases (Mild); and 23 healthy blood donors with samples collected in October 2019. Demographic, clinical, and laboratory data were collected from these patients. Anti-IgE antibodies against Spike (S) protein were detected using a homemade ELISA, where the plate was sensitized with the RBD of recombinant S protein. The cutoff point was established to achieve an assay specificity of 91.3% based on levels in healthy donors.
Results: Among 64 SARS-CoV-2 infected patients, 28.1% tested positive for IgE isotype antibodies against S protein RBD, whose prevalence was similar across severity groups: Mild 23.8%, Severe 28.6%, and Critical 31.8% (p=0.842). Patients with IgE response exhibited higher levels of LDH compared to non-IgE responders, with a 40% increase (p=0.031), and a non-statistically significant increase in other inflammatory markers. The frequency of obesity, dyslipidemia, and intensive care needs were twice as high in non-IgE respondents, with a statistically significant difference observed in dyslipidemia (p=0.027). Conversely, IgE responders were more frequently treated with Remdesivir (4% vs 25%) and twice as high for Tocilizumab, with only the former showing statistical significance (p=0.048).
Conclusion: In SARS-CoV-2 infection, roughly a fourth of patients develops an IgE isotype response. Although this response is homogeneous across different severity groups of patients in the studied cohort, there is an association trend with higher levels of inflammatory parameters, especially LDH and receiving treatment with Remdesivir.

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1759 – P3.12.43

Fluoxetine treatment improves the clinical outcome of age-dependent severe COVID-19

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Severe coronavirus disease 2019 (COVID-19) continues to pose a significant threat, particularly among vulnerable populations such as the elderly and immunocompromised individuals. Coagulopathy and dysregulation of the coagulation cascade are recognized as key risk factors associated with the progression of SARS-CoV-2 infection to severe COVID-19. Additionally, recent studies have shed light on the potential role of platelets in modulating the antimicrobial immune response in various respiratory infection models through the uptake and conversion of serotonin. Notably, fluoxetine treatment, an inhibitor of serotonin uptake in platelets, has shown promise in improving clinical outcomes in COVID-19 patients.
In light of these findings, our study aims to investigate the impact of fluoxetine treatment in an age-dependent severe COVID-19 preclinical model. Specifically, we infected both old and young adult C57BL/6 mice with mouse-adapted SARS-CoV-2, resulting in distinct clinical outcomes based on the age of the mice. Old mice exhibited exacerbated clinical parameters and impaired pulmonary functions, which correlated with increased platelet activation and diminished infiltration of monocytes in the respiratory airways. However, upon preventive fluoxetine treatment, old mice demonstrated an ameliorated clinical outcome comparable to that observed in young adult mice. This phenotype was characterized by reduced weight loss, improved respiratory functions, and a concomitant reduction in platelet aggregation alongside enhanced monocyte infiltration. Results from this study suggest that fluoxetine treatment reverses the age-dependent severity of COVID-19 typically observed in elderly C57BL/6 mice following MA-CoV-2 infection. Our preliminary data indicate that this treatment leads to a reduction in platelet aggregation and activation, along with an increased infiltration of monocytes, which is typically associated with improved COVID-19 outcomes.
In future investigations, our focus will be on elucidating the cellular and molecular mechanisms underlying the observed improvement in clinical outcomes in fluoxetine-treated elderly C57BL/6 mice. Specifically, we aim to investigate whether a potential platelet-monocyte axis may underlie the observed phenotype.

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In-depth analysis of Respiratory Syncytial Virus-specific Fc-mediated antibody effector functions in children and adults

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Respiratory syncytial virus (RSV) infections are a major cause of bronchiolitis and pneumonia in infants and older adults, for which there is no known correlate of protection. Increasing evidence suggests that Fc-mediated antibody effector functions play an important role in protection, but much remains unknown about these functions in different age groups. In light of future vaccination strategies, a clear view of the immunological background and differences between various target populations is of crucial importance. We have previously shown that RSV-specific antibodies of adults are better inducers of antibody-dependent NK cell activation(ADNKA) than those of children, which is not explained by antibody levels. To further investigate this, the current study aims for a further in-depth analysis of RSV-specific serology in children and adults.
Here, we have assessed different aspects of RSV post-F-, pre-F-, and N-specific serum antibodies, including IgG/IgA levels, IgG subclasses, IgG avidity, neutralization titers, antibody-dependent complement deposition(ADCD), cellular phagocytosis(ADCP), and ADNKA. Samples were collected cross-sectionally in 2-year-old children and adults (n=46 per group) in the winter of 2015/2016.
We found that RSV-specific serum IgG levels were generally comparable between children and adults. In contrast, RSV-specific IgA levels and IgG avidity were significantly higher in adults. Additionally, several differences were found in antigen-specific IgG subclasses between children and adults. Furthermore, adults generally show increased antibody functionality (neutralization, ADNKA, and ADCP) compared to children, even after correction for antibody levels. Despite a strong correlation between Fc- effector functions and IgG1 levels, antibody levels alone do not fully explain the observed differences in functionality. IgG avidity does not correlate with Fc-effector functions *within* age groups, but does seem to influence the increased antibody functionality in adults compared to children. Surprisingly, IgG3, known for its high potency in inducing Fc-effector functions, also did not correlate with functionality and was generally lower in adults than children.
Together, these data provide an overview of the functional landscape of RSV-specific serum antibodies in these age groups, highlighting significant differences between children and adults. This knowledge may support the rational design of future vaccination strategies.

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The pathogenesis of MIS-C involves a distinct T cell profile compared to pediatric COVID-19 cases

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Purpose: Multisystem inflammatory syndrome in children (MIS-C) presents with fever, inflammation, and multi-organ damage subsequent to infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Despite the typically mild course of SARS-CoV-2 infection in children, the factors contributing to the development of MIS-C remain ambiguous. Recent investigations into COVID-19 have highlighted the pivotal involvement of T cells in orchestrating the immune response. Our study aims to elucidate the potential contributions of cytotoxic T cell subsets (CTL) and regulatory T cells (Tregs) in the pathogenesis of MIS-C.
Methods: 17 MIS-C, 17 pediatric COVID-19 cases and 17 healthy control were included in the study. Flow Cytometry evaluation was performed with a 10-color MoAb panel from peripheral blood samples.
Results: It was observed that effector memory T cells (EM; $CD3^{+}CD8^{+}CD45RA^{-}CCR7^{-}$) were decreased in MIS-C compared with pediatric COVID-19 cases and healthy control group. Effector memory 1 (EM1; $CD45RA^{-}CCR7^{-}CD27^{+}CD28^{+}$) CD8+ T cells increased in MIS-C compared to pediatric COVID-19 cases. Effector memory 2 (EM2; $CD45RA^{-}CCR7^{-}CD27^{+}CD28^{-}$) $CD8^{+}$ T cells decreased in MIS-C compared with pediatric COVID-19 cases and healthy controls. Exhausted CD8+ T cells ($PD\text{-}1^{+}$), naive ($CD45RA^{+}FoxP3^{low}$), activated affector ($CD45RA^{-}$ $FoxP3^{high}$), and nonsuppressive ($CD45RA^{-}$ $FoxP3^{low}$)Treg cells, decreased in MIS-C cases compared with pediatric COVID-19 cases and healthy controls.
Conclusions: The observed decline in the frequency of CD45RA-CCR7- effector memory (EM) CD8+ T cells within peripheral blood, known for their tropism towards non-lymphoid tissues, alongside an augmentation in the rapidly activated CD8+ T cell subsets (EM1, EM2) expressing the co-stimulatory molecule CD28, as well as elevated levels of effector (E) CD8+ T cells, signify notable immunological alterations in patients with MIS-C. Furthermore, the re-expression of CD45RA in antigen-induced senescent T cells, coupled with the downregulation of CD28 and CD27 expression, alongside a reduced abundance of exhausted CD8+ T cells and Tregs, collectively suggest a potential association with sustained inflammatory responses and subsequent multi-organ injury in MIS-C patients.

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1813 – P3.12.48

TLR7 deficiency accelerates severe COVID-19 through reduced IRF7 activation and interferon production

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TLR7 deficiency-accelerated severe COVID-19 is associated with reduced production of interferons (IFNs), a key innate immunity. However, the underlying mechanisms remain elusive. Here, we utilized *Tlr7* and IFN regulatory factor 7 (*Irf7)* deficient mice, single-cell RNA seq, and bone marrow chimerism studies to define the Tlr7 pathway in vivo. The deficiency of *Tlr7* globally or in immune cells accelerated severe COVID-19 via impaired IFN activation in both immune and non-immune cells, leading to an increased lung viral load from 2 to 14-day-post-infection (DPI). IRF7 is specifically activated by TLR7 activation for regulating IFN production at 2 DPI. Despite higher amounts of lung viral antigen, *Tlr7* or *Irf7* deficiency resulted in substantially reduced production of antibodies against SARS-CoV-2, thereby delaying the viral clearance. These results highlight the importance of the activation of *Tlr7* and *Irf7* leading to the IFN production on both the development of innate and adaptive immunity against COVID-19.

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1817 – P3.12.49

Immunological Profile of Long COVID Patients Reveals Persistent Virus-Specific T Cell Response

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Purpose: We aimed to explore the pathogenetic mechanisms underlying Long COVID (LC).
Methods: We conducted a cross-sectional study at a post-COVID-19 center in Brazil from September 2020 to February 2021, involving non-vaccinated individuals with confirmed SARS-CoV-2 infection that were fully recovered or developed LC symptoms. Standardized medical evaluations were performed to collect comprehensive sociodemographic, clinical, and quality of life data, ensuring a well-characterized population. IgG for SARS-CoV-2 RBD, EBV and CMV were measured and Peripheral Blood Mononuclear Cells were obtained and cryopreserved within four hours post-collection. After 24-hour of stimulation with SARS-CoV-2 antigens, CD4+ T cell populations were sorted for single-cell sequencing (scRNAseq) of whole-genome and T-cell receptor (TCR). Pseudobulk analysis was performed to account for dependency across cells of the same individual.
Results: scRNAseq was performed for 18 LC cases and 5 recovered individuals. In naïve CD4+ T cells, we identified a gene expression profile distinctive to LC, that may be potentially useful as a biomarker of LC. In AIM+ CD4+ T cell, 9 genes were significantly upregulated in LC, including IFITM1, a gene that codes interferon-induced transmembrane proteins, implicated in facilitating SARS-CoV-2 invasion. Transcriptional analysis of TCRs revealed that SARS-CoV-2-specific AIM+ CD4+ T cells were more frequently observed in LC patients compared to recovered (median: 8.3% vs 5.1%, p=0.03). However, TCRs specific to other pathogens like CMV and EBV did not show significant differences between the groups, suggesting a selective, ongoing immune response in LC specifically targeted towards SARS-CoV-2. No difference was observed in the serologic analysis of IgG anti- SARS-CoV-2 RBD, EBV and CMV of 76 LC patients and 25 recovered.
Conclusion: These findings suggest a persistent and specific immune activation in individuals with LC, marked by distinct gene upregulation and a prevalence of virus-specific T cells. The absence of a generalized hyperactivation of the immune system suggests a targeted immunological dysregulation in LC. The improved comprehension of T-cell-mediated responses in LC provides new opportunities for therapeutic intervention, potentially guiding precision medicine approaches to manage and treat the long-term sequelae of COVID-19. Further research is essential to explore the utility of these molecules as potential biomarkers for LC.

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1908 – P3.12.50

A new perspective on cellular complement and how opsonized HIV 1 enhances dendritic cell maturation and survival via Mcl-1 stabilization regulated by anaphylatoxin receptors

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The complement system is an ancient component of innate immunity. Its functions were previously believed to be limited to recruiting other immune cells and recognizing/destroying invading pathogens. However, recent studies have revealed the system's multiple roles in immune regulation and its interaction with other cellular effector systems. Recent evidence indicates that complement has additional functions within the immune system beyond pathogen recognition and immune recruitment.
Monocyte-derived dendritic cells (moDCs) were infected with HIV or complement-opsonised HIV (HIV-C) in the presence or absence of specific inhibitors for 24 hours. The expression levels of complement factors and cathepsins were measured, along with intra- and extracellular anaphylatoxin generation, regulation of anti-apoptotic Bcl-2 family members, as well as cell stress, maturation, and survival.
It was demonstrated that HIV-C promotes elevated anaphylatoxin production, maturation, and increased survival by stabilizing the anti-apoptotic Bcl-2 family member Mcl-1 through phosphorylation at threonine 163. This stabilization is regulated by the interaction of anaphylatoxin C5a and its receptor (C5aR) since inhibition of C5aR impeded this stabilization. The upstream pathways that control this specific phosphorylation event are dependent on MAP kinase and, to a lesser extent, protein kinase C. Inhibition of either pathway resulted in reduced Mcl-1 stabilization. This work demonstrates a complement-dependent mechanism that improves DC survival and maturation. This could represent a novel approach for enhancing antigen presentation and protective immunity against viral infections.

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1916 – P3.12.51

Latent human cytomegalovirus infection is not associated with decreased vaccine effectiveness against SARS-CoV-2 in patients with stage 5 chronic kidney disease

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Purpose: Human cytomegalovirus (CMV) is associated with immunosenescence and contributes to greater morbidity and mortality. The association with reduced vaccine effectiveness is inconclusive. Recent reports suggest that latent CMV infection decreases vaccine-induced serologic response. Additionally, patients with stage 5 chronic kidney disease experience accelerated senescence and vaccine hyporesponsiveness. We aimed to analyze the association between latent CMV infection in the population with CKD stage 5 and the SARS-CoV-2 infection-free survival after completion of the primary vaccination series.
Methods: We conducted a retrospective, single-center analysis of the time to first confirmed SARS-CoV-2 infection in patients with stage 5 CKD. The study included a healthier subset of patients who were candidates for renal transplantation and whose CMV serostatus was available. Patients with prior SARS-CoV-2 infection and unavailable vaccination data were excluded. Time to first SARS-CoV-2 positive test and vaccine type were recorded, irrespective of the symptoms. Patients were censored at death or transplantation.
Results: We included 263 patients. CMV-negative patients were younger (median 47 vs. 56 years), but otherwise did not differ in terms of sex, diabetes, dialysis vintage, atherosclerosis, and smoking history. While time to first infection was expectedly lower for unvaccinated individuals (log-rank, $p<0.0001$), we observed a better protection with ChAdOx1 vaccine (log-rank, $p = 0.02$). The CMV serostatus was not associated with better vaccine effectiveness (log-rank, $p = 0.61$). In a Cox regression analysis, adjusted for age, latent CMV was not associated with a different risk of infection. Interestingly, when comparing survival curves before VOCs circulated, there was an apparent lower risk of infection in CMV-negative patients. However, the numbers were small and the association was not significant ($p = 0.088$). The effect of booster vaccination was not analyzed.
Conclusion: In our cohort, we found no evidence that latent CMV infection reduced the effectiveness of the anti-SARS-CoV-2 vaccines. Interestingly, there seems to be a non-significant difference in the period before the emergence of VOCs, but the number of cases was small. Given the retrospective design and limited sample size, and not-per-protocol testing, our results remain inconclusive and highlight the need for further research.

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1935 – P3.12.52

Flow cytometric immune cell subset analyses for the diagnosis of post-COVID syndrome and myalgic encephalomyelitis/chronic fatigue syndrome

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Introduction: Post-COVID syndrome (PCS) is a heterogeneous, multisystemic clinical picture that occurs in 5 - 10 % of patients after mild to moderate Coronavirus disease 2019 (COVID-19). Symptoms persist for over three months after severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection and include fatigue, postexertional malaise (PEM), respiratory distress, pain, and cognitive dysfunction. A subset of PCS patients meets the Canadian Consensus Criteria (CCC) or the Institute of Medicine (IOM) criteria for the diagnosis of myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS). Evidence suggests immunological dysregulation with studies having identified inflammation, antibody-mediated autoimmunity, vascular inflammation and SARS-CoV-2 persistence as potentially involved pathomechanisms. The lack of diagnostic tests and the prevalence of the disease made this project urgent and important.
Objective: To assess the state of research and own results of single-cell RNA-sequencing (scRNA-seq) analyses, cellular signatures were analyzed by flow cytometry comparatively in PCS/ME/CFS patients and convalescent healthy controls (CHC).
Methods: The suitability of flow cytometric immunophenotyping was investigated by comparison of staining of "untouched" lysed whole blood (WB) and isolated and cryopreserved peripheral blood mononuclear cells (PBMCs) from 9 PCS/ME/CFS patients and 10 CHC 6 - 12 months after SARS-CoV-2 infection. Several panels to characterize immune cell subsets, inflammation and activation markers were applied.
Results: Immunophenotypical alterations were found in PCS/ME/CFS patients, including higher frequencies of CD4+ effector memory T cells and decreased frequencies of CD69+ and CD16+ subsets. Furthermore, frequencies of $CD56_{dim}$ NK cells and CX3CR1+ NK cell subsets were decreased. Frequencies of CXCR4+ monocytes were increased. While the different staining procedures had no impact on immune cell subset alterations of T cells, NK cells, and monocytes, they impacted detection/analysis of activation markers such as CD69 and chemokine receptors CX3CR1 and CXCR4.
Conclusion: Various alterations in immune cell subset frequencies and activation markers were found in PCS/ME/CFS. Further we found that processing procedures impact the expression of activation markers and chemokine receptors, which should be considered in future analyses. The here identified immunological dysregulations in a small cohort of PCS/ME/CFS patients provide a basis for future biomarker research.

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1993 – P3.12.53

An IFNγ-dependent immune-endocrine circuit lowers blood glucose to potentiate the innate anti-viral immune response

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Viral infection makes us feel sick. The extent of these changes to our metabolism are relative to the severity of disease. Whether blood glucose levels are subject to infection-induced modulation is largely unknown. Here we show that strong, non-lethal infection restricts systemic glucose availability which promotes the antiviral IFN-I response. Following systemic viral infection of mice, we find that IFNγ produced by γδ T cells directly stimulates pancreatic β-cells to increase glucose-induced insulin release. Subsequently, hyperinsulinemia lessens endogenous glucose output by the liver. Glucose restriction enhances type-I interferon production by curtailing lactate-mediated inhibition of IRF3 and NF-κB signaling. Induced hyperglycemia constrained IFN-I production and increased mortality upon infection. Our findings identify glucose restriction as a physiological mechanism to bring the body into a heightened state of responsiveness to viral pathogens. This immune-endocrine circuit is disrupted in hyperglycemia, which explains why patients with metabolic disease are more susceptible to viral infection.

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2003 – P3.12.54

Myeloperoxidase and Xanthine oxidase in association with T cell subsets of people living with HIV on cART with sustained viral suppression

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Objectives: Xanthine oxidase (XO) and Myeloperoxidase (MPO) play a crucial role in inflammation and oxidative stress in many pathological conditions.
The aim of this study was to evaluate the levels of plasma MPO and XO of people living with HIV (PLHIV) on combined antiretroviral therapy (cART), with sustained viral suppression and to analyze their association with T cell subsets.
Material and Methods: Peripheral blood samples (Li-heparin) were collected from PLHIV (n=27) on cART and age and sex-matched HIV-negative donors (HC, n=11). Plasma HIV viral load was determined by Abbott Real time HIV-1 PCR (LLOD 40 copies/mL). CD4 absolute count (AC), the share of naïve (CD45RA+CCR7+), central-memory (CD45RA-CCR7+), effector-memory (CD45RA-CCR7-), terminal effector (CD45RA+CCR7-) and exhausted (CD57+) CD8+ and CD4+ T were determined by multiparameter flow cytometry. Plasma concentrations of MPO and XO [mU/ml] were measured by fluorometric method.
Results: Despite undetectable viral load (< 40 copies/mL) and similar CD4 AC between groups (903±283 vs. 1057±237, $p>0.05$), CD4/CD8 ratio were significantly lower in HIV+ individuals (1.31±0.46 vs. 2.28±0.71, $p<0.05$).
The difference in MPO concentrations did not reach statistical significance (70±25 vs. 20±15, $p>0.05$) as opposed to XO levels (387±448 vs. 42±33, $p<0.01$).
In PLHIV group a positive correlation between MPO and the shares of terminal effector (CD45RA+CCR7-) CD4, and exhausted CD57+CD8+T cells ($R= 0.5$, $p<0.01$, for both) were observed. Furthermore, negative correlations between MPO and central-memory (CD45RA-CCR7+) CD8+ and CD4+T cells were established ($R=-0.6$, $p<0.03$, for both).
Conclusion: These data suggest that XO and MPO plasma levels may have an important role in the pathways of the regulation on T-cells and immune homeostasis maintenance.

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2029 – P3.12.55

Interference with Co-stimulatory molecule ICOSL by human herpesviruses: impact on antigen presentation and immune evasion

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Co-signaling molecules play a pivotal role in shaping cellular adaptive immune responses by regulating T-cell activation. Pathogens, in turn, have developed mechanisms to disrupt their signals, dampening host immune responses to facilitate their replication. The inducible co-stimulator (ICOS, CD278), primarily expressed on activated T cells and on T follicular helper cells, enhances T-cell activation and promotes optimal germinal center (GC) formation through binding to its natural ligand ICOSL (CD275). ICOSL is constitutively expressed on antigen-presenting cells such as dendritic cells, macrophages, and B cells, and is upregulated by inflammatory stimuli. Our previous work has shown that the murine cytomegalovirus (MCMV) protein m138/fcr-1 interacts with ICOSL, preventing its cell-surface expression, thereby limiting MCMV-specific T-cell responses and viral control during acute infection.
Here, we demonstrate that human cytomegalovirus (HCMV) infection of primary monocyte-derived macrophages or PMA-differentiated THP-1 cells results in significant and specific downregulation of ICOSL, a process that requires viral gene expression. Kinetic assays performed in THP-1 cells indicated that by 24 hours post-infection ICOSL levels were already drastically reduced. While HCMV lacks an ortholog of m138, performing experiments with GFP-expressing HCMV deletion mutants, we identified one mutant, HCMVΔUS14-US22, which was unable to significantly downmodulate ICOSL in THP-1 cells. The region deleted in this viral mutant encompasses most members of the US12 family of HCMV, encoding distantly related proteins predicted to have seven transmembrane segments and to be related to G-protein coupled receptors. Flow cytometric analysis revealed that cells transfected with US16-GFP exhibited a significant reduction of cell surface ICOSL as compared to untransfected cells, thereby evidencing that the viral protein was capable to mediate these effects by itself, outside the infectious context. US16 is an early protein that localizes intracellularly in the viral assembly compartment and is important for shaping virion composition, affecting HCMV tropism. This viral molecule perturbs cellular ICOSL levels through a lysosomal degradation-independent process. Furthermore, we observed a similar depletion of ICOSL on differentiated THP-1 cells during infection with two α-herpesviruses, HSV-1 and HSV-2. These findings underscore the critical role of the ICOS:ICOSL axis in host defense against various herpesviruses.

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2030 – P3.12.56

Dynamism of anti-SARS-CoV-2 vaccine efficacy studied in mouse model

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Purpose: Our development had several objectives: to produce a stable monoclonal antibody with high affinity and high virus neutralizing capacity for research, as well as for diagnostic and potential therapeutic applications. Another objective was to study the time kinetics of the immune response induced by the mRNA-based vaccine in immunized mice.
Methods: We used commercially available recombinant antigens including the spike protein, and an mRNA-based vaccine (Pfizer-BioNTech Comirnaty Covid 19), to immunize 6-8 week old female BALB/c mice. Hybridomas were produced and selected on usual way. Characterization of the clones were made immunserologically by ELISA technique and by Western-blot. The affinity measurement performed by Octet K2 System. Virus neutralization capacity was tested on VeroE6 *in vitro* cultured SARS-CoV-2 virus holding cells.
Results: A significantly higher number of positive (mostly IgM-producing) clones were found among hybridomas from mice immunized with S protein antigens than after mRNA vaccine-based immunization. Among the hybridomas after mRNA immunization, the clones with good affinity head IgG (mostly IgG2a) isotype subclass. Based on the affinity measurements and specific viral neutralizing capacity, subclone 3/H8-H1-D4 (IgG2b) was selected for further studies. The time dynamics of mRNA-based vaccine induced immune reaction was tested immunoserologically and by characterization of hybridomas produced 6 weeks and 30 monts after the immunization. Specific anti-SARS-CoV-2 antibody production was detected in high titer during the whole investigation period, however, hybridomas specific immunoglobulin producing were not able to select from the 30 monts old mice.
Conclusion: Both the S protein antigen and the mRNA vaccine were able to induce intense immune response with high specificity, but the isotype profiles were significantly different after immunization by these two antigens. For testing the long-term efficacy of SARS-CoV-2 vaccines we used BALB/c mice that had been vaccinated with an mRNA-based vaccine two years earlier. Although a high specific anti-SARS-CoV-2 antibody titer was detected in the serum, we were unable to isolate clones with specific antibody production by repeated cloning from the hybridomas obtained by somatic cell hybridization of splenic lymphocytes. Presumably, the long-term memory B cells in old mice are no longer in the spleen but in the bone marrow.

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2071 – P3.12.57

Viral and immune kinetics of non-persistent HPV genital infections in young adult women

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Purpose: Human papillomavirus (HPV) infections drive one in twenty new cancer cases. Despite the potential for improving treatment, screening, and vaccination strategies, little is known as to why most HPV infections clear spontaneously within two years.

Methods: To untangle the dynamics of these non-persisting infections, we performed a combined quantitative analysis of virological, immunological, and clinical data from an original longitudinal cohort of 189 women with high temporal resolution.

Results: We find that HPV viral load reaches a plateau within two months, and clears within a median time of 14 months. Furthermore, we identify immune correlates associated with infection clearance, especially TCR-gamma-delta cells.

Conclusions: Our results open new perspectives for understanding the frontier between acute and chronic infections and for controlling HPV-associated diseases.

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2077 – P3.12.58

Inflammatory signature as an early predictor of severe respiratory viral infection

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Purpose: The aim of this study is to analyse the inflammatory signatures associated to infection caused by emergent and re-emergent respiratory viruses. Host response to viral pathogens may drive an exacerbated inflammatory response, leading to detrimental tissue damage in a dysregulated effort to fight the invading pathogen. Our purpose is to set up *ex vivo* and *in vitro* models to identify predictive biomarkers of pathogenic inflammation induced by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), Respiratory Syncytial virus (RSV) and Influenza virus (IV).
Methods: *Ex vivo* experiments were performed on whole blood samples from uninfected adults aged 65 and older visiting the Geriatric Clinic at Agostino Gemelli University hospital, Rome. Exclusion criteria other than an ongoing infection included immunodeficiency/immunodepression, autoimmune disease, cancer, diabetes, chronic obstructive pulmonary disease, obesity, hypertension/coronary artery disease, metabolic syndrome, and inflammatory conditions. Whole blood was stimulated with UV-inactivated RSV (subtype A), IV (H1N1), and SARS-CoV-2 (Wuhan). The expression of 22 genes involved in inflammation and immune response was assayed by RT-PCR array plates.
In vitro models to study respiratory viral infections were established by using the respiratory epithelial cells A549 infected by RSV-A and IV (H1N1) and the A549-ACE-2 cells infected by SARS-CoV-2 (Wuhan). RNA was extracted at different time points to evaluate gene expression.
Results: Whole blood experiments showed the induction of a common type1-IFN response by the three inactivated respiratory viruses in elderly subjects. RSV stimulation was characterized by a unique IL-15 upregulation and by higher chemokines expression. *In vitro* infections of A549 cells showed that IV and RSV share the ability to activate a set of pro-inflammatory genes comprising TNF-α, IL-6, CCL5 and ICAM-1. Experiments on the induction of inflammatory mediators driven by SARS-CoV-2 infection of A549-ACE-2 cells are in progress.
Conclusion: Our results highlighted the induction of a core of common inflammatory genes by three emerging and re-emerging respiratory viruses, albeit virus-dependent disparities in the host response occur. These results are instrumental to wider investigations pointing out to the association between the onset of severe disease and the activation of a divergent immune response.

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2092 – P3.12.59

Human immunodeficiency virus based pseudotype assays overestimate severe acute respiratory syndrome coronavirus 2 neutralising activity in sera from areas of high human immunodeficiency virus prevalence

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Purpose: Estimating functional humoral immunity requires the measurement of neutralising antibodies. As live virus assays require high-containment level laboratories, pseudotyped virus neutralisation assays (PVNAs) offer a practical, low-containment alternative. While human immunodeficiency virus (HIV)-based PVNAs are used widely due to their ease of manipulation and high dynamic range, their application in high HIV prevalence populations is limited. We assessed the use of HIV-based PVNAs to quantify SARS-CoV-2 neutralisation in HIV-infected sera.
Methods: HIV(SARS-CoV-2) pseudotypes bearing B.1 (ancestral), B.1.351 (Beta), B.1.617.2 (Delta) and BA.1 (Omicron) spikes were used to assess SARS-CoV-2 neutralising activity in sera from a longitudinal cohort in Malawi. Self-reported HIV prevalence in cohort was 4.9% (n=99), 82 of which reported receiving antiretroviral therapy (ART). Sera from HIV-infected participants were re-tested using vesicular stomatitis virus (VSV)-based SARS-CoV-2 pseudotypes (VSV(SARS-CoV-2)). HIV(VSV-G) pseudotypes, bearing the VSV-G protein, were used to confirm the target of any inhibitory activity. Neutralising activity in sera from HIV-infected participants, as measured with HIV- and VSV-based SARS-CoV-2 PVNAs, was then compared with activity estimated using live virus assays. Ethical approval was provided by the Malawi College of Medicine Research Ethics Committee 9P11/20/3177) and the University of Glasgow College of Medicine, Veterinary and Life Sciences Research Ethics Committee (200200056).
Results: Seroprevalence measured using HIV(SARS-CoV-2) PVNAs was significantly higher in HIV-infected participants (85.5-93.9%) compared with HIV-uninfected participants (8.1-54.5%) ($p=0.006$). VSV(SARS-CoV-2) PVNAs suggested a lower seroprevalence (5.6-65.2%) in HIV-infected individuals, indicating potential overestimation by HIV(SARS-CoV-2) PVNAs. HIV-infected participant sera inhibited HIV(VSV-G) pseudotypes (observed in 75.0-87.9% of HIV-infected samples), confirming interference with the HIV vector system. VSV(SARS-CoV-2) PVNA results correlated well with live virus assays in HIV-infected individuals ($r>0.5$), while HIV(SARS-CoV-2) PVNAs showed poor correlation ($r<0$). Among HIV-infected participants, there was no significant difference in HIV- or VSV-based seroprevalence by ART status ($p>0.05$).
Conclusions: HIV(SARS-CoV-2) PVNAs overestimated neutralisation seroprevalence from HIV-infected individuals, independent of ART usage. HIV-based pseudotyped virus assays should therefore not be used in high HIV prevalence cohorts. Protective immunity to many viruses estimated by HIV-based assays is likely over-reported in high HIV prevalence populations.

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2215 – P3.12.60

High titer against OC43 seasonal coronavirus enhances susceptibility to SARS-CoV-2 infections

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Several recent studies reported significant associations between the antibody levels against seasonal human coronavirus (hCoV) OC43 and susceptibility to SARS-CoV-2 infection. While in some studies these cross-reactive antibodies were protective, in others they were detrimental, i.e. increased suceptiblity to SARS-CoV-2. Here we studied their potential role using samples from two independent clinical cohorts.
In the first study, we longitudinally tracked 50 participants comprising university staff and students along 9 months. Both symptomatic and asymptomatic viral infections were identified through weekly symptom questionnaires and oronasal swabs. Cornonavirus viral infections, including SARS-CoV-2 and the four seasonal hCoVs (HKU1, OC43, NL63, 229E), were diagnosed using qRT-PCR. Monthly blood samples were collected to facilitate analyses of peripheral blood mononuclear cells (PBMCs), cytokines, and antibodies. Baseline serum samples were sorted according to their IgG or IgA titers to each of the seasonal hCoVs, and SARS-CoV-2 infection rates were compared in the highest and lowest quartiles. We observed a stark contrast between participants in the highest and lowest quartiles for OC43 IgG: All (100%) individuals in the highest quartile were infected with SARS-CoV-2, whereas the infection rate in individuals in the lowest quartile peaked at 50% (Fisher's exact test: OR = 0.000, p = 0.01373). In support of these findings, we found that the baseline and pre-infection anti-OC43 IgG antibody levels of individuals who were subsequently infected with SARS-CoV-2 were significnatly higher than those of individauls who were not infected with SARS-CoV-2 (p=0.019 and p=0.002, respectively).
We then used samples from a multi-center study of 606 healthcare workers folowed over a seven-month period, who were monitored for symptomatic SARS-CoV-2 infections via qRT-PCR. Remarkbly, we observed a similar pattern: Only 52% of individuals with higher anti OC43 IgA titers (highest quartile) were subsequently symptomatically infected with SARS-CoV-2, and 37.5% of individuals in the lowest quartile were infected with SARS-CoV-2 (Fisher's exact test: OR = 0.554, p = 0.0152).
Our findings suggest a potential correlation between elevated OC43 antibody titers in the serum and increased susceptibility to SARS-CoV-2 infections. These observations underscore the intricate interplay between different coronaviruses and shed light on factors influencing susceptibility to viral infections.

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2265 – P3.12.61

Differing systemic and mucosal antibody profiles to SARS-CoV-2 in a vaccinated University cohort- revealing increased male salivary anti-Spike IgA and anti–Spike IgG.

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Background: Immunity to SARS-CoV-2 induced by RNA vaccines has increased study into the antibody response to both the virus and vaccine targets. We compared the antigen specificity of antibody types in a vaccinated University cohort using rapid and conventional tests to determine systemic and mucosal immunity.
Methods: 472 participants were enlisted and consented over a 3 month period and asked to declare exposure to SARS-CoV-2 in the past 6 months. Serum & Saliva samples were subsequently obtained, and serum screened on site using rapid point of care (POC) tests detecting antibodies to viral nucleocapsid and spike antigens. Subsequent ELISA analysis was used to confirm results and to characterise serum and salivary IgG and IgA.
Results: The survey revealed 54 % (245) of participants believed they had been exposed to SARS-CoV-2 in the past 6 months. However, nucleocapsid antibody POC results revealed that 95% (431) had exposure to SARS-CoV-2 in the past 6 months. Neutralising anti- Spike levels were also assessed using POC tests and ELISA revealing varying levels of protection. Anti-Spike antibody POC images were digitally scored and compared to titrated antibody levels in competitive binding neutralisation assay ELISA revealing a detection level of 62.5 ng/ml equating to 55% competitive binding. Comparison of the serum and saliva by ELISA revealed increased anti-Spike IgA and anti–Spike IgG in male salivary samples compared to female volunteers (186 males vs 286 females) with no difference in serum (systemic) anti-Spike antibody levels.
Conclusions: Detection of antigen specific antibody profiles to SARS-CoV-2 can identify recent exposure and possible waning neutralising immunity. Further study of the differential gender specific antibody immune response at the mucosa is warranted in SARS-CoV-2 biology.

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P3.13 VISUALIZING IMMUNE RESPONSE

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650 – P3.13.01

Mathematical modeling of IL-6 driven T lymphocyte cross-talk for the selection of treatment modality in rheumatoid arthritis patients

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Mathematical models of immune mediated disorders provide an analytic platform in which we can address specific treatment choice in the vast personalized manner. Our interdisciplinary team has previously reported on the development of a mathematical model of rheumatoid arthritis pathogenesis by determining the progression of the disease over time using a system of non-linear differential equations. The mentioned model considers the inter-relating variables between B and T lymphocytes and, accordingly the level of cartilage damage in the course of the disease.
Objective of the present study was to create the model for rheumatoid arthritis management, in which the different modalities of treatment can be addressed. Still using the approach of non-linear differential equations, we issued the system explaining IL-6 driven cross-talk of pro- and anti-inflammatory CD4+ T cell subsets in the disease course and, based on it, we created the disease treatment model, in which the modalities of treatment with methotrexate and tocilizumab in a separate or combined scheme are considered. In this treatment model, the corresponding Cauchy problem was posed and its solution has been found.
In conclusion, we propose a novel mathematical model that best describes the readouts of the outcomes of different treatment modalities in patients with rheumatoid arthritis and, therefore, may take a rapid pace towards its individualized testing schemes.

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766 – P3.13.02

Using bioorthogonal chemistry to measure single immune cells metabolic fluxes

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Purpose: Immunometabolism research is advancing our understanding of how cellular and whole-body metabolism shapes immune responses. Immune cells exhibit highly dynamic physiology, whereby their metabolic and biosynthetic capacities are dramatically regulated in response to immune stimulation and cell activation. The nutrient uptake capability reflects the functional performance of immune cells in diseased environments. However, it is becoming clear that many nutrient uptake discoveries performed using immune cells cultured *in vitro* do not accurately translate to *in vivo* cellular metabolism features. Consequently, there is an urgent need to devise a methodology capable of accurately measuring the nutrient uptake capacities of immune cells at disease sites and visualizing their metabolic characteristics.
Method: Our research introduces a novel metabolic assay utilizing bioorthogonal, or 'click' chemistry that attach fluorophores to the different nutrients after it has been transported into cells. Subsequently coupled with flow cytometry sorting, this approach can be used to investigate how individual cells take up nutrients including amino acids, and long-chain fatty acids, at these sites of disease. This is highly informative because the uptake of nutrients is the first limiting step for cellular metabolism.
Results: The data show that this technology enables us to simultaneously measure the uptake of amino acid into individual immune cells *ex vivo* (Single-Click), as well as effectively assess the new protein synthesis capacity of cells after combining with OP-Puromycin（Double-Click）. Our findings also suggest promising *in vivo* applications of select bioorthogonal nutrient probes, enabling the visualization of nutrient uptake at the disease site with single-cell resolution. Furthermore, our investigations validate the utility of this technique in measuring fatty acid uptake into individual immune cells *ex vivo*. We aim to advance this methodology by developing the 'Triple-Click,' which will offer comprehensive metabolic flux information in up to four dimensions for each analyzed cell.
Conclusion: We are currently employing this approach to enhance our understand of immune cell metabolism across various disease models. By generating the detailed footprint of metabolic alterations in immune cells at the disease site, this research aims to produce novel insights driving innovation towards novel immunotherapies.

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925 – P3.13.03

Exploring Small Vulnerable Newborn Immune Development in Urban Burkina Faso: Novel Biospecimen Collection Procedures for Flow Cytometery in Low-Income Settings

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Purpose: Newborn mortality remains a key issue worldwide, particularly in low-income contexts. While high-income countries benefit from robust infrastructure and advanced technology to explore infants' immune system development, limited resources hinder similar research in low- and middle-income countries. Our study aims to address this gap by combining local expertise with modern diagnostic tools from high-income countries, allowing effective collection and analysis of fragile biospecimens from urban Burkina Faso.
Methods: In the DenBalo study, 140 pregnant women are be followed up from the third trimester postpartum and divided into control (mothers with full-term neonates) and cases (mothers with small vulnerable newborns) groups after delivery. During the first two months of neonates' lives, non-invasive methods such as finger-prick sampling for mothers and heel-prick sampling for neonates are used to collect ≤ 500 µl blood samples at specific time points. These samples are be stained, preserved in Burkina Faso and shipped to Belgium for advanced analysis including cytokine multiplexing and flow cytometry. The study aims to provide valuable insights into the relatively unexplored area of immune system development in small vulnerable newborns. Immune cell types including dendritic, natural killer, T, B, and Mucosal-Associated Invariant T (MAIT) cells will be examined for their activation, immature, or memory states, along with the exploration of pro-inflammatory cytokines, chemotaxis cytokines, and other immune regulatory cytokines.
This research is a part of a broader study that investigate the interplay between immune system, gut microbiome and breastmilk (ClinicalTrials.gov ID NCT05730569).
Results: Based on the input of expert partners and results from a pilot immunostaining analysis which showed strong signals, standard protocols for blood collection, preservation and immunostaining were created.
Conclusion: The purpose of this presentation is to introduce the scientific community to a novel approach for studying neonates' immune system in low- and middle-income countries through standardizing biospecimen collection and processing, thereby allowing repeatability and reproducibility of experimental results.

This study has been reviewed and approved by the ethical committee of the Ghent University Hospital (B670201734334) and the Burkinabe ethics committee (050-2022/CEIRES). This work is supported by the Bill & Melinda Gates Foundation [INV-004773].

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952 – P3.13.04

Linking effector CD4+ T cell dynamics and response to pathogen proliferation

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Effector $CD4^+$ T cells are confronted with complex environments when recruited to an infection site. In *Leishmania* skin infections, the situation is even more complex. Monocyte-derived phagocytes, the usual T cell activators, provide the niche for the parasite. This results in heterogenic phagocyte subpopulations with different activation states and pathogen proliferation rates. Whether effector $CD4^+$ T cells can detect the different pathogen proliferation rates and differentially address these cell populations is currently unknown. To spatial and temporally unravel this interplay by we use intravital 2-photon microscopy (IV-2PM) of the infection site and single cell RNAseq.
IV-2PM revealed decreased $CD4^+$ T cell speeds in close proximity (<10µm) to cells harbouring low-proliferating parasites compared to cells carrying high-proliferating pathogens. This suggests are preferred and more stable interaction of effector $CD4^+$ T cells with this subpopulation. Paradoxically, slow replicating *Leishmania* are mainly observed in host cells with an anti-inflammatory transcriptomic profile, while high-proliferating pathogens are prevalent in phagocytic cells exhibiting a pro-inflammatory gene expression.
By exploring on the molecular mechanism of this phenomenon, we focused on genes specifically expressed in such anti-inflammatory pathogen-harbouring phagocytes. Among the highest hits of individually expressed genes was Cathepsin B (CTSB), a protein involved in intracellular processes as well as in remodelling of the extracellular matrix. We demonstrated that knocking out CTSB in monocytes leads to efficient T cell engagement of infected phagocytes and increase their Th1 response. Implying that upregulation of CTSB in host cells result in an immunomodulation in favour of the parasite.
Together the data indicate that in an acute *Leishmania* skin infection it is possible to link pathogen proliferation to the dynamics and activation of effector $CD4^+$ T cells. Insights like this provide a start for informed host-directed therapies to counteract pathogen persistence and ultimately to control infection diseases.

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1011 – P3.13.05

Role of regulatory T cells in the cellular immune response to influenza vaccine in older adults

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Purpose: Influenza significantly affects the health of older adults, associated with a decrease in the effectiveness of the vaccine. Previous studies have noted increased regulatory T cells (Tregs) potentially limiting vaccine efficacy. However, conclusive evidence remains lacking. Herein, we evaluate the effect of the Treg response in young and old before and after vaccination and its effects on the Th1 and CD8 T response.
Methods: Tregs' influence on influenza vaccination responses in both young and older participants, administered with influenza vaccine, was examined. Flow cytometry was employed to assess their impact on T-helper and CD8+ T cell responses. This analysis involved individuals vaccinated during the 2023/2024 campaign.
Results: After vaccination, older individuals displayed a heightened response of central memory Tregs, indicated by increased frequency in peripheral blood. Both age groups exhibited augmented suppressive activity of Tregs (CD39+) post-vaccination, while older individuals showed reduced response of Helios+ Tregs, suggesting a peripheral induction origin. Conversely, young individuals demonstrated increased proliferation of CD8 T cells (Ki67+). Notably, older individuals maintained a baseline activation profile (Ki67+) in CD8 T cells post-vaccination, implying a response to antigens other than the vaccine. Furthermore, an inverse correlation was observed between effector memory Tregs and the post-vaccination Th1 and CD8 TemRA cell response, mainly in older adults.
Conclusion: These results highlight the adverse influence of Tregs on the vaccine-triggered response of TCD8 and Th1 lymphocytes. Considering the importance of these cell populations in the anti-influenza response, this suggests a detrimental effect of Tregs on the effectiveness of influenza vaccines. Further research is underway to explore additional effects of Tregs on these groups, including specific antibody and cytokine responses.

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1162 – P3.13.06

Establishment of a highly sensitive live-cell imaging-based cytotoxicity assay for functional validation of rare epitope-specific CTLs

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Recognition of pathologically altered cells by cytotoxic T lymphocytes (CTLs) is a prerequisite for successful disease clearance. Thus, many immunotherapeutic approaches, e.g. therapeutic vaccination or adoptive T cell transfer, rely on the identification of specific epitopes. Possible epitopes can be identified *in silico* or by *in vitro* T cell immunogenicity assays. However, their functional validation in cytotoxicity assays can be challenging and often requires the generation of epitope-specific T cell clones. Here, we describe a highly sensitive image-based cytotoxicity assay that allows functional analysis of low-frequency T cells, e.g. from peripheral blood.
To obtain sufficient numbers of effector cells, peptide-specific T cell 14 day-expansion lines are generated from PBMCs, through addition of the peptide of interest and cytokines to support APC activation and T cell proliferation. For the assay setup, $CD8^+$ T cells are enriched by MACS.
In the live-cell imaging-based setup, transient red labeling of the target cells combined with a green caspase 3/7 probe allows reliable measurement of the frequency of apoptotic target cells. Moreover, the time course analysis enables monitoring of subtle differences. To detect the peptide-specific cytotoxicity executed by a small CTL fraction within the population of $CD8^+$ T cells, the frequency of apoptotic target cells in co-culture with specific T cell lines is compared to this frequency observed in co-culture with unspecific T cell lines. Thereby, recognition of naturally presented epitopes can be validated. Alternatively, if the peptide of interest is not presented on the target cells, the same T cell line can be tested in coculture with peptide-loaded and unloaded target cells.
The assay is very flexible and can – due to utilizing a transient dye - easily be adapted to the use of different target cells. In validation experiments with EBV- and CMV-specific CTLs, titration of the fraction of peptide-specific CTLs in the T cell line demonstrated that killing mediated by 0.5% peptide-specific T cells can still be detected. The assay was successfully applied to functionally validate HPV16 epitopes in the context of several HLA-types.
Overall, this cytotoxicity assay setup provides a straightforward approach to assess the cytotoxic ability of rare CTL populations.

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1288 – P3.13.07

Human iPSC-derived Cerebral Organoids as Neuroinflammation Models

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Neuroinflammation is a multifaceted process involving various cell types and signalling pathways. This complexity makes it challenging to model, overall, including in organoids, which are three-dimensional miniature organs that mimic the structure, organization, and functionality of human organs. One of the challenges has been finding standardized protocols that inherently give rise to microglia, the immune component of the brain that plays a crucial role in neuroinflammation. At ACROBiosystems, we have developed novel, hydrogel-free human iPSC-derived cerebral organoid systems. These organoids inherently contain neurons, astrocytes, oligodendrocytes, microglia, and vascular cells, as verified by RNA-seq and marker immunolabeling. We have functionally validated these organoids in terms of physiological electrical activity, both intracellularly via patch clamp and extracellularly via MEA and silicon probes. Upon LPS stimulation of the cerebral organoids, increased IL-6 cytokine levels were observed. To study neuroinflammation associated with neurodegenerative diseases, we created non-genetic 3D models of Alzheimer's and Parkinson's disease by treating the cerebral organoids with Tau and Alpha-synuclein pre-formed fibrils (PFFs), respectively. Additionally, a small molecule drug, TRx0237, was able to alleviate the high phosphorylation of the Tau upon addition of Tau PFFs. Furthermore, these organoids can be used for AAV-mediated transgene delivery to modify gene expression or investigate therapeutic applications in diseases associated with neuroinflammation. We utilized the three following AAV serotypes to evaluate transgene efficiency using the cerebral organoid model: AAV5-wt, as negative control, IVB-1, and IVB-2. IVB-2 was the most efficient serotype for delivering the eGFP transgene. In conclusion, we outline the use of commercially available ACROBiosystems human iPSC-derived cerebral organoids as improved models to study neuroinflammation. This may help provide better guidance for neuroinflammation research, gene therapy development, disease modeling and drug development.

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1552 – P3.13.08

Predict the response to CD3xBCMA bispecific immunotherapy in multiple myeloma

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Background: Recently, the development of engineered bi-specific antibodies opened a new era in immunotherapy. In the context of Multiple Myeloma (MM), bi-specific antibody treatment brings together T cells (CD3 antigen) and tumor cells (BCMA antigen), leading to tumor cells lysis by T cells. Recently, T cells receptor (TCR) analysis in MM patients treated with BCMAxCD3 bi-specific antibodies reveals a clonal expansion of T cells, suggesting an anti- tumoral T cells response induce by this treatment. Overall, these data clearly demonstrate that MM can be targeted by immunotherapy, but treatment efficiency needs to be improved. Therefore, identification and characterization of tumor antigens specific T cells, represent an important axis of research in MM to improve patients' response (e.g. Immune check point inhibitor antibodies, vaccination with tumor antigens...) in combination with the current treatment (i.e. CD3xBCMA bi-specific treatment).
Objectives: Identification and characterization of anti-tumor T lymphocytes in patients at diagnosis and remission, to determine whether the identification of these populations could constitute a predictive marker for the response to this treatment.
Methods: We used mass-cytometry approach to characterize T cells populations.
Results: Our data shows much greater complexity in the CD4 and CD8 T cell population than previously appreciated at early time point. Moreover, we observed a non-uniform pattern of variations across patients tested, which highlights a broad diversity of these cells as previously described. These observations, particularly with respect to markers associated with T cells exhaustion, may help to explain heterogeneity in clinical outcomes following various forms of immunotherapy.
Conclusion: Great diversity of CD4 and CD8 T cells between patients and across time-point following CD3xBCMA bi-specific treatment.

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1582 – P3.13.09

Spatially resolved single-cell metabolic profiling of the melanoma tumor microenvironment using Multiplexed Ion Beam Imaging (MIBI)

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Purpose: Melanoma tumors are heterogeneous ecosystems composed of different cell types, including cancer, immune, epithelial and stromal cells. Constant metabolic interactions between these cells within the tumor microenvironment have been linked to the formation of an immunosuppressive niche, poor prognosis, and the development of therapeutic resistance. Despite its relevance, metabolic profiling of human tumors in general is hampered by technological limitations of existing approaches, such as lack of true single-cell resolution, spatial information, and scalability to larger patient cohorts.
Methods: To address this issue, our group employs multiplexed ion beam imaging (MIBI), a novel antibody-based highly multiplexed imaging platform. In MIBI, heavy metal conjugated antibodies are used to measure the expression of up to 40 targets directly in formalin-fixed, paraffin-embedded (FFPE) samples, while providing subcellular resolution over a linear dynamic range of four orders of magnitude. By measuring the expression of carefully curated rate-limiting metabolic regulators as a surrogate for metabolic activity, our approach enables spatially resolved metabolic characterization of individual cells alongside their functional phenotypes in situ.
Results & Conclusions: In the present work, we perform spatially resolved single cell metabolic profiling on a cohort of metastatic melanoma patients (n = 27 patients) stratified for immune checkpoint inhibition. To this end, lineage annotations were thoroughly curated for a dataset of 500,000 cells spanning 12 cell types, and bioinformatic analyses revealed pronounced metabolic heterogeneity among cell types and patients. In ongoing work, we are in the process of compiling a large feature set containing various aspects of metabolic and spatial information. Using this feature set as input for machine learning models, we aim to identify features that predict therapeutic response. Such insights may in the future help to stratify patients into appropriate treatment regimens.

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1599 – P3.13.10

Characterizing the immunometabolism of both the tumor microenvironment and tumor cells at the single-cell level in leukemia

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Purpose: Metabolism holds pivotal significance in immunity, serving as the cornerstone of cellular functionality. Distinct metabolic traits delineate various immune cell types and their activation statuses. Nonetheless, the intricacies of immunometabolism remain enigmatic. Targeting metabolic pathways emerges as a promising avenue in immunotherapy, particularly in T lymphocytes, owing to their pivotal role in anti-cancer immunity.
Methods: We devised an antibody panel comprising 23 surface markers for discerning cell subpopulations (including CD4, CD8, exhausted, and senescent cells) alongside 16 metabolic markers and 5 histone marks, delineating the activity of diverse pathways. Mass cytometry facilitated comprehensive data acquisition.
Results: Our findings underscore the efficacy of our methodology in scrutinizing metabolism and histone mark expression at the single-cell level across immune cell subpopulations. Moreover, our assay underwent validation using bone marrow samples from acute myeloid leukemia patients, revealing discrete immune cell and leukemic blast cohorts harboring distinct metabolic profiles.
Conclusion: The potential for metabolic therapeutic interventions to modulate T cell functionality and enhance adaptive metabolism is apparent. However, the translational utility of metabolic therapeutics hinges upon a comprehensive understanding of T cell metabolism under physiological conditions and during immune responses. The outcomes of this study stand to significantly advance our comprehension of T cell metabolic dynamics, thereby fostering advancements in therapeutic research endeavors.

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1783 – P3.13.11

Multiplex immunoassays to visualize immune responses in large population-based cohort studies to increase pandemic preparedness, support disease modelling/eradication and define prevalence of climate sensitive infections

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Capturing protective immunity or providing differential immunity estimates on population level as part of large epidemiological cohort studies is challenging as currently available methods are not able to capture complex aspects of B-cell driven immune responses such as different isotype or antigenic responses profiles after infection or vaccination
By combining our expertise in infectious disease epidemiology, virology, immunology and assay development, we have developed a comprehensive multiplex immunoassays portfolio to provide such differential immunity estimates in particular for HZI-managed epidemic panels like MuSPAD/RESPINOW or prospectively any other (long-term) cohort study to aid translational (public) health research.
Prime efforts of our work are MULTICOV-AB™ a SARS-CoV-2 immunoassay with over 40 coronavirus antigens including multiple VoCs RBDs and a complementary surrogate neutralization assay RBDCoV-ACE2. Both assays were used to screen more than 100.000 samples and have supplied valuable real-time data to support decision-making in the COVID-19 pandemic by providing seroprevalence estimates and characterizing SARS-CoV-2 (VoC) humoral immunity after infection and vaccination in multiple populations such as children, adults, in indigenous or “difficult to vaccinate”-individuals such as dialysis patients or between mild and severely ill individuals.
A RSV assay shows potential to define population estimators of re-infections to provide input for informed dynamic scenario modelling predicting disease burden, hospitalizations and impact of non-pharmaceutical interventions. A mPOX assay, which includes vaccinia virus antigens, can be used to determine population immunity from previous small pox immunizations towards emerging zoonotic mPOX strains as wells as to assess mPOX prevalence in population-based cohorts. In case of measles, differentiation of vaccination versus infection-induced antibodies could support global elimination efforts, while an assay developed to measure antibody levels towards all currently known hepatitis viruses can support public health efforts to eliminate hepatitis virus-induced diseases and deaths which particularly effect low and middle income countries in a resource-efficient manner. Last, with the on-going climate change and the associated alterations in transmission dynamics, geographic spread, we plan to develop further assay to assess the (re)emergence of vector-borne diseases such as dengue or west nile virus, while an existing borrelia assay already allows to monitor increases in subtype-specific seroprevalence estimates.

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1826 – P3.13.12

Biomechanics of macrophages during interaction with etxracellular stimuli

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Purpose: Macrophages are involved in every stage of the immune responses in the body tissues, including the resolution of the inflammatory reaction. The modulation of macrophages' behavior is closely linked with extracellular environment and physicochemical stimulation. Within these stimuli, macrophages are especially sensitive to surface properties, such as mechanical and geometrical characteristics, directly influencing adhesion, migration, and consequently their immune response.
Methods: In this investigation, we combine atomic force microscopy (AFM), finite element modeling (FEM) and quantitative biochemical approaches in order to understand the mechanotransduction from the extracellular matrix (ECM) and phagocytic particles into cellular response. The mechanical cues from the substrate are transduced into the cells through the formation of integrin-regulated focal adhesions and cytoskeletal reorganization, which in turn modulate cell biomechanics by decreasing cell stiffness.
Results: Surface topography and consequent biomechanical response impact the overall behavior of macrophages by increasing movement and phagocytic ability, without significantly influencing their inflammatory behavior. Along with ECM modification, the shape and stiffness of phagocytic particles also influenced the actin cytoskeletal organization, a fundamental process during phagocytosis. In particular, macrophages can exert internal forces able to bend and curl elongated particles with a stiffness of 150 Kpa or less. Finally, we explored the biomechanical mapping of macrophages as a novel classifier to distinguish their activation status. Applying AI algorithms to different data maps we realize that mechanical data layer is very efficient to automatically distinguish macrophages' phenotypes.
Conclusion: Our investigations suggest a strong potential of biomechanics as stimuli for the regulation of macrophage functions, as well as a novel classifier to determine their physiological status.

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1930 – P3.13.13

Antiviral immunity revealed by B cell ImmunoSpot informs immunity to future viral exposures

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Viruses circulating in the human population such as SARS-CoV-2 are subject to immune-mediated selection pressure (naturally-acquired or vaccine-mediated) that drives the emergence of antigenic variants, some of which are variants of concern (VOC) due to increased virulence. Such variants can cause reinfections in immune individuals due to their ability to evade neutralizing antibody activity. In the face of declining effective antibodies, viral-specific pre-existing memory B cells (B_{mem}) serve as a durable secondary wall of humoral defence. While the B_{mem} may initially possess a reduced affinity for the new viral variant, they are maintained at a stable precursor frequency. Moreover, such B_{mem} can be re-recruited at a future time into germinal centre reactions where they undergo further rounds of proliferation and acquire somatic mutations to refine both the specificity and affinity of their antigen receptors and ultimately their secreted antibodies. Identification of such cross-reactive B_{mem}, together with a measure of their affinity, indicates a person's ability to mount an effective recall response upon viral re-encounter and thus could inform vaccination regimens. Owing to the scarcity in blood of antigen-specific B_{mem}, and technical difficulties associated with their identification, assessment of B_{mem} reactivity and affinity against emerging SARS-CoV-2 variants in large donor cohorts has been largely neglected. Using recent innovations in the B cell ImmunoSpot platform, including the ability to assess affinity and cross-reactivity of cultured B_{mem} at the single cell level, our study evaluated B_{mem} reactivity in subjects infected early in the pandemic with the prototype Wuhan virus (Hu-1) for recognition of Receptor-Binding Domain (RBD) antigens representing the Delta (B.1.617.2) and Omicron (BA.1) VOC. Fluorescent spots in the assay revealed B_{mem} secreting specific antibodies of varying affinity for the VOC antigens based on spot size and intensity. This study was further able to demonstrate B cell cross-reactivity against key antigens from VOCs. Collectively, this study demonstrates the feasibility of performing B cell ImmunoSpot assays to efficiently, and with limiting cell input numbers, identify cross-reactive B_{mem} and their affinity, at single-cell resolution. This assay is therefore a useful addition to the current immune monitoring toolbox in the battle against VOC.

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2156 – P3.13.14

Designing in vitro platforms to study transendothelial T cell migration in colorectal cancer

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Over the years, a better understanding of the tumor microenvironment (TME) in colorectal cancer (CRC) has highlighted its critical role in tumor development and progression. The infiltration of cytotoxic T lymphocytes into the tumor is one of the most predictive factors of prognosis in CRC. Therefore, great interest has been focused on understanding the ability of T lymphocytes to cross the endothelial barrier, navigate the stroma and access the tumor, and how the TME affects this migration. However, studying this process in vivo is extremely difficult and costly, and standard in vitro models are too simplistic to fully recapitulate the journey of T cells from the blood stream to the tumor. Recent advances in biofabrication such as 3D bioprinting have opened new avenues to obtain three-dimensional in vitro models mimicking the in vivo TME. To this end, we use a tiered approach developing increasingly complex in vitro models to dissect the mechanisms underlying transendothelial migration (TEM) and T cell motility within the stromal microenvironment. Specifically, we investigate distinct barriers using Transwell models (i) coated with adhesion molecules (ICAM1 or Fibronectin) or (ii) cultured with endothelial cells mimicking the leukocyte endothelial transmigration; (iii) primary T cells embedded in hydrogels together with primary fibroblasts, mimicking the migration through the stromal compartment, and (iv) the extravasation model that combines the stromal compartment and endothelial barrier to effectively recapitulate the transendothelial and stromal T cell migration dynamics. In order to recapitulate the signalling cascades present during the early stages of CRC, we use APC-/- intestinal organoids cultured on monolayers. Each of these models will help understand the effect of the different barriers and TME in the T cell migration, providing a toolbox to study the mechanical and biochemical signals during immune cell recruitment within a complex tissue-like CRC model.

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