Abstract-Book

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Oral Presentations

Session 1: Immunobiology

S1.02

Prostaglandin E2 controls the metabolic adaptation of T cells to the intestine

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Immune cells must adapt to different environments during the course of an immune response. We studied the adaptation of CD8+ T cells to the intestinal microenvironment and how this process shapes their residency in the gut. CD8+ T cells progressively remodel their transcriptome and surface phenotype as they acquire gut residency, and downregulate expression of mitochondrial genes. Human and mouse gut-resident CD8+ T cells have reduced mitochondrial mass, but maintain a viable energy balance to sustain their function. We found that the intestinal microenvironment is rich in prostaglandin E_2 (PGE_2), which drives mitochondrial depolarization in CD8+ T cells. Consequently, these cells engage autophagy to clear depolarized mitochondria, and enhance glutathione synthesis to scavenge reactive oxygen species (ROS) that result from mitochondrial depolarization. Impairing PGE_2 sensing promotes CD8+ T cell accumulation in the gut, while tampering with autophagy and glutathione negatively impacts the T cell population. Thus, a PGE_2-autophagy-glutathione axis defines the metabolic adaptation of CD8+ T cells to the intestinal microenvironment, to ultimately influence the T cell pool.

S1.03

TRPM7 ion channel and kinase drives AKT signaling and immune cell activation

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Ion channels of the TRP family are crucial for cellular homeostasis. TRPM7 is a unique member with its dual function as ion channel-coupled protein kinase, and a ubiquitous expression pattern localized to the plasma membrane. Besides its fundamental ability to provide divalent cations such as Mg2+ and Ca2+, it drives intracellular signaling processes by a constitutively active kinase domain. The TRPM7 channel and kinase are interdependent in their function, and have been identified as modulators of immune homeostasis, cell activation, proliferation and differentiation. In previous work we have demonstrated a role of TRPM7 kinase in T-cell signaling, Th17 differentiation and gut immunity. In a murine model, TRPM7 kinase facilitated induction of acute graft-versus-host disease. SMAD2 thereby serves as TGF-beta dependent cellular substrate of TRPM7 kinase, driving

proinflammatory signals and Th17 lineages. Besides a number of cell-specific interaction partners, we recently identified the AKT signaling hub downstream of TRPM7 kinase, facilitating activation of neutrophils and T cells. In human and murine neutrophils, we pinpointed this to AKT/mTOR-mediated induction of oxidative burst and directed cell migration. In T cells, TRPM7-dependent Mg2+ conductance is crucial for cellular survival and proliferation, and the protein facilitates T-cell-receptor-mediated Ca2+ flux. We could show that TRPM7 is required for T-cell activation machinery, involving induction of AKT dependent pathways. In vitro, we identified a direct interaction of TRPM7 kinase being able to phosphorylate AKT substrate, confirming previous findings. Altogether, our data suggest TRPM7 kinase as potential target in inflammatory and also malignant diseases, due to its interconnection with AKT signaling and pro-inflammatory cellular responses.

S1.04

The interplay between inflammation and oxidative stress supports autistic-related behaviors in mice

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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 particular 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 mutant mice. Oxidative stress and inflammation in the cerebellum, as well as pro-inflammatory conditions in the peripheral blood, 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. Unexpectedly, social impairments as well as cerebellar and systemic inflammation were induced in NAC-treated Cntnap2+/+ mice. Taken together, our findings suggest that the interplay between oxidative stress and inflammation may support the pathogenesis of ASD-related behaviors in mice.

S2.02

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, 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 MS. We first showed that MS patients express higher PKM2 levels than control individuals in circulating T cells, with differential expression depending on the MS forms. We then evaluated whether limiting PKM2 moonlighting activity with the PKM2 allosteric activator TEPP-46 may impact the inflammatory profile of T cells from MS patients. We found that TEPP-46 inhibits T cell proliferation and decreases the production of pro-inflammatory cytokines by T cells, suggesting that PKM2 may control T cell pathogenicity in MS patients. Overall, our data indicate that PKM2 may represent a novel biomarker for disease activity, and that targeting PKM2 moonlighting functions may be of relevance in MS. PKM2 allosteric activators thus represent promising pharmacological tools for MS treatment.

S2.03

The role of metabolic secretome of macrophages in maintaining tissue homeostasis.

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Tissue-resident macrophages can tune their metabolism to alter their immunomodulatory functions and promote tissue homeostasis. Recently, we showed that mTORC1–activated colon macrophages in a DSS-induced colitis model secrete polyamines like spermine and spermidine that can influence the proliferation and differentiation of colonic epithelial cells. This indicates that macrophages can produce and secrete novel metabolites that promote tissue homeostasis. A secretome-based non-targeted metabolomic analysis of bone marrow-derived macrophages (BMDMs) was done to investigate what metabolites are secreted. Several metabolites involved in key metabolic pathways

were revealed. Phosphocreatine, an energy compound formed from creatine and ATP, was an interesting metabolite secreted at predominant levels by steady-state BMDMs that was also confirmed by targeted metabolomics. We showed that phosphocreatine release by BMDMs occurs in the presence of creatine which was either taken up from the serum or was made from the precursors – arginine and glycine. Moreover, IL–4 stimulated M2-like BMDMs showed increased phosphocreatine secretion. Blocking of creatine uptake using β–guanidinopropionate, a creatine analog, reduced phosphocreatine secretion and intracellular ATP levels in BMDMs. Independently, colon organoids treated with phosphocreatine showed increased proliferation and higher intracellular ATP levels. Overall, our findings suggest a commensal role played by macrophages in metabolically maintaining and supporting their surrounding tissue environment.

S2.04

Atypical chemokine receptor 4 is expressed in glomerular parietal epithelial cells and mitigates the severity of experimental glomerulonephritis

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Background: Cell migration contributes to the innumerable physiological and pathological processes driven and directed by chemotactic cytokines (chemokines), small structurally homologous proteins signalling via their cognate GPCRs on motile cells. In addition to GPCRs, chemokines importantly interact with atypical chemokine receptors (ACKRs) that are characterised by expression in various stromal cells and distinctive ligand specificities. ACKR4 binds CCL19, CCL20, CCL21, CCL22 and CCL25, chemokines involved primarily in adaptive immune responses. Accordingly, ACKR4 is prominently expressed in the primary and secondary lymphoid organs, thymus, bone marrow, lymph nodes and spleen where it regulates distinct cell migratory steps driven by its cognate ligands. Furthermore, ACKR4 is expressed by dissimilar cell types in multiple parenchymal organs, the heart, the liver and the gut. Currently it is not entirely clear how chemokine scavenging by ACKR4 in these organs might affect their pathophysiology.

Here we investigated the expression of ACKR4 in murine kidney and assessed its contribution to nephrotoxic serum nephritis (NTSN), an experimental murine model of immune complex glomerulonephritis. The pathomechanisms in NTSN are known to rely on the contribution of multiple chemokines and their receptors and functions of Th1 cells, Th17 cells, neutrophils and macrophages exerting pathogenic effects in both lymphoid organs and the kidney.

Methods: The expression of renal ACKR4 was evaluated in healthy ACKR4-eGFP reporter mice as well as after the induction of NTSN. To investigate the contribution of ACKR4 to NTSN, ACKR4-deficient mice and WT controls were subjected to an anti-basal membrane immunization protocol and the parameters of immunopathogenesis and the ensuing kidney disease were evaluated at 7 and 14 days after the immunization.

Results: Multicolour immunofluorescence confocal microscopy revealed that ACKR4 is expressed in the kidney exclusively in the glomeruli by a discrete subset of parietal epithelial cells localising adjacently to the vascular glomerular pole. In mice with NTSN, the overall proportion of ACKR4 expressing cells in glomeruli at the vascular pole did not change as compared to healthy mice, however ACKR4+ cells were not detected in glomeruli corresponding with the increased abundance of alpha-SMA, a marker of renal fibrosis. The ACKR4-deficient mice showed delayed antibody response following immunisation and reduced readouts of cellular immunity. However, despite this,

disease parameters of NTSN, including albuminuria, PAS-score and crescent formation were significantly increased in ACKR4-deficient mice as compared to the WT controls. Renin, a hormone secreted by the cells adjacent to ACKR4+ cells in the kidney was significantly increased in serum of nephritic ACKR4-/- mice.
Conclusion: We report for the first time that ACKR4 is expressed in the kidney by a small subpopulation of parietal epithelial cells. Reduced parameters of humoral and cellular immunity in ACKR4-deficient mice contrast with a more severe manifestations of NTSN seen in these mice, suggesting a tissue-specific functional bias of ACKR4 in the kidney that limits the development of NTSN. Furthermore, the immediate proximity of ACKR4 expressing cells to the juxtaglomerular apparatus and the observed increased renin secretion in ACKR4-/- mice suggest a potential contribution of ACKR4 to the regulation of renin-driven pathways, by a yet unclear mechanism.

Session 3: Infections, Immunodeficiencies and Vaccines

S3.02

Absence of TYK2 enhances host resistance to C. albicans skin infection

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Tyrosine kinase 2 (TYK2) is a Janus kinase that mediates signal transduction by multiple cytokines. While TYK2 is recognized for its essential role in protecting against viral and bacterial infections in humans and mice, its contribution to immune defense against fungal pathogens remains largely unexplored. The recent approval of the first TYK2 inhibitor for psoriasis treatment highlights the need to better understand the role of TYK2 in skin immunity.
To study the role of TYK2 in the immune defense against fungal infections, we used a mouse model of skin infection with C. albicans, the most common human fungal pathogen. We found that TYK2-deficient mice (Tyk2-/-) and mice expressing kinase-inactive TYK2 (TYK2K923E) are more resistant to cutaneous candidiasis than WT mice, as evidenced by a reduced fungal burden in the skin and reduced C. albicans dissemination into the kidneys. Mechanistically, we found that skin-infiltrating neutrophils form a non-proliferating layer around C. albicans in Tyk2-/-and TYK2K923E mice but not in WT controls, which correlated with a strongly reduced invasion of fungi into the deep skin. RNA-seq analysis of skin-infiltrating neutrophils showed a downregulation of interferon-stimulated genes (ISGs) in the absence of TYK2. In line with this, we found impaired upregulation of IFNγ in the infected skin of Tyk2-/- and TYK2K923E mice. IFNγ-receptor-knockout mice grossly phenocopied Tyk2-/- and TYK2K923E mice, indicating that the TYK2 promotes fungal dissemination through the upregulation of IFNγ. Using mice deficient for T cells and B cells and conditional Tyk2-knockout mice we provide collective evidence that TYK2 signaling in non-NK, non-NK T and non-γδ T cells induces IFNγ production and promotes fungal dissemination into kidneys.
Taken together, our results reveal a kinase-dependent detrimental role of TYK2 in the host defense against cutaneous candidiasis and shed new light on the immune response to fungal infections in the skin.

3.03

Functional heterogeneity of stromal cells directs immune lineage specification and survival

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Interleukin 15 (IL-15) has been identified to be crucial for NK cell development and memory CD8 T cell survival in the bone marrow (BM), yet it remains elusive which cell types locally provide the IL-15 signal. While myeloid cells have long been considered to be the major source of IL-15, they seem to play a minor role in supporting IL-15 dependent immune cells in the BM. Thus, the spatial and functional organization of IL-15 producing cells in the BM remains elusive.
Therefore, we aimed to characterize the heterogeneity of IL-15-producing stromal cells and to identify distinct BM niches that regulate the development and maintenance of NK, NKT and memory CD8 T cells.
Using our IL-15-EGFP reporter mice, we performed single-cell RNA-seq and flow cytometry, and found that IL-15 is produced by various stromal cell subsets, in particular Lepr+ mesenchymal stromal cells, sinusoidal endothelial cells and chondrocyte-lineage cells.
Conditional deletion of IL-15 or IL-15Rα in specific stromal cell subsets using five different Cre lines characteristically shaped the NK, NKT and CD8 T cell landscape in the BM.
We thus could show that heterogenic, functionally specialized cytokine-expressing stromal cells provide contextualized, non-redundant signals which are required to guide and tightly control immune lineage specification and survival in the BM. Our study therefore highlights the in vivo role of the stromal-immune architecture in the BM and provides insights into the immunomodulatory capacity of stromal cell subsets, which may also be relevant in inflammatory and malignant diseases.

S3.04

Impaired vaccine response to SARS-CoV-2 in anti-TNF-α-treated IBD patients is associated with lack of cTfh1 cell activation and reduced B memory cell formation

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Background: Patients with inflammatory bowel disease (IBD) and healthy controls received primary SARS-CoV-2-mRNA vaccination and a booster after six months. Anti-TNF-α-treated patients showed significantly lower antibody (Ab) levels than α4β7-integrin-antagonist recipients and controls. This study aimed to elucidate the underlying mechanisms on the basis of circulating T-follicular helper cells (cTfh) and B memory cells.
Methods: We measured SARS-CoV-2-specific Abs against Wuhan and Omicron variants, B- and T-cell subsets at baseline and kinetics of Spike (S)-specific B memory cells along with distributions of activated cTfh cells before and after 2-dose primary and booster vaccination.
Results: Anti-TNF-α treated IBD patients showed lower levels and faster waning of Wuhan- and Omicron-specific IgG. This was associated with reduced numbers of total and naïve B cells vs. expanded plasmablasts prior to vaccination. Along with low Ab levels in anti-TNF-α-treated IBD patients, reduced S-specific B memory cells were established after the 2nd dose which declined to non-detectable after 6 months. This was in contrast to high responder α4β7-integrin-antagonist treated IBD patients and controls, who mounted high S1-specific Ab levels and B memory cells up to 6 months post primary vaccination. Booster vaccinations induced a strong increase of both Abs and S-specific B memory cells in these groups. This was not the case in anti-TNF-α treated IBD patients, who also showed reduced neutralizing capacity against Omicron variants. Of note, activation of cTfh1 cells significantly correlated with the induced Ab levels and S-specific B memory cells, particularly after booster vaccination.
Conclusions: The reduced magnitude, persistence, and neutralizing capacity of SARS-CoV-2 specific Abs after vaccination in anti-TNF-α-treated IBD patients were associated with impaired formation and maintenance of S-specific B memory cells due to absent activation of cTfh1 cells after primary vaccination. This most likely led to only short-lived extrafollicular immune responses and diminished B memory cell diversification. These observations have implications for patient-tailored vaccination schedules/vaccines in anti-TNF-α-treated patients, irrespective of their underlying disease.

Session 4: Immunology in Pediatrics + Pulmonology

S4.02

Salivary antibodies protect against SARS-CoV-2 BQ.1.1 in a 3D respiratory model

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Background: The emergence of SARS-CoV-2 Omicron subvariants BF.7 and BQ.1.1 worldwide has made it crucial to assess current immunity against novel variants of concern. While most studies focus on antibody titers and neutralization capacity in serum, little is known about mucosal immunity.
Methods: This study evaluates SARS-CoV-2 specific antibody titers and neutralizing capacity of sera and saliva from triple vaccinated individuals boosted with an adapted bivalent COVID-19 vaccine or recovered from BA.4/BA.5 infection. In addition, personalized protective effects of serum and saliva were assessed in a human 3D respiratory model.
Results: Analysis of SARS-CoV-2-specific antibodies showed increased serum IgG in vaccinated individuals, while IgA levels remained comparable. Cohorts showed similar serum neutralization against SARS-CoV-2 wildtype and Omicron BA.4/BA.5, but critically reduced for BQ.1.1 and BF.7. Salivary neutralization against BA.4/BA.5 was increased in convalescent individuals compared to

vaccinated, while salivary neutralization against BQ.1.1 and BF.7 remained comparable in these groups. In the human 3D respiratory model, personalized protective effects revealed strong salivary neutralization against different Omicron subvariants accompanied with reduced inflammation. Conclusion: Vaccinated and vaccinated/recovered individuals show similar neutralization capacity against SARS-CoV-2 sub-lineages with increased salivary protection of recovered individuals against the variant that caused the natural infection. Low neutralization titers against BQ.1.1 and BF.7 suggest a need for updated vaccine strategies. Personalized protection assays highlight the importance of serum and saliva-mediated protection, especially salivary protection against different SARS-CoV-2 variants.

S4.03

Mitochondrial oxidative stress promotes an IL-4 producing phenotype in human T cells

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Background: Numerous studies have indicated that the production of reactive oxygen species (ROS) influences intracellular signaling pathways and thereby shapes effector functions of T cells. One major source of ROS is the superoxide production by mitochondria. Yet, the exact contributions of different ROS levels on immune cell function are only incompletely understood.
Methods: Human CD4+ and CD8+ T cells were activated with anti-CD3/anti-CD28 antibodies in the presence of the Coenzyme Q10 analogue Mitoquinone-mesylate (MitoQ), which increases superoxide levels in a dose-dependent fashion. In parallel, CRISPR/Cas9-mediated knockout of Superoxide Dismutase 1 (SOD1) in primary human T cells was performed, leading to disabled ROS scavenging and ensuing higher superoxide levels. Subsequently, in both models, effector functions were measured by flow cytometry, multiplex ELISA and qPCR. Similarly, RNAseq from human CD4+ T cells activated in the presence of MitoQ was performed.
Results: Activation of CD4+ and CD8+ T cells in the presence of MitoQ led to a marked decrease of most effector functions including proliferation, expression of activation markers and cytokine production. Of note, IL-4 production was initially also decreased but showed a marked rebound at later time points of activation. This was observed at the protein level as well as the transcription level, indicating that superoxide stress leads to an altered expression dynamic of IL-4. This observation was replicated in the SOD1 knockout Supernatants from MitoQ treated T cells led to a strong Th2 polarization when transferred to naïve CD4+ T cells. As first insights into the mechanism, RNAseq data revealed gene signatures relating to the ER stress response thus potentially indicating a link between oxidative stress and IL-4 production.
Conclusion: Our data strongly indicate that oxidative stress induces a distinct IL-4 producing phenotype. This may especially be of interest as novel mechanism triggering Th2 polarization and allergic sensitization.

S4.04

Single-cell profiling uncovers regulatory programs of pathogenic Th2 cells in allergic asthma

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Background: Lung pathogenic T helper type 2 (pTh2) cells are important drivers of allergic asthma, but fundamental questions remain regarding their molecular regulation and heterogeneity. The differentiation and effector functions of immune cells are tightly regulated by epigenetic processes. Histone deacetylase 1 (HDAC1) is an important epigenetic regulator of T cells, but its role in pTh2 cells is yet to be determined.
Methods: Here we investigate immune regulation in allergic asthma by single-cell RNA sequencing (scRNA-seq) in mice challenged with house dust mite, in the presence and absence of HDAC1 function.
Results: Our analyses reveal two distinct subsets of lung pTh2 cells: pathogenic effector Th2 (peTh2) and pathogenic Th2 tissue-resident memory (Th2 Trm) cells. Both pTh2 cell subsets are highly proinflammatory and exhibit distinct transcriptional and phenotypic signatures as compared with other lung Th subsets. Based on our scRNA-seq analysis, we identify conditions to generate pTh2 cells in vitro and confirm that these in vitro generated pTh2 cells have a similar transcriptional profile as lung peTh2 cells. Using our new in vitro model, we demonstrate that the p38 mitogen-activated protein kinase pathway is critical for interleukin-5 (IL-5) and IL-13 expression in pTh2 cells. Our data further underline the importance of HDAC1 in limiting the pathogenicity of lung and in vitro pTh2 cells and in the formation of lung Th2 Trm cells.
Conclusion: In summary, we have generated novel insights into pTh2 cell biology and established a new in vitro model for investigating pTh2 cells that will be useful for discovering molecular mechanisms involved in pTh2-mediated allergic asthma.

Session 5: Next generation immunologists

S5.02

Microenvironmental and cell intrinsic factors governing human cDC2 differentiation and monocyte reprogramming

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Background: BMPR1a/ALK3 signaling in DCs is functionally required for resolving established skin inflammation. This signaling axis is aberrantly induced in the BMP7^{HI} psoriatic epidermis, coinciding with the neo-appearance of blood-derived epithelial DCs.
Objective: cDC2s occur abundantly in inflamed tissues, and exhibit specific characteristics dependent on their anatomical sites. However, the local signals instructing their phenotype and function remained poorly understood.

Methods: Quantitative immunofluorescent analysis of healthy and psoriatic skin; blood cDC2 and monocyte differentiation in vitro; generation of cDC2s from human hematopoietic progenitor cells; $CD4^+$ Th cell polarization; extensive transcriptomic and gene pathway analysis using the Ingenuity pathway analysis (IPA) platform and GENEVESTIGATOR.
Results: We demonstrated that strong BMP7-BMPR1a signaling cooperates with additional epithelial lesional signals for promoting the differentiation of Axl^+ regulatory cDC2s from blood cDC2s. Moreover, these cells further differentiated into Langerhans cells (LCs) upon TGF-β1 stimulation, marking the resolution phase of inflammation. Consistently, psoriatic epidermal lesions harbor a spectrum of $Axl^+CD1c^{(+)(-)}CD207^{(+)(-)}$ cells, and Axl^+cDC2/LC transition is also evident from a novel serum-free differentiation model of progenitor cells. The high responsiveness of blood cDC2s to TGF-β/BMP ligand signaling is correlated with absence of the monocyte identity factor KLF4, known to antagonize TGF-β signaling. Nevertheless, blood cDC2s can acquire $KLF4^{HI}$ pro-inflammatory-type monocyte-derived characteristics.
Conclusion: BMP7 signaling, aberrantly induced in the enlarged psoriatic epidermis, promotes the presence of regulatory Axl^+cDC2s that adopt LC characteristics upon decreased BMP signal strength. Moreover, cDC2s are reprogrammed to monocyte-derived cells in response to inflammatory signals.

S5.03

Purification and characterization of poppy seed allergens

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Background: Poppy seeds (Papaver somniferum L) are used as ingredients and garnishing of cakes and bread. Poppy seeds contain 45–50% oil, which can be used as a source of high amount vitamin E and a moderate amount of phytosterols as compared to other plant oils. Poppy seed allergy is rare, however cases of poppy seed allergy were reported. It has been shown, poppy seed allergic patients are at risk to cross react with tree nuts. The aim of this study was to purify and characterize IgE-binding proteins from poppy seed.

Methods: Poppy seeds were obtained from a local store, ground, defatted and dried at room temperature. Afterwards, proteins were purified from poppy seed extract applying a combination of different chromatographical methods. Physiochemical characterization of purified proteins was performed by circular dichroism (CD) spectroscopy and mass spectrometry. Allergen specific IgE was evaluated by ELISA and Western blot using 46 sera from poppy seed allergic patients.
Results: Proteins were purified according to established protocols for seed storage proteins and were checked for purity and molecular mass. CD spectrometry confirmed α-helix and β-sheet based structures that corresponds to legumin- and vicilin-like proteins. Mass spectrometry indicated theoretical molecular masses for cupin proteins: 46 and 49 kDa. In addition, an unknown protein was isolated from poppy seed extract with a molecular mass about 10-12 kDa.
ELISA and Western blot confirmed IgE recognition of all three purified proteins. When testing 46 poppy seed sensitized patients' sera, 40 sera were positive for poppy seed extract. Out of those, 37 and 34 were positive for legumin-like and vicilin-like proteins, respectively. In addition, 26 sera showed IgE-binding to an unknown protein.
Conclusion: Our study provides novel data on poppy seed proteins and their IgE-binding capacity. In addition, the mass spectrometry data showed the similarity between poppy seed proteins and nuts, particularly almond. Allergenicity and the cross reactivity of poppy seed proteins will be further investigated.

S5.04

FceRI expression profile on basophils as marker for hymentoptera venom allergy

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Background: IgE mediated mastcells and possibly also basophil granulocyte activation are thought to be the main driver of the anaphylactic reaction. Until today no biomarker has been established to reliably predict the severity of anaphylaxis upon sting reaction. We therefore examined if the high affinity IgE-receptor (FceRI) expression on basophils could serve as marker for hymenoptera venom allergy.
Methods: Blood was drawn from 31 patients with diagnosed bee or wasp allergy and five healthy control persons. Total and free IgE-receptor densities on basophils were assessed via FACS analysis. Basophil activation was assessed with a standardized basophil activation test (BAT) kit. Clinical parameters including anaphylaxis grading (Ring & Messmer) and routine blood tests were used for correlation analysis.
Results: FceRI expression density on patients' basophils ranged from about 35.000 to 700.000 receptors/cell. Unoccupied FceRI numbers were comparably low (max. 20.000 per cell). In the mean, higher levels of total-FceRI and lower levels of IgE-free FceRI expression on basophils were seen compared to healthy controls. High expression of total FceRI was highly correlated with low expression of IgE-free FceRI in our patient cohort. Total FceRI expression was significantly correlated with total IgE and with sIgE to bee venom but inversely correlated with anaphylaxis grading. Anaphylaxis grading was also inversely correlated with total IgE levels, but positively correlated with high BMI and baseline tryptase levels. In our patient cohort the BAT results were not a satisfying biomarker for anaphylaxis.
Conclusion: To our knowledge this is the first report that a low total IgE-receptor density on basophils was associated with increased severity of anaphylaxis in insect venom allergic patients. This marker should be further assessed in larger cohorts for its relevance and capability for risk assessment in sensitized patients. Furthermore, changes in FceRI expression should be studied throughout the course of specific immunotherapies.

Session 6: Cancer and Tumor Immunobiology

S6.02

Molecular mapping of immune checkpoints reveals CD80 as antagonist of MHC-I/PD-L1 clusters

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The activation and function of immune cells is guided by signals encountered on the surface of their interaction partners. In the case of T cells, activatory (costimulatory) and inhibitory (checkpoint) molecules engaged on the surface of dendritic cells during initial T cell activation dictate whether T cells become effectors, while the relative ratios of these molecules determine if effector T cells can subsequently kill their targets, such as tumor cells. Despite the importance of these molecules in T cell immunoregulation, little is known about the spatial organization and surface architecture of these proteins. To address this knowledge gap, we employed multiplexed, super-resolution DNA-PAINT imaging on the surface of individual mouse cDC1s and melanoma cells to simultaneously map the single protein distributions of 6 different proteins: the co-stimulatory molecules CD80 and CD86, the immune checkpoints PD-L1 and PD-L2, and MHC-I and MHC-II. Across the course of cDC1 activation, we validated previously reported, in addition to identifying multiple unreported, protein-protein interactions, with the DC surface enriched for smaller protein nano-clusters. In striking contrast to cDC1s, melanoma spatial organization was dominated by large-scale PD-L1 and MHC-I aggregates. Critically we identify CD80 as a key factor dictating whether cells adopt a “melanoma-like” or “DC-like” surface architecture. CD80 ablation from cDC1s precipitated melanoma-like PD-L1/MHC-I surface aggregates, while CD80 over-expression on melanoma cells led to a dispersed, DC-like surface organization. Collectively, these data reveal unappreciated and complex spatial organization associated with immune activation vs repression, and identify CD80 as a key “remodeling” factor that disrupts formation of immunosuppressive surface complexes. Our study is thus an important stepping stone towards the much higher plex, bona fide “spatial proteomics” technologies required to comprehensively address the many unanswered fundamental biology questions surrounding cell surface organization.

S6.03

The NLRP3/eIF2 axis in acute myeloid leukemia

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The NLR family pyrin domain containing 3 (NLRP3) inflammasome has lately attracted great attention in various biomedical fields, as excessive activation of the NLRP3 inflammasome plays an important role in many different pathologies including hematologic diseases. In this context, our study highlights a novel function of the NLRP3 inflammasome in Acute Myeloid Leukemia (AML). We demonstrate that NLRP3 inflammasome components and the inflammasome-related cytokines IL-18 and IL-1b are overexpressed in AML patients, resulting in poor survival rates. Using shotgun proteomics, we could identify the eIF2 pathway as an important novel NLRP3 target. Genetic knockdown or pharmacological inhibition of NLRP3 results in enhanced eIF2a phosphorylation, which in turn was linked to the inhibition of cell cycle progression and apoptosis in vitro and in vivo. Additionally, a strong decrease in the cyclin-dependent kinases CDK4 and CDK6 was observed, also accompanied by an upregulation of the CDK inhibitor p21 (CDKN1A), resulting in pronounced cell cycle arrest in the G0/G1 phase and increased apoptosis. Taken together, these findings suggest that NLRP3 overexpression, as observed in many AML patients, blocks apoptosis of leukemic cells by dysregulating the eIF2 signaling pathway. Our data suggest that the NLRP3/eIF2 axis acts as a novel driver of cell cycle progression in AML. As AML is a disease with extremely low survival rates and limited treatment options, a better understanding of the molecular mechanism linking inflammation to excessive cell proliferation may open new paths for novel treatment strategies.

S6.04

G Protein-Coupled Receptor 55 in Pancreatic Cancer: An Immunomodulatory Role

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The G protein-coupled receptor 55 (GPR55) is a part of the expanded endocannabinoid system (ES). It has been shown to have pro-tumorigenic effects in different cancer models, including models of pancreatic cancer. Cells of the tumor microenvironment (TME) express cannabinoid receptors and thus can be influenced by the ES. However, the role of GPR55 in the TME and its involvement in tumor growth is not well understood. Knowing that pancreatic cancer is characterized by low immune infiltration and poor treatment response, it is important to uncover the role of GPR55 in tumor immunity.

KPCY cells (mouse pancreatic ductal adenocarcinoma with a high [T cell high; TCH] or low [T cell low; TCL] T cell response) were subcutaneously injected into GPR55 wild-type and knock-out mice. Flow cytometry and in situ hybridization were used to phenotype cells within the tumors, while cytokine array, ELISA, and qRT-PCR were used to determine the expression levels of proteins and cytokines. In vivo assays were conducted with mouse neutrophils to elucidate their behavior in the TME. To access the function of GPR55 in tumor cells, GPR55 knock-in TCH and TCL cell lines were produced.
GPR55 knock-out mice injected with TCH KPCY cells had smaller tumors than the wild-type mice. Additionally, they showed higher CD8+ T cell and dendritic cell infiltration with higher CCL21 expression, but lower infiltration of neutrophils when compared to wild-types. In the TCL model, tumor weight was higher in the knock-out group compared to wild-types. The TCL GPR55 knock-outs also showed higher infiltration of neutrophils, suggesting that neutrophils could be important regulators of the TME in the KPCY tumor model.
Our data indicates that the knock-out of GPR55 in the TME of mouse pancreatic cancer models leads to differing immune cell infiltration, which could be important regarding future immuno-therapies of pancreatic cancer.

Session 7: Immunology in Dermatology + ENT

S7.02

Targeting of Th17-related cytokines in patients with Darier Disease

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Darier disease (DD) is a rare, inherited multi-organ disorder associated with mutations in the ATP2A2 gene. In DD patients, the skin is frequently affected, characterized by malodorous, inflamed skin and recurrent, severe infections. Therapeutic options are limited and inadequate for the long-term

management of this chronic disease. Using NanoString technology, we performed high-throughput immunoprofiling of genes expressed in lesional skin of six DD patients. Furthermore, we examined Th17-, Th1-, and Th2-related cytokines in DD patients by qRT-PCR. Overexpression of cytokines was confirmed at the protein level in immunofluorescence stainings of skin sections. Gene set enrichment analysis of the gene expression data revealed that IL-17-signaling was enhanced in the skin of DD patients compared with the skin of healthy controls. Further analysis of Th17-, Th1-, and Th2-related cytokines by qRT-PCR showed significantly increased IL17A expression compared to HC skin. Overexpression of Th17-related cytokines was confirmed in immunofluorescence stainings of skin sections. Due to these results we administered monoclonal antibodies targeting the overexpressed cytokine to three treatment-refractory patients and observed a clinical improvement of skin manifestations. We demonstrate for the first-time enhanced expression of Th-17-related genes in DD and provide new options for the long-term management of skin inflammation in these patients.

S7.03

EGFR protects from scarring alopecia by securing the stem cell immune privilege

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Scarring hair loss is a distressing adverse event of long-term anti-cancer treatment with epidermal growth factor receptor (EGFR) inhibitors, which can result in dose reduction or cessation of therapy. Generally, inflammatory cicatricial alopecia, which can eventually lead to permanent hair loss, is a challenging and poorly understood condition.
To investigate the mechanisms underlying EGFR inhibitor-induced alopecia, we used mouse models with hair follicle-specific deletion of EGFR. By analyzing the transcriptional profile of the bulge stem cells before the onset of hair loss using fluorescence-activated cell sorting and RNA sequencing, we identified a burst of hyper-proliferative stem cells followed by a complete loss of the bulge stem cells and fibrotic scarring alopecia. Upregulation of the antigen presentation machinery through activated cell intrinsic JAK-STAT1 signalling indicates the collapse of the bulge stem cell immune privilege. Single-cell profiling and cell depletion studies identified interferon gamma-expressing natural killer and CD8+ T cells as the driver of this chronic inflammatory cascade. Therapeutic inhibition of JAK signalling was able to re-establish the immune privilege and induce activation of the remaining stem cells to elicit novel hair growth. Translational studies using skin biopsies of patients treated with an EGFR inhibitor as well as of patients with folliculitis and cicatricial alopecia revealed activation of the STAT1 pathway in the hair follicle. Finally, in a case study of folliculitis decalvans, we could successfully treat progressive hair loss, and perifollicular erythema with the JAK1/2 inhibitor Baricitinib.
Overall, our findings highlight the critical role of EGFR in protecting hair follicle quiescence and bulge stem cell immune privilege during inflammatory tissue destruction. This provides new insights into the mechanisms underlying scarring alopecia and provides a mechanism-based therapeutic option for this challenging condition.

S7.04

Beyond skin inflammation: cell-specific immune-modulatory functions of S100A8/A9 alarmins

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Background: Skin diseases are among the most common health conditions affecting over 900 million people. Inflammatory skin diseases (ISDs) comprise a series of complex pathologies with unknown aetiology, such as Atopic Dermatitis (AD), which accounts for the largest ISD burden. Despite major efforts gaining insights in the cellular and molecular mechanisms underlying AD, an effective treatment is currently lacking. AD is accompanied by skin microbiota dysbiosis characterized by Staphylococcus aureus (SA) colonization. Furthermore, S100A8 (A8), S100A9 (A9) and their heterocomplex Calprotectin (CP) mainly released by keratinocytes and neutrophils are deregulated in AD and can act as antimicrobial peptides. However, the cell-specific A8/A9 contribution to SA infection and systemic inflammation associated with AD is still elusive.
Methods: A genetically engineered mouse model (GEMM) of AD, based on constitutive epidermal loss of JunB (JunBΔep), was crossed to A9-/- (JunBΔepS100a9-/-) or conditional A9 mice (JunBΔepS100a9Δep). JunBΔep mice with A9-deficient neutrophils were established by bone marrow transplantation of mice with Mrp8-Cre mediated A9 deletion.
Results: Global A9 inactivation in JunBΔep mice ameliorated AD-like skin lesions, reduced SA colonization, neutrophil recruitment and decreased inflammatory mediators in the skin including A8, IL-17, G-CSF and Neutrophil elastase. Furthermore, JunBΔepS100a9-/- mice developed aggravated systemic inflammation with prominent swollen digits and SA penetration, bone erosion and local A8 upregulation. In contrast, JunBΔepS100a9Δep mice displayed worsened skin lesions, increased SA colonization, and elevated neutrophil infiltrate and A8/A9-positive immune cell recruitment in the skin. Importantly, A9-deficiency in neutrophils improved skin lesions with reduced A8 and A9, whereas CP levels were elevated compared to control bone marrow chimeras. Ongoing experiments using new GEMMs suggest a pro-inflammatory role of keratinocyte-derived A8 involved in AD.
Conclusion: Epithelial- and immune cell-specific functions of A8/A9 were identified that modulate local and systemic inflammation in AD-like disease with potential therapeutic relevance.

Session 8: Molecular Allergology

S8.02

The cross-blocking activity of immunotherapy-induced antibodies accords with allergen homology

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Background: We recently reported that sublingual immunotherapy (SLIT) with recombinant (r) Betv1 (rBetv1-SLIT) or its homolog in apple, Mald1 (rMald1-SLIT), resulted in differing types of IgE-blocking antibodies (Abs). rBetv1-SLIT-induced Abs blocked IgE-reactivity to Betv1 but not to Mald1 whereas rMald1-SLIT-induced Abs blocked IgE-reactivity to Mald1 but not Betv1. Here, we employed Cora1 from hazelnut (highly homologous to Betv1), Pruav1 from cherry (highly homologous to Mald1), and Dauc1 from carrot to assess the cross-reactivity, cross-blocking capacity and avidity of SLIT-induced Abs.
Methods: Allergen-specific IgE, IgG1, and IgG4 levels were determined by ELISA in pre- and post-SLIT samples of 20 rMald1-SLIT and 17 rBetv1-SLIT-treated individuals, respectively. To assess IgE-blocking, allergens were incubated with pre- and post-SLIT samples from 7 individuals of each group prior to their use in basophil activation tests. Avidity was compared by challenging the binding of SLIT-induced Abs to plate-bound allergens with acidic buffers. Potentially shared epitopes of SLIT-induced Abs on the different allergens were assessed by competition ELISA. Shared surface areas of the allergens were identified by using an in-house designed script based on structural alignments.
Results: rBetv1-SLIT significantly enhanced IgG1 and IgG4 responses to all studied food allergens except Dauc1. rMald1-SLIT significantly increased IgG1 levels specific for Mald1, Cora1, and Pruav1, and IgG4 levels specific for Mald1 and Pruav1. Post-rBetv1-SLIT sera displayed higher blocking activity and avidity for Betv1 whereas post-rMald1-SLIT sera showed higher blocking activity and avidity for Mald1 and Pruav1. Competition ELISA suggested that rMald1-SLIT-induced IgG1 Abs share more epitopes with Pruav1 than those following rBetv1-SLIT. Accordingly, highest surface identities were found between Pruav1 and Mald1.
Conclusion: Our results with Betv1-related allergens indicate that IgE-cross-blocking depends on the homology of allergens. These findings are relevant to understand better why therapy with Betv1 has limited effects on associated food allergies.

S8.03

Allergen-laden virus-like nanoparticles (VNP) with Th1-priming activity

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Background: Virus-like nanoparticles (VNP) are a well-established platform for vaccination and immunomodulation, which can be loaded with allergens inside and decorated with functional cytokines on their surface. We have analyzed whether surface decoration of allergen-laden VNP with IL-12 would improve their immunomodulatory properties.
Methods: The major mugwort pollen allergen Art v 1 was encased within VNP by N-terminal fusion to the viral matrix protein MAp15 of MoMLV. For surface expression, single-chain (p35::p40) mIL-12 was C-terminally fused with the minimal CD16b GPI-anchor acceptor sequence. 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: IL-12+ allergen-expressing VNP induced high numbers of IFN-g+CD4+ T cells in sorted naïve allergen-specific T cells from humanized mice in the presence of BM-DCs, similar to classical Th1 polarizing conditions. Induced Th1 cells remained stable after re-stimulation with allergen in the presence of BM-DCs. In splenocyte cultures of humanized allergy mice, IL-12+ allergen-expressing

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. While IL-12+ VNP moderately inhibited T cell proliferation, the proportion of IFN-g+CD4+ T cells was significantly increased. Among the CD3+CD4+IFN-g+ T cells an additional population co-expressing clear-cut levels of IL-10 was induced, indicative of a Tr1 cell-like phenotype. IL-12+ VNP strongly inhibited the expansion of allergen-specific, IL-13+ Th2 cells of humanized allergy mice in vitro. In vivo, prophylactic intranasal treatment with IL-12-decorated allergen-laden VNP before exposure to allergen aerosol reduced expansion of IL-4+ and IL-13+ CD4+ T cells compared to control VNP decorated with an constitutively 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.

S8.04

Potential of prophylactic adoptive cell therapy in IgE mediated allergy

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Background: Prophylactic techniques to prevent allergy development via building tolerance remain an unmet medical need. Recently, cell therapy approaches, showed promising results in prophylactic allergen-specific tolerance induction. We created a protocol for adoptive cell transfer that uses the transplantation of allergen-expressing bone marrow cells to induce allergen-specific tolerance. In this study, we investigated whether the transfer of allergen-expressing lymphocyte subsets is able to induce allergen-specific tolerance.
Methods: Allergen-expressing CD19+ B cells were isolated from Phl p 5-transgenic BALB/c mice and transferred to naive BALB/c recipient mice. Recipients received a pre-treatment with a short course of rapamycin and anti-CD40L antibody. Molecular chimerism levels in the peripheral blood were analyzed by flow cytometry. Tolerance was assessed upon 3 subcutaneous sensitizations with Phl p 5 and a control allergen, Bet v 1. Phl p 5 and Bet v 1-specific IgE and IgG_1 antibody responses were analyzed in serum samples by ELISA. Whole body plethysmography was performed to investigate the allergen-induced lung inflammation.
Results: Transfer of $10*10^6$ Phl p 5-expressing purified CD19+ B cells induced B cells chimerism for up to 2-3 months. No Phl p 5-specific IgE and IgG_1 antibody response were detected in chimeric mice for the length of the so far follow-up period of 15 weeks. Upon nasal Phl p 5 challenge, lung function was substantially better in CD19+ B cell-treated mice compared to sensitized mice, which had not received cell therapy.
Conclusion: We showed allergen-specific tolerance induction through the transfer of Phl p 5-expressing CD19+ B cells and highlighted the potential of prophylactic adoptive cell transfer, for inducing tolerance in IgE mediated allergy.

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Molecular Allergology & Clinical Allergology

P1.02

High affinity IgE receptor expression on basophils – Is there a pathophysiological role in the early phase of tolerance induction?

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Background
Specific immunotherapy (SIT) is the typical treatment for the most common cause of anaphylactic reactions in adults: bee and wasp venoms. Until today, little is known about the immunomodulatory mechanisms during early tolerance induction of SIT. As the allergic reaction is mainly driven by mastcells and possibly also basophil granulocytes, we investigated the allergic patients' basophils during the early SIT phase.

Methods: From 19 patients, blood was drawn before and six hours after the initiation of SIT. A standardized basophil activation test (BAT) to hymenoptera venom was performed. The surface densities of total and IgE-free high affinity IgE-receptors (FceRI) were assessed via FACS analysis. An ELISA-kit was used to detect changes in soluble IgE-receptor (sFceRI) concentration in the serum. Clinical parameters and routine blood examinations served for statistical analysis.

Results: Patients with preactivated basophils (>10% activation in negative control before the start of SIT) show a higher tolerance in the BAT against insect venom after 6h of SIT. Total FceRI density shows a tendency towards decreasing surface levels 6 hours after SIT-start in bee and wasp allergic patients, although not significant. The IgE-unoccupied FceRI density increases significantly in bee allergic patients. Interestingly, this was correlated with patients´ age. The higher the age of the patient, the less IgE-unoccupied receptors were found on basophils after 6 hours compared with the initial free-FceRI density. In our patient cohort sFceRI decreased after six hours in the serum in 12 of 13 tested patients.

Conclusion: Early tolerance induction does not lead to dramatic changes on the basophil level, regarding reactivity and total FceRI density. However, small but significant changes like the increase of IgE-free FceRI density on basophils in bee allergic patients were detected. We conclude that the principle leading to early tolerance most likely involves also other still unknown mechanisms.

P1.04

Differential diagnosis of genuine Blomia tropicalis sensitization using recombinant Blomia tropicalis allergens

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Background: Around 30% of the general population is affected by house dust mite (HDM) allergy and it causes a wide range of allergic manifestations such as asthma, allergic rhinitis and atopic dermatitis. Blomia tropicalis (Blo t) is a house dust mite species of tropical and sub-tropical areas in South America, Africa and Asia which belongs to the superfamily of Glycyphagidae. Our goal was the isolation and characterization of allergens from this mite species to provide component resolved diagnosis in the form of a microarray chip.
Methods: To determine the clinical relevance of the individual Blomia tropicalis allergens, 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 BL21 (DE3) using the pET17b plasmid. Once expressed and purified, these recombinant allergens were characterized by SDS PAGE for purity and by circular dichroism for fold. Sera from patients sensitized to HDM and/or Blo t were tested on the microarray chip to determine the frequency of IgE recognition of the individual allergen molecules.
Results: Our results showed that Blo t 5, Blo t 21 and Blo t 2 are the most important allergens for Blo t sensitized patients and that Blo t sensitised patients also show IgE reactivity to Der p allergens, whereas Der p sensitised patients mainly react to Der p allergens.
Conclusion: With the panel of allergens produced, it is possible to achieve component resolved differential diagnosis between HDM (Der p and Der f) and Blomia tropicalis.

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P1.05

Obesity increases allergic airway inflammation that can be successfully treated by oral immunotherapy

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Background: Obesity and allergy have become significant health problems, particularly in the westernized world. It is unclear whether obesity enhances respiratory allergy and whether tolerance can be efficiently induced in obese.
Methods: C57BL/6 male mice fed with a high-fat diet (HFD) or standard chow diet (STD) for nine weeks were immunized/sensitized and then challenged with ovalbumin (OVA). To induce tolerance, mice were orally treated with OVA before sensitization. Metabolic parameters and allergen-specific antibodies were measured in serum. Differential cell counts were performed in bronchoalveolar lavage and cytokine measurements, FACS analysis, and immunofluorescence staining in the lung. Fourier transform infrared spectroscopy was used to investigate the molecular composition of the gut.
Results: HFD-fed animals exhibited twice the body weight and significantly higher leptin levels than STD-fed animals. Sensitization and challenge with OVA resulted in upregulating allergic parameters, such as eosinophil counts, Th2 cytokines, and OVA-specific IgE levels, which were significantly higher in obese compared to lean animals. Oral tolerance induction led to a non-allergic phenotype. The

respective diet, but not tolerization, significantly influenced the cellular biochemical components in all experimental groups. In obese, this was associated with maintaining the M1 polarized macrophages in the lungs of tolerized animals, indicating that this cell type might have a role in counteracting the allergic phenotype.
Conclusions: We demonstrated that obese and lean mice showed a robust allergic response, albeit more substantial allergic inflammation in obese. Interestingly, the oral tolerance was not impaired and efficiently attenuated the increased allergic reaction in the obese mice. We suggest that pulmonary M1 macrophages are at least partly responsible for this effect in tolerized obese mice.

P1.06

Micro-arrayed PR10 proteins and peptides for studying antibody reactivity profiles in allergic patients and non-allergic individuals

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The major birch pollen allergen Bet v 1 and structurally related pathogenesis-related class 10 (PR10) proteins represent a family of cross-reactive allergens. Aim of the study was to establish chips containing micro-arrayed PR10 allergens and peptides thereof for studying antibody reactivity profiles in patients allergic to PR10 proteins and in non-allergic subjects exposed to PR10 allergens. Recombinant PR10 allergens (Bet v 1, Mal d 1, Aln g 1, Cor a 1.01, Cor a 1.04, Que a 1, Pru p 1, Gly m 4, Act d 8, Dau c 1, Api g 1) were expressed as hexahistidine-tagged proteins in E.coli, purified by Nickel affinity chromatography and characterized by SDS-PAGE, circular dichroism, spectroscopy and mass spectrometry. Two unfolded Bet v 1 fragments comprising the first and second half of Bet v 1 were obtained by E.coli expression. Six Bet v 1- and Mal d 1-derived peptides were prepared using solid phase synthesis, purified by HPLC and checked by mass spectrometry. Antigens were immobilized on glass slides using SciFlexArrayer S12.
We demonstrate the utility of the PR10 allergen micro-array to study the extent of IgE cross-reactivity between Bet v 1 and PR10 allergens in birch allergic patients, the natural PR10-specific antibody response in non-allergic subjects and to verify that IgE antibodies of Bet v 1-allergic patients react exclusively to folded PR10 allergens but not to unfolded Bet v 1 fragments or PR10 allergen-derived peptides. Contrarily, IgG from allergic and non-allergic subjects react with conformational and unfolded epitopes.
Our results demonstrate that IgE and IgG antibodies from birch pollen allergic patients recognize unrelated epitopes and seem to origin from clonally unrelated B cells. The PR10 allergen micro-array is useful for diagnostic purposes to reveal IgE cross-reactivity in PR10-allergic patients and to monitor antibody responses and blocking effects of IgG on IgE binding during AIT.

P1.07

Particulation of Bet v 1 decreases allergen-specific Th2 responses in mice

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Background: Although many common features of allergens have been proposed, up to date, no causative relationship between protein intrinsic features and IgE-inducing capacity has been identified. One common feature for aeroallergens is a high aqueous solubility causing a fast release from the allergenic source. The current study aims to investigate the concept of solubility as a causative feature of allergenicity.
Methods: To alter solubility, the major birch pollen allergen Bet v 1 was covalently coupled to silica particles. To investigate the sensitization potential of particulate Bet v 1 compared to soluble Bet v 1, Balb/c mice were subcutaneously immunized with either Bet v 1, particulate Bet v 1 or silica particles without Bet v 1. Aluminium hydroxide was used as an adjuvant in all three groups. Specific IgE serum levels were analysed using a mediator release assay. Specific IgG1 and IgG2a levels were analysed by ELISA. T cell polarization was analysed via flow cytometry in splenocytes after restimulation with Bet v 1.
Results: Sera from the particulate Bet v 1 group were significantly less reactive in the mediator release assay, indicating a lower level of specific IgE antibodies. In line with that, splenocytes of the particulate Bet v 1 group had significantly lower frequencies of Gata3+, CD4+ T cells. Specific IgG1 and IgG2a levels were comparable between both groups. T-bet+, CD4+ T cell frequencies were also lower in the particulate Bet v 1 group, however, the difference was less pronounced.
Conclusion: Our results demonstrate that particulation of allergens decreases Th2 polarization while Th1 and Treg responses are not affected. This supports the hypothesis that solubility of proteins is an important prerequisite for becoming an allergen. These findings might open opportunities for altering the solubility of allergens in vaccine preparations in order to increase the efficacy of allergen-specific immunotherapy.

P1.08

Macropinocytosis is the main uptake mechanism for allergen-specific virus-like nanoparticles

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Background: Virus-like nanoparticles (VNP) are regarded as a safe vaccination platform and have proven to be suitable for allergen-specific immunotherapy in preclinical models. In fact, we previously showed that VNP derived from the Moloney murine leukaemia virus (MoMLV), expressing a shielded version of the major mugwort pollen allergen Art v 1 (MA::Art v 1 VNPs) are hypoallergenic and induce allergen-specific immunotolerance. While our study revealed efficient uptake of VNP by lung APC in vivo, the exact uptake mechanism(s) operative were not elucidated as of yet.
Methods: Herein we tested a collection of inhibitors for their impact on VNP uptake by APC. Firstly, the uptake of MA::Art v 1 VNP was examined with murine DC-2.4 dendritic and human leukemic THP 1 monocytic cells by a split luciferase reporter-system. In parallel, classical fluid phase markers and fluorescently labelled VNPs were used as controls to confirm results obtained with the split reporter-system by flow cytometry. Subsequently, different fluorescently labelled VNP (FITC, cell-mask orange, mCherry) were used to study VNP uptake by primary APC types isolated directly from mouse lungs and spleens in vitro. These organs were selected to provide insights into the future prospects for success of i.t.- and i.v.-based VNP application routes.
Results: The combined results of this study indicate that MA::Art v 1 VNPs are taken up into APC primarily by macropinocytosis, as confirmed by experiments performed with bona fide APC lines and primary APC. VNP uptake could be blocked with sucrose (300 - 500 mM) and Rottlerin (1 - 10 µM), both substances are well-established inhibitors of macropinocytosis.

Conclusions: We have established a high-throughput split-luciferase reporter system for the screening of substances that may affect VNP uptake in APC and we have confirmed obtained results using fluorescently labelled VNP. With the help of these screenings, we have identified macropinocytosis as being the main mode of VNP uptake by APC in vitro, which may pave the way to further improve VNP-based applications in the future.

P1.09

Allergy tests in patients with chronic urticaria- the allergologist´s point of view

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Introduction: According to the current “The EAACI/GA(2)LEN/EuroGuiDerm/APAAACI guideline for the definition, classification, diagnosis and management of urticaria”, allergy tests should not be performed regularly in the routine workup of urticaria patients. The guideline is based on studies suggesting that allergies are rarely causing chronic spontaneous urticaria. Our study is challenging this statement and asking how often allergies can be detected as an underlying cause in patients suffering from chronic urticaria.
Methods: All patients seen by one clinician at the allergy outpatient clinic “Floridsdorfer Allergie-Zentrum” in the year 2018 were included in this study. Three diploma students established a database using the Excel software based on 5857 patients’ reports. Excel and SPSS were used for data analysis, and calculation.
Results: For this study, we analyzed the subgroup of 554 patients (38.6 ± 18.8 years) with chronic urticaria. Of them, 31.4% (n=174) were male, 68.2% (n=380) female. 332 patients suffered from chronic spontaneous urticaria. For the 222 patients with chronically inducible urticaria, the following physical triggers were identified: 164 dermatographism (29.6%), 19 cold urticaria (3.5%), 16 cholinergic (2.9%), 7 pressure (1.3%), and 7 heat urticaria (1.3%), 2 others (0.4%), with overlaps occurring. In 7.9% (n = 44) a type I allergy was detectable as the eliciting trigger. Of these, 33 suffered from immunological contact urticaria, in which wheals developed after direct contact with allergens, e.g. grass or cat saliva. The remaining 11 patients (2.0%) had positive allergy tests to foods that could be present in multiple sources, e.g., alpha-gal, LTP, mugwort-celery-spice syndrome, or peanut.
Of all 554 patients with chronic urticaria, 23 patients (4.2%) met the criteria of anaphylaxis according to the classification of Müller I-IV. The eliciting allergens were insects, food, and drugs.
Furthermore, 97 patients of all 554 patients with chronic urticaria (16.4%) had a clinical relevant sensitization against inhalant allergens.
Conclusion: In approximately 8% of cases, a previously unrecognized allergy (food allergy or anaphylaxis) was detected as the cause of the chronic urticaria through routine allergy testing. Furthermore, the assessment in the skin prick test revealed a high incidence of dermatographism as an inducible physical urticaria. Lastly, irrespective of the type of urticaria, a considerable proportion of patients exhibited a clinically significant inhalant allergy, leading us to conclude that an allergological baseline testing is advisable in patients with chronic urticaria.

P1.11

Development of a protocol to separate high and low affine allergen-specific antibodies

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Background: One immune mechanism of allergen immunotherapy (AIT) is the induction of IgE-blocking antibodies. We have recently shown that high IgE-blocking activity accorded with high affinity. Moreover, studies on passive immunotherapy of allergic patients with allergen-specific monoclonal antibodies (mAbs) assigned the clinical success to high affine IgE-blocking antibodies. The in vivo levels of such blocking antibodies are unique to each AIT-treated individual and may change in the course of therapy. Our first goal in the process of characterizing this individual variability in more detail was the establishment of an affinity purification protocol for allergen-specific antibodies from sera. For this purpose, we employed the major birch pollen allergen and a panel of mAbs of differing binding strength to Bet v 1.
Methods: Recombinant Bet v 1 was coupled to NHS-sepharose beads and incubated with four specific mAbs with diverse affinity as assessed by surface plasmon resonance and a non-specific control mAb. After centrifugation, the supernatants were collected (=flow through) and the beads were washed intensely. Finally, mAbs were eluted with glycine-HCl, pH 2.4, and their recovery was assessed by immunodot blot and BCA.
Results: Antibodies of high affinity to Bet v 1 were almost completely recovered with minute amounts detected in the flow-through. Antibodies of low affinity were retained in the flow-through and their recovery by elution was very low. The non-Bet v 1-specific mAb did not bind to the beads confirming specific purification.
Conclusion: Our affinity purification protocol is applicable to separate Bet v 1-specific mAbs of high and low affinity. Thereby separated fractions of polyclonal antibodies from sera of AIT-treated individuals will now be tested for their IgE-blocking activity to study whether only high affine Abs are relevant for therapeutic efficacy of AIT.

P1.12

A modified ELISA to assess the binding strength of allergen-specific antibodies in serum samples

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Background: Allergen immunotherapy (AIT) induces allergen-specific IgE-blocking antibodies. Using human allergen-specific monoclonal antibodies (mAbs), we recently showed that strong IgE-blocking accorded with high affinity. To better characterize the features of AIT-induced IgE-blocking antibodies, the avidity of polyclonal IgG in post-AIT sera should be studied. However, avidity measurements in serum are problematic due to several technical obstacles. Here, we tested whether acidic disruption of antibody-allergen complexes is applicable for this purpose. A modified ELISA protocol was established with mAbs of known affinities and then applied to sera collected after AIT with either recombinant Betv1 or Mald1, which induced differing IgE-Mald1 blocking activities.
Methods: Recombinant allergens were coated to microplates. Next, specific mAbs, diluted in PBS or serum, were incubated. The immune complexes were exposed to buffers of increasing acidity up to pH 3.4. Remaining mAbs were expressed as the percentage of those bound at neutral pH normalized to 100%. Avidity indexes (AIs), reflecting the pH at which 50% of antibodies dissociate, were calculated and compared to dissociation constants (KD) measured by surface plasmon resonance (SPR). Finally, Mald1-specific IgG1 antibodies in sera taken after AIT with Mald1 (n=8) or Betv1 (n=9) were tested.

Results: The chosen pH range disrupted immune complexes while retaining allergen integrity. Resulting AIs of mAbs were reproducible and agreed with SPR analyses. They were independent of epitope specificity and the presence of serum proteins did not influence assay performance. Mald1-AIT-induced IgG1 showed higher avidity to Mald1 than Betv1-AIT-induced IgG1, which corresponded with a higher IgE-blocking activity of post-Mald1-AIT sera.
Conclusion: The modified ELISA accurately estimated the binding strength of mAbs in serum. Thus, resistance to acidic disruption of antibody-allergen complexes is useful for further studies on antibody avidity in the context of AIT. A first analysis of AIT-induced polyclonal serum antibodies showed that avidity correlated with IgE-blocking.

P1.13

RNA-seq analysis of nasal mucosa following nasal provocation in birch pollen-allergic individuals

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Birch pollen (BP) directly interacts with airway epithelial cells resulting in cellular responses, which may lead to allergic sensitization in certain individuals. We aim to characterize the cellular responses upon BP nasal provocation (NP) in BP-allergic and non-allergic individuals, using a systems biology approach. BP-allergic (n=11) and non-allergic individuals (n=12) were recruited, informed consent was obtained. Participants were subjected to NP with saline solution (N1) as baseline and an aqueous BP NP solution (N2), on two separate days. Nasal scrapings (NS) were obtained 15 minutes after N1. Participants were assigned to four different time points to obtain NS after N2. Total RNA was isolated from NS, sequencing was performed and gene expression profiles between N1 and N2 were compared. Nasal secretions were obtained at N1 and N2 from all participants to detect cytokine levels. At time points 15, 30, 60 and 120 minutes, 8, 123, 13 and 21 DEGs in allergic patients and 13, 3, 21 and 7 DEGs in non-allergic subjects, respectively, between N1 and N2, were obtained. Preliminary gProfiler over-representation analysis revealed significantly enriched cytokine signaling pathways in allergic patients and keratinization related pathways common in both allergic and non-allergic groups. Ingenuity pathway analysis with combined DEGs identified 92 significant Canonical Pathways and 3122 Upstream Regulators in allergic patients. Enrichment analysis determined activation of biological processes like chemotaxis, cytokine mediated signaling pathways and keratinization in allergic patients. Significant increase in levels of IL-7, IL-16, MIP-3a, IL-33, TARC and Eotaxin-3 was observed in allergic patients compared to non-allergic participants after birch pollen nasal provocation. Our results demonstrate activation of cellular pathways related to innate immune responses, chemotaxis and cell adhesion in allergic patients after NP, which do not occur in non-allergic participants. These DEGs and dissected pathways may open new perspectives to understand the key players in BP allergy.

P1.14

Biophysical and immunological characterization of Arginine kinases from House dust mite species and other invertebrates

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House dust mites (HDM) are among the most common triggers of allergy worldwide. Allergenicity is found for different HDM species, predominantly for Dermatophagoides pteronyssinus and Dermatophagoides farinae, which represent the majority of WHO/IUIS-reported HDM allergens. To date, 40 HDM allergen groups have been classified. Arginine kinases are classified as group 20 and minor HDM allergens. They belong to guanidine phosphotransferases that catalyze the reversible transfer of phosphoryl group from ATP to arginine, forming ADP and N-phosphoarginine, which is a form of energy storage in various invertebrates. Some invertebrate arginine kinases were shown to be cross-reactive allergens associated with respiratory and food allergies. Arginine kinases are monomeric proteins that share high sequence and structural similarities among numerous invertebrate species. Within mite species, they exist in 2 isoforms, namely Der p 20 and Der p 20_like in Dermatophagoides pteronyssinus. To our knowledge, the functional and molecular background of the existence of two arginine kinase isoforms within HDM species has not been shown so far. Based on homology search we screened for arginine kinases that have a high sequence identity with HDM arginine kinases originating from D. pteronyssinus (Der p 20, Der p 20_like) and D. farinae (Der f 20, Der f 20_like) and selected itch mite, Sarcoptes scabiei (Sar s 20), black tiger shrimp, Penaeus monodon (Pen m 2), and grasshopper species, Schistocerca americana (Sch a). Recombinant arginine kinases from respective species were cloned, expressed in E. coli and purified as histidine-tagged proteins and by using size-exclusion chromatography. Arginine kinases were biophysically characterized and kinase activity was measured and compared among homologs. In silico epitope prediction analysis, using BepiPred and DiscoTope, suggested possible linear and conformational B-cell epitopes, which showed to be similar among chosen homologs. Furthermore, immunochemical techniques were used to investigate arginine kinase allergen cross-reactivity within selected invertebrates.

P1.15

Expanding the nanobody toolbox: Bispecific nanobody designs for topical application in birch pollen allergy

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Background: Mucosal surfaces in nose and eyes are prominent entry sites for pollen allergens and therefore represent an opportunity to intervene with allergen uptake and subsequent local effector cell activation. Intercellular adhesion molecule 1 (ICAM-1) expression on these surfaces is upregulated in allergic individuals and was identified as promising target to anchor antibody-based constructs that bind both, ICAM-1 and the allergen. We sought to generate bispecific nanobodies targeting ICAM-1 and the major birch pollen allergen Bet v 1 and investigate their ability to inhibit allergen penetration.
Methods: Bet v 1- and ICAM-1-specific nanobodies were analyzed for their binding characteristics by ELISA, flow cytometry and immunofluorescence microscopy. DNA sequences coding for Nb32 (Bet v

1-specific) and Nb44 (ICAM-1-specific) were connected using a standard GS-linker, and equipped with a His-tag, or a spacer sequence, HA-tag and His-tag (-hHH). Sequences were codon-optimized, synthesized and ligated into pMES4. Bispecific constructs were expressed in E. coli, purified by affinity chromatography, and specificities against both recombinant antigens were tested by ELISA. Binding to ICAM-1 expressed by the human bronchial epithelial cell line 16HBE14o- was studied by flow cytometry.
Results: Nb32 bound to Bet v 1 and cross-reactive allergens. Nb44 recognized ICAM-1 on cell surfaces and was not internalized during the study period (24h). We designed three bispecific constructs: Nb32-44, Nb44-32 and Nb32-44-hHH. Only Nb32-44 and Nb44-32 were sufficiently expressed and investigated further. Both designs simultaneously bound to Bet v 1 and ICAM-1 in ELISA, but to different extends. This binding behavior was also reflected in recognizing ICAM-1 expressed on cells (Nb32-44: 82.0 ± 6.6% vs. Nb44-32: 36.2 ± 0.8% of alive cells).
Conclusion: The bispecific nanobody candidate Nb32-44 showed favorable binding characteristics by recognizing both antigens and binding to cell surfaces. Further cell-based assays will determine its potential in blocking allergen penetration through epithelial cell layers.

P2.03

Immunodeficiency Newborn screening in Austria – 2 years' experience & outcome

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Background: Severe combined immunodeficiencies (SCID) are defined as having the risk to present as a life-threatening pediatric emergency situation. In general, newborn screening (NBS) for critical diseases enables early postnatal presymptomatic diagnosis and prompt treatment. Nowadays, in developed countries, NBS is regarded as a state-of-the-art prerequisite for lifesaving therapeutic management also for severe combined IDs. In June 2021 NBS for the entire Austrian birth cohort was expanded for significant T-cell and B-cell deficiencies and for spinal muscular atrophy by adding an 'one-stop-shop' unique PCR approach.
Methods: After having performed a pilot study from 2019-2020 by comparing different combined PCR kits for T-cell receptor excision circles (TRECs) and kappa-deleting recombination excision circles (KRECs) quantification, a CE-IVD certified kit for simultaneous analysis of TRECs, KRECs as well as the SMA-causing deletion in exon 7 of SMN1 was established for the screening of the entire Austrian newborn cohort (n = 165.000/ 2021-2023).
Results: 54 newborns were identified with significant T- and/or B-cell-cytopenia, requiring further evaluation. 11 of identified newborns were confirmed to have an immunological disorder, some being classified as primary ID/IEI. Others showed improved results upon further investigation due to postnatal maturation, or are still under a diagnostic process. 3 newborns were diagnosed with SCID and underwent successful stem-cell transplantation, 1 newborn was diagnosed with inborn Agammaglobulinemia type 2 and is under continuous care.
Conclusion: Combined screening by PCR approach for Severe (Combined) Immunodeficiencies and Spinal Muscular Atrophy in Austria was proven to be technically feasible and successful. Regarding the development of novel treatment options, including gene therapy, this combined screening represents a backbone of targeted medicine. Its follow up from 2021 – 2023 has demonstrated to be successful in respect of prompt introduction of personalized therapeutic approaches.

P2.04

TET2-mosaicism in human is associated with lymphoproliferation, autoimmunity, immunodeficiency, and hematologic malignancy

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Background: The TET2 gene encodes ten-eleven translocation methylcytosine dioxygenase 2 (TET2). TET2 is an epigenetic regulator that converts 5-methlycytosine to 5-hydroxymethylcytosine and also interacts with histone-modifying enzymes and transcription factors. Somatic mutations in TET2 are early events in clonal expansion and are found in association with myeloid and lymphoid

haematological diseases. Germline mutations in TET2 have only been described in 3 children of consanguineous parents. These mutations led to immunodeficiency and lymphoma and early mortality in childhood.
Objectives: To assess the clinical and immunological consequences of a combination of a germline and a somatic mutation in TET2.
Methods: Genetic analysis was done by whole exome sequencing. An in-depth immunological analysis was performed.
Results: Clinical phenotype:
We studied a patient of non-consanguineous parents that presented at the age of 38 with HLA-B27-positive axial spondyloarthritis. Apart from trace homogeneous fluorescence on the HEp2 cell his immunologic work-up was unremarkable. Due to a secondary loss of effect of NSAIDs he was started on a TNF-inhibitor. Subsequently, he developed persisting low-grade fever, serositis, interstitial lung disease, peripheral arthritis and a pronounced lymphadenopathy and splenomegaly. His condition improved after the TNF-inhibitor was changed to an IL-17 inhibitor combined with oral prednisone (up to 40 mg/d) but lymphadenopathy persisted and the patient recurrently suffered fom low-grade fevers, fatigue and pleurisy. Several lymphnode biopsies showed no signs of malignancy. During that time the patient had recurrent episodes of pancytopenia that spontaneously improved after few days and developed two episodes of pneumonia that required hospitalisation. Furthermore, he suffered from recurrent episodes of herpes zoster.
Immunological results: On immunologic work-up the patient had a polyclonal hypergammaglobulinemia and autoantibody testing revealed a homogenous ANA (1:1600), and positive anti-nucleosomen-, anti-PM-Scl75-, anti-SRP-, anti-PL-12-, anti-phospholipid-, anti-dsDNA- and anti-MPO-autoantibodies. Despite of increased amounts of immunoglobulins the patient developed a progressive and persistent loss of B cells, with an increased expression of CD80/86 on the remaining memory B cells. Within the T cell compartment double-negative T cells were increased.
Results of whole exome sequencing: Whole exome sequencing revealed a germline mutation in TET2 with the variant c3641G>A; pArg1214Gln in the heterozygous state (NAF 0.53; NM_001127208.3). This mutation affects a phylogenetically conserved amino acid and is classified as predominantly pathogenic in the in silico prediction. Another variant identified is the mutation c.1864C>T; p.Gln622, which leads to the emergence of a stop codon (NAF 0.41). This variant was only detectable as a low-grade mosaic (5-10%) in the buccal mucosa in the control, possibly as a result of lymphocytic infiltration into the buccal mucosa. In leukocytes, this mutation was detectable in 83% of cells in the heterozygous state. Both mutations have a low frequency in the population (1-2/125000-150000; gnomAD).
Hematogical malignancy: 5 years after the progessive loss of B cells had started, the patient was diagnosed with a follicular B cell lymphoma and shortly after with AML with myelodysplasia-associated changes (AML-MRC). .
Conclusions: Combined heterozygous germline and somatic mutations in TET2 are associated with a complex phenotype combining autoimmunity, lymphoproliferation and hematological malignancy, may present in adulthood and thus clinically differ from combined heterozygous germline mutations with early onset in childhood.

P2.05

Determination of Aged-Associated CD4+ T cell Distribution throughout Different Organs by Spectral Flow Cytometry

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The improvement of healthcare systems and medical advances increase the lifespan rising up a demographic aging that augments the incidence of age-associated diseases such as cardiovascular, neurodegenerative or metabolic diseases. Recently, the low-grade chronic inflammation known as "inflammaging" has been related to the development of age-associated multimorbidity, promoting aged-associated diseases and immunosenescence.
Upon aging, thymus involution affects the distribution of T cell populations, reducing the naïve T cell pool and increasing the effector T cell pool. The development of the single cell RNA sequencing (scRNAseq) has allowed to identify and characterize several populations of aged-associated T cells (Taas) among this effector T cell pool that have an important contribution to the development of inflammaging.
We have developed a cytometry panel of spectral flow cytometry, based on the scRNAseq results, that allows us to identify the same Taas populations in a faster and more economical manner. Using such panel, we have determined the different distribution of Taas in several tissues both lymphoid (spleen and bone marrow) and non-lymphoid (white adipose tissue, liver, brain or colon) and we have observed that some Taas accumulate preferentially in specific tissues. Among the different Taas we have identified a population of CD4+ Tcells that accumulates in liver with age with a cytotoxic profile, Th17 like, that harbors mitochondrial and DNA damage, that are similar to those identified in pathologies such as EAE.
This data will allow us to design strategies to study the functional and pathological implication of these Taas in each tissue as well as its contribution to their homeostasis.

P2.06

Novel N-glycome analysis with conserved sialic acid residues for biomarker detection in post-viral fatigue

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Evaluation of N-glycosylation pattern is gaining interest in immunology as it plays a crucial role in the structure and function of molecules relevant for the immune function. N-glycans can modulate the activity and specificity of antibodies, the complement system as well as pathogen-host interactions influencing the ability of binding to antigens and of eliciting an immune response. Alterations in N-glycan structures have implication in a large variety of diseases such as rheumatoid arthritis, cancers and viral infection. Thus, a comprehensive picture of the N-glycosylation profile is pivotal for better understanding of mechanisms associated with poorly understood disorders such as post-viral fatigue. Sialic acids in antennary positions of glycans were stabilized through modification and derivatization of the carboxylic acid group using 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM) and methylamine. The glycosylated proteins themselves served as a solid phase support for glycan derivatization and purification from excess reagents, as well as the for hydrolysis of the glycans.
After purification and concentration via HILIC microextraction, the N-glycome of biological fluids from Long-COVID patients were analysed using MALDI-MS in positive mode. MS spectra were analysed using the Glycoworkbench software and a user definite database allowing detection of custom made modifications and annotation of the single N-glycans.
In patients' sera more than 10 weeks after COVID infection we observed significant changes in the N-glycome profile. High molecular weight glycans were reduced significantly in patients with persistent fatigue development after COVID infections being associated with elevated levels of non-sialyated

glycans. This was in contrast to the pattern detected in healthy controls indicating a persistent change of the glycome on serum proteins after viral infection.
Our method allows a rapid, accurate and cost-effective identification of N-glycans in biofluids opening up future developments in diagnostics identifying new biomarkers, since distinct N-glycosylation pattern have been observed in many pathophysiological conditions.

P2.07

The fitness of human CD8+ T cells is set by the coordination of actin remodeling and metabolic programs

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Background: Cytotoxic T lymphocytes (CTL) expressing the co-receptor CD8+ are a subset of immune cells, which are specialized in eliminating infected cells and tumor cells. CTL execute their cytotoxic function by relying on a high capability to migrate in most parts of the organism, a very specific ability to recognize antigens at the surface of target cells and a sophisticated cytotoxic machinery. Each of the dynamic steps ruling the activities of CTL, from migration to recognition and killing of target cells are extremely dependent on the remodeling of the actin cytoskeleton. Although actin network dynamics is recognized as a major engine for the different steps ruling CTL function, its energetic burden has not been assessed. We here investigated how the energy expenditure related to actin remodeling might condition the fitness of human cytotoxic T cells.
Methods: To assess the interplay between actin remodeling and metabolic status of CTL, we applied a combination of high content cell imaging pipeline, flow cytometry based assessment of metabolic status (SCENITH), functional assays (confined migration, cytotoxicity) and bulk RNA sequencing to primary human CD8+ T-cells.
Results: We first established that the spreading ability of CTL cells in conditions of LFA-1 and TCR engagement mirrored the cytotoxic potential of these cells. Morphological and functional fitness were both potentiated by IL-2, which co-stimulated the transcription of glycolytic enzymes, actin isoforms and ARP2/3 subunits. This molecular program scaled with F-actin content and cell spreading. Blockade of glycolysis reduced F-actin density at the lamellipodium and impaired chemokine-driven motility and synaptic adhesion. T cells naturally deficient for the ARP2/3 subunit ARPC1B and its activators WASP and HEM1 presented with morphological and functional defects that could only be partially alleviated by IL-2, positioning ARP2/3 mediated actin polymerization as a major drain for ATP production via glycolysis.
Conclusion: Our study reveals multiple layers of relationship between the glycolytic activity of effector T cells and their ARP2/3-mediated ability to migrate, to assemble the immunological synapse and ultimately to kill target cells.

P2.08

Elevated concentrations of sodium chloride rewire macrophage metabolism to potentiate the proinflammatory phenotype

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Background: A high intake of dietary salt (NaCl) has been implicated in the development of hypertension, chronic inflammation, and autoimmune diseases, as it leads to Na+ accumulation in certain tissues above the serum levels and alters the function of resident immune cells. However, whether increased Na+ is present in synovial tissue of rheumatoid arthritis patients and affects the immune microenvironment is largely unknown.
Methods: Concentrations of selected ions, including Na+, were measured in synovial fluids of patients with active arthritis. To study how changes in extracellular Na+ influence mononuclear phagocytes, which are crucial drivers of arthritic inflammation, we differentiated human blood monocytes to macrophages in media containing different concentrations of NaCl. Macrophages were also activated to the proinflammatory M1 phenotype and characterized by qPCR, cytokine analysis, flow cytometry, and their effect onto T cells was assessed in coculture experiments.
Results: We show that synovial fluids of arthritis patients exhibit elevated concentrations of both Na+ and Cl-. Subsequent in vitro experiments demonstrated that macrophages responded to the elevated extracellular NaCl by modulating expression of several cytokine and metabolic genes and accumulated intracellular ATP. High salt-treated macrophages more potently stimulated autologous T cells in coculture experiments than their isotonic counterparts, and skewed the T cells towards a Th1/Th17 phenotype. Observed effects were even more pronounced when macrophages were polarized to the M1 type.
Conclusions: Our results indicate that increased salt concentrations trigger metabolic rewiring in macrophages with possible implications in the immunopathology of rheumatoid arthritis. In the future, we aim to uncover the precise molecular pathway that links sensing of excess extracellular salt to changes in macrophage energy metabolism.

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P2.09

The fate of dietary immuno-modulating glycan along the gastrointestinal tract

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Background: Glycan structures, which are bound to the surface of proteins and lipids, are involved in numerous physiological and pathological processes including cell-cell interaction, antibody receptor binding, cellular infection by viruses and many other. Codified in the glycan structure, each nutrient contains a specifc glycosignature depending on the dietary source. Moreover, dietary glycans might influence human health due to potential incooperation of foreign glycans in the human glycome. Thus, we aimed to determine the glycan composition of different food sources and of samples collected along the gastrointestinal tract for a better understanding of the fate of dietary glycans.
Method: The N-glycome of different food products and oro-gastrointestinal samples was characterized by MALDI mass spectrometry in positive mode after purification and derivatization with methylamine. After labelling with DMBA, total and specific sialic acid content was quantified by HPLC-RP-FL.
Results: We observed substantial variations in N-glycome and monosaccharide composition depending on the source of the food: vegetable, fungal, meat, milk (human vs animal milk), and cheese (fermented milk). Starting from the mouth throught the stomach and the intestine, different degree of gylcan hydrolysis was observed, with mainly the sialic acid residues being cleaved from the glycan structure.
Conclusion: Our results clearly confirm the species-specific glycan pattern reflected by a distinct N-glycome and sialic acid amount and composition in different food, but also in gastrointestinal samples. As the dietary glycan composition influences human health, immuno-nutritional effects should be taken into consideration.

P2.10

Lung tumor neutrophils undergo heterogeneic metabolic reprogramming during NSCLC

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Background: Lung cancer is among the most common and deadly cancers, and neutrophils are a key immune cell found in high abundance in lung tumors. Neutrophils are generally pro-tumoral but display heterogeneity, signifying a need for further studies of their roles in the lung tumor microenvironment (TME). Immunometabolism, which deciphers how metabolism of immune cells dictate their function, is not well-understood in neutrophils, especially within the TME, where signaling molecules and nutrient levels are altered.
Methods: Flow cytometry was performed on neutrophil populations of human lung and tumor tissue surgically removed from non-small lung cancer (NSCLC) patients. In vitro experiments testing metabolic inhibitors and fuels were performed with neutrophils from healthy and NSCLC individuals. Mitotracker Deep Red and Seahorse measured mitochondrial function in these neutrophils ex vivo and in vitro. Metabolic pathways in neutrophil subsets in NSCLC were analyzed using single-cell RNA sequencing data.
Results: Neutrophils displayed altered mitochondrial metabolism upon infiltration into the tumor, and subpopulations of lung TME neutrophils had varied expression in metabolic pathways, including glycolysis and oxidative phosphorylation. Neutrophil populations with CXCR2hi and CXCR2lo expression had modified mitochondrial membrane potential and metabolic gene expression. In vitro,

human neutrophils showed enhanced mitochondrial function when treated with NSCLC tumor supernatants, consistent with our ex vivo human data. Neutrophils increased mitochondrial function upon treatment with IL-8, a CXCR2 ligand, revealing a potential axis between IL-8/CXCR2 in mitochondrial metabolic remodeling. Further, human neutrophils had varied functional responses, including ROS production and degranulation, upon treatment with glycolytic and mitochondrial inhibitors in combination with tumor supernatants and IL-8.
Conclusion: These results reveal that neutrophils and their subsets undergo specific metabolic changes, including in mitochondria, within the lung TME during NSCLC. This metabolic remodeling mediates functional changes in tumor neutrophils. Mitochondrial pathways in neutrophils may provide a potential future therapeutic for lung cancer.

P2.13

The influence of αKG supplementation in obesity

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Background: Alpha-ketoglutarate (a-KG), a key tricarboxylic acid cycle metabolite and derivative of dietary glutamine and glutamate reportedly extends lifespan and decrease body-weight gain of HFD challenged aged mice primarily through effects on adipocyte browning, associated with improved metabolic homeostasis.
Methods: Here, we investigated the immune-modulatory and metabolic effects of a-KG supplementation on younger HFD challenged animals in a short term (4 weeks) and long term (16 weeks) context.
Results: In contrast to previous findings reporting that the obesity prevention effects of a-KG were specific for aged animals we find that 8 week old animals receiving a-KG supplementation in flavoured water exhibit a dose-dependent weight loss post short-term HFD with an attenuation of both subcutaneous and visceral adiposity. These effects were related to reduced food intake. Contrastingly, in animals challenged with prolonged HFD, a-KG improved glucose and insulin tolerance, independent of effects on food and water intake, energy expenditure, activity as well as adipose browning.
Conclusion: To conclude, these findings show that a-KG is able to prevent negative obesity-related effects. We further hypothesize that aKG is able to influence the global metabolic landscape and thus promote an anti-inflammatory phenotype.

P2.14

Regulation of tissue residency in the skin after hematopoietic stem cell transplantation

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Tissue-resident memory T cells (TRM) regulate immune defense and homeostasis at barrier sites. Factors determining human TRM development and function in specific tissues are understudied, as

adequate human models are lacking. In the skin of patients undergoing hematopoietic stem cell transplantation (HSCT), host skin TRM survive myeloablative conditioning, while donor T cells can home to the skin to form a new TRM pool, which exists beside host TRM. To discriminate host and donor origin of immune cells and study pathways involved in the development of TRM, we performed a longitudinal analysis on HSCT patient samples. We performed single-cell RNA and T cell receptor (TCR) sequencing on skin and blood-derived CD45+ and CD45- cells of patients before and after HSCT (days -7, 0, +14, +100) to uncover changes in their transcriptional state, define dynamics of T cell clonality in parallel in two compartments and assess cell-cell interactions occurring in the skin.
We recovered leukocytes and structural cells of all longitudinal samples. Immune cells after transplantation were annotated to host and donor origin based on single nucleotide polymorphisms. Distinct T cell subsets displayed time-dependent expression patterns related to TRM function and metabolism, indicating formation of a specialized local immune memory. Few skin T cells of donor origin were detected at day 14 but dominated the skin T cell pool by day 100. Computational cell-cell communication analysis between immune and structural cells predicted several interactions not previously related to TRM function, such as CD97-CD55. We could show upregulation of CD97 on T cells in response to co-culture with CD55-expressing keratinocytes along with other TRM markers, such as CD69 and CD103, suggesting involvement in TRM phenotype regulation. We are currently exploring the functional role of CD97 upregulation in skin TRM. Our study uncovers new mechanisms of tissue-instructed T cell function in human skin, which in turn can instruct new therapeutic approaches for skin diseases.

P2.15

Enhanced Tissue Regeneration and Function 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 CD8α-/- 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 CD8α-/- mice from AKI.
Collectively, our findings suggest that IL-15 treatment enhances the regeneration of renal TECs following IRI and ameliorates AKI. 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.

P2.16

Diversely polarized tissue macrophages contribute to distinct stages of human graft-versus-host disease

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Graft-versus-host disease (GvHD) is a major life-threatening complication of allogeneic hematopoietic stem cell transplantation (HSCT), limiting the broad application of HSCT for hematologic malignancies. Cutaneous GvHD is described as post-transplant inflammatory reaction by skin-infiltrating donor T cells and remaining recipient tissue-resident memory T cells. Despite the major influence of lymphocytes on GvHD pathogenesis, the detrimental role of mononuclear phagocytes (MNP) in tissues affected by GvHD is increasingly appreciated. Using single cell RNA sequencing and multiplex tissue immunofluorescence, we identify increased abundance of MNP in skin and blood from patients with acute cutaneous GvHD. We show that acute GvHD lesions harbor expanded CD163+ tissue-resident macrophage populations with regulatory and tissue remodeling properties. In individuals after sex-mismatched transplantation, we use expression of X-linked genes to detect rapid tissue-adaptation of newly recruited donor MNP resulting in similar transcriptional states of host- and donor-derived macrophages in GvHD skin lesions. Notably, macrophage polarization in chronic lichenoid and chronic sclerotic GvHD types drastically differed from acute GvHD, supporting the notion of distinct cellular players in clinical GvHD subtypes.

P3.01

Myeloperoxidase enhances cell migration and invasion in human choriocarcinoma JEG-3 cells

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Background:
The importance of the immune system in pregnancy and placenta development is well described. Contrary to other leukocytes, the role of neutrophils in this context has been unnoted for a long time. However, since the presence of neutrophils at the maternal-fetal interface was confirmed, their function in placentation has attracted more and more attention. Myeloperoxidase (MPO) is one of the most abundant proteins in neutrophils. By catalyzing the production of reactive oxygen species it plays an important role in host defense. Additional to its antimicrobial function, MPO has also been shown to influence endothelial and cancer cell behavior. As placental extravillous trophoblasts have some shared communalities with tumor cells, such as cell migration and invasion, it was hypothesized that trophoblasts which become exposed to MPO, released by activated neutrophils at the maternal-fetal interface alter their function. In this study, we determined the effect of MPO on JEG-3 human choriocarcinoma cells as a model of extravillous trophoblasts (EVTs) during early pregnancy.
Methods: JEG-3 cell proliferation, apoptosis, migration, and invasion were assessed in vitro and MPO uptake was analyzed using western blot, flow cytometry and fluorescence microscopy.
Results: MPO is present at the feto-maternal interface during first trimester pregnancy, where neutrophils are abundant. We found that MPO was internalized by JEG-3 cells and localized to the cytoplasm and nuclei. MPO internalization and activity enhanced JEG-3 cell migration and invasion, whereas theses effects were impaired by treating cells with heparin, to block cellular uptake, or by the MPO-activity inhibitor 4-ABAH.
Conclusion: This study identifies a novel mechanism for the effect of MPO on EVT function during normal pregnancy and suggests a potential role of MPO in abnormal pregnancies.

P3.02

Farm effect: Beta-lactoglobulin counteracts allergic inflammation via lipocalin-interacting membrane receptor (LIMR) on innate immune cells

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Background: Allergy-protection from the farm effect is mainly mediated via drinking raw cow's milk and inhaling cattle stable dust. Both factors contain beta-lactoglobulin (BLG) as a central protein. We previously demonstrated that the immunomodulatory capacity of BLG depends on ligand load. In dust and ambient air of cattle farms, we identified zinc as the natural binding partner of BLG. Here we aimed to analyze the role of lipocalin-interacting membrane receptor (LIMR) for BLG uptake and a cellular pathway for BLG.
Methods: Expression of LIMR was evaluated on subsets of healthy donor PBMC using flow cytometry. On monocytic THP-1 cells, changes in cellular surface LIMR expression was determined after stimulation with (i) purified BLG, (ii) raw milk as cognate matrix of BLG, or (iii) differently processed

milk samples in titrated concentrations. NFκB-activation after stimulation with BLG, BLG-zinc or different milks was assessed in a monocytic THP-1 NFκB-reporter cell line (THP1-Lucia™).
Results: Within the human PBMC subpopulations, LIMR was exclusively expressed by CD56+ NK cells and CD14+ monocytes, with the highest expression on CD56+dim NK cells. LIMR expression on THP-1 cells inversely correlated with BLG concentration used for stimulation. Incubation of THP-1 with raw milk modified LIMR expression in a concentration-dependent manner. When stimulating THP1-Lucia™ cells, NFκB activation was significantly lower with BLG-zinc compared to BLG alone, and significantly lower when stimulated with raw cow's milk compared to ultra-high-temperature-treated milk.
Conclusion: The putative BLG-receptor LIMR is predominantly expressed on human CD56+dim NK cells, known to highly release the anti-Th2 cytokine IFN-γ. We propose that BLG counterbalances allergic immune responses by dampening the pro-inflammatory NFκB-pathway via LIMR on innate immune cells, thereby contributing to the allergy-protective farm effect mediated via stable dust and raw milk.
Support: Austrian Science Fund FWF (SFB F4606-B28); Biomedical Int. R+D GmbH, Vienna, Austria; Danube Allergy Research Cluster (DARC), project #08 of the Karl Landsteiner University, Krems, Austria. HM was supported by AllergyCare®, Vienna, Austria.
CoI: EJJ is shareholder in Biomedical Int. R+D GmbH, Vienna, Austria and inventor on patent EP 2894478 A1.

P3.03

The allergy-protective milk protein beta-lactoglobulin – from its discovery in stable dust to its source in bovine organs

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Introduction: Numerous studies have shown that life on a farm and consumption of raw milk has a protective effect against atopic sensitization, lasting to adulthood. Among farms, cowsheds have been shown to provide the most beneficial environments. We previously discovered the bovine protein beta-lactoglobulin (BLG), best known from milk, in high quantities in cattle farm dust. We also revealed that this protein is excreted via urine independently of bovine sex. BLG carries micronutrients, originally dedicated for the calf. Depending on the presence of such ligands, BLG may also exert immunomodulatory effects. Until now, the only known production site of BLG is the bovine udder, providing no proper explanation for its presence in high quantities in stable dust and cattle urine.
Aim: In this study we intended to search potential production sites of BLG (apart from udder) in tissue of both sexes in cattle.
Methods: Different bovine organs (udder, kidney, adrenal gland, testis) were tested for the presence of BLG via immunohistochemical staining, ELISA, immunoblot, and RT-PCR.
Results: BLG, apart from the mammary tissue, was detected also in other female bovine organs (kidney, adrenal gland). The expression of BLG could also be demonstrated in male bovine organs (kidney, testis, adrenal gland).
Conclusion: The occurrence of the milk protein BLG in bovine tissue/organs other than the mammary gland questions BLG to be a mere nutritive protein for the calf. We therefore hypothesize that bovine BLG has an innate immune-regulatory function in the animals. In addition, its excretion into the urine

facilitates its environmental distribution. Therefore, BLG might contribute to the allergy-protective farm effect.
Support: Austrian Science Fund FWF (grant SFB F4606-B28 to EJJ); Danube Allergy Research Cluster (DARC#08 of the Karl-Landsteiner University, Krems, Austria, to EJJ). SA was supported by a grant from the Ministry of Higher Education, Egypt.

P3.06

TRPM7 in Ca2+ signaling and T-cell activation

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Ion homeostasis and signaling on a cellular level occurs via ion channels and requires tight regulation. Ca2+ is one of the most prominent signaling ions, playing an important role in all cell types and in immunological functions. In T cells, Ca2+ influx triggers T-cell activation, differentiation, proliferation and expression of cytokines, such as IL2.
TRPM7, a member of the TRP channel family, is a highly conserved, ubiquitously expressed cation channel conducting Mg2+, Ca2+ and Zn2+. Its bifunctional role as an ion channel connected with an intracellular kinase domain makes TRPM7 an interesting signaling molecule. The channel and kinase domain of TRPM7 work interdependent from each other and have been identified as important modulators of immune homeostasis. Previously, we have demonstrated a role of TRPM7 in proinflammatory pathways, also shaping gut immunity. In T cells, TRPM7 acts as crucial regulator of Mg2+ homeostasis, thereby contributing to cellular survival and proliferation. In the past, TRPM7 was extensively discussed in regulated Ca2+ mediated T-cell activation. Indeed, our recent data suggest an involvement of TRPM7 in T-cell-receptor mediated Ca2+ influx, yet it is still unclear how this is aided at a molecular level. We aim to investigate the contribution of TRPM7 kinase in this context in CRISPR/Cas9-engineerd human T cell lines, applying state of the art methods such as mass spectrometry to identify potential target proteins, imaging methods, such as FRET and proximity ligation assays to determine a protein-protein interaction network between TRPM7 kinase and identified candidates, as well as electrophysiological approaches. Ultimately, we aim to translate our findings to primary human T cells.
With our work we aim for a better understanding of the role of TRPM7 in Ca2+ signaling, immune cell homeostasis, immune responses and ultimately in pro-inflammatory diseases, such as autoimmunity.

P3.07

Histone lysine methyltransferase NSD2 as interrelated partner of AID and Ki67 in germinal centers

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The adaptive immune response is a powerful arm of the immune system to combat various pathogens. Secondary lymphoid organs contain lymphoid structures with active germinal centers (GC) which represent highly organized immunological structures. There the critical B-cell-associated immune responses develop and occur, with activation-induced cytidine deaminase, AID, being the master regulator. To understand better the AID-associated mechanisms that drive lymphoid

structure formation and function, we applied an integrative systems biology approach using Tissue Image Cytometry (TissueFAXS platform) and compendium-wide analysis of transcriptomic data sets (GENEVESTIGATOR). Immunostaining of tonsil tissue sections for AID and Ki67 – nuclear protein with complex biology associated with proliferation – with the follow-up single cell-based quantitative image analysis revealed similarity in staining patterns with pronounced dark zone staining of GC B cells. We next aligned the top 200 co-expressed genes for AICDA, encoding AID, and MKI67, encoding Ki67, using microarray data sets attributed to lymphoid tissues. We found 151 overlapping genes. Among overlapping genes is NSD2, encoding the histone lysine methyltransferase. Comprehensive transcriptomics analysis revealed that NSD2 is highly expressed in different immune cells and tissues, especially in different subsets of B cells including tonsillar B cells. Being predicted through bioinformatics, quantitative analysis of staining patterns indeed showed that NSD2-positive cells dominate within the same pre-defined follicular compartments as AID and Ki67. Our data suggest specific role for NSD2 during GC reaction. Overall, we nominate NSD2 as a potential novel marker of tonsillar GC.

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P3.09

Immunity of lipid-lowering drugs: how statins and PCSK9-inhibitors shape immune 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 phenotype with functional outcome, i.e. calcium fluxing and cytokine production. Furthermore, we analyse various biomarkers, including circulating hormones, hepatic fat composition, skeletal microarchitecture, and grey matter composition. 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.

P4.01

Developing of Canine Checkpoint Inhibitors: Caninized Anti-PD1 and Anti-PD-L1 Monoclonal Antibodies with IgG1 and IgG4 Canine Constant Regions for Treating Cancer in Dogs

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Background: While dogs and humans share similar cancer types, cancer-related morbidity, and mortality, canine-specific checkpoint inhibitors targeting PD-1 and PD-L are currently unavailable to treat canine cancer. However, PD-1 and PD-L1 are expressed in dogs, with a 66.2% and 75.7% amino acid similarity to humans, respectively. Therefore, this study aimed to generate caninized versions of IgG1 and IgG4 anti-PD1 and anti-PD-L1 checkpoint inhibitors and evaluate their effects on canine cancer cell lines.
Methods: Canine antibodies were expressed by transfecting Expi293F and ExpiCHO cells with vectors containing canine IgG1 or IgG4 constant regions and the variable regions of the humanized monoclonal antibodies pembrolizumab (anti-PD-1) and atezolizumab (anti-PD-L1). Recombinant canine antibodies were purified from supernatants using affinity chromatography and analyzed using SDS-PAGE. The labeled antibodies, unlabeled antibodies, and a secondary antibody rabbit anti-dog IgG F(ab')2 PE were applied in flow cytometry to stain dog cancer cell lines D17, CF33, and CF41.
Results: Expi293F and ExpiCHO cells successfully produced atezolizumab IgG1 with a yield of 0.4 mg/ml, and the antibody assembly was confirmed through SDS-PAGE analysis. Humanized pembrolizumab and atezolizumab recognized the canine homologous PD-1 and PD-L1 in all tested cancer cells (D17, CF33, and CF41). Furthermore, the purified recombinant caninized atezolizumab IgG1 specifically detected PD-L1 on D17, CF33, and CF41.
Conclusions: Atezolizumab and pembrolizumab recognized PD-1 and PD-L1 molecules expressed by canine cancer cell lines. It was also confirmed that the newly developed caninized atezolizumab IgG1 could bind to PD-L1 expressed by canine cancer cells. Further investigation into atezolizumab IgG1 functional properties will be conducted. The findings suggest that checkpoint inhibitors are an innovative cancer treatment option for dogs.

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P4.02

Myeloperoxidase alters lung cancer cell function to benefit their survival

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Background/Aims:

Neutrophils are an abundant immune cell type in non-small cell lung cancer (NSCLC) that accounts for 85% of all lung cancer cases. Cytoplasmic granule components of neutrophils, such as myeloperoxidase (MPO), are considered to contribute to tumour development, but the involved mechanisms are unknown. MPO is a heme-containing peroxidase and converts hydrogen peroxide and chloride ions to hypochlorous acid (HOCl). Upon neutrophil activation, MPO is secreted into the extracellular milieu where it can modify proteins, lipids, or internalise into neighboring cells. Most of the evidence suggests that MPO supports tumour initiation and progression. Reports demonstrated that MPO can influence proliferation, apoptosis and migration of cancer cells. In our laboratory we showed that MPO-deficient mice have up to 40% reduced tumour size compared to MPO wild-type (WT) mice. To further understand the role of MPO in the setting of lung cancer, we aimed to investigate whether MPO can alter the function of tumour cells in vitro.
Methods:
Changes in proliferation, apoptosis and MPO activity were assessed using flow cytometry. MPO uptake by cancer cells was analysed with western blot and fluorescence microscopy.
Results:
MPO treated human lung cancer cells (A549) show increased proliferation and reduced apoptosis when compared to untreated cells. We found that MPO internalises A549 cells and preserves its enzyme activity as we detected HOCl after exposing cells to MPO. Blocking MPO internalisation with heparin or additional treatment with an MPO specific inhibitor (ABAH) reduced or abolished MPO effects on lung cancer cell function. Lastly, MPO WT mice treated with ABAH developed smaller tumours when compared to vehicle treated mice.
Conclusion:
In conclusion, our results demonstrate that MPO can directly alter cancer cell function in-vitro, in a mechanism involving both; binding to the cell surface and enzymatic activity. The exact mechanism of action needs to be further investigated.

P4.03

The avian chorioallantois membrane as a 3R model to study immunoncology

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Background
The 3Rs principle (Replacement, Reduction, and Refinement), is a fundamental concept in biology and animal research that promotes the ethical and humane use of animals in scientific experiments. The chicken chorioallantoic membrane (CAM) assay is considered a replacement for traditional animal models because it can provide valuable insights into biological processes without using a large number of mammals. It also helps to reduce the number of animals used in research by providing an alternative testing platform. Here we show CAM assay applications in immunoncology.
Methods
Fertile eggs from White Lohman are used in this study. Eggs are incubated for three days, cracked in a sterile dish and incubated for further seven days. Well-defined human cell lines (six hematopoietic malignancies as well as 18 different carcinoma cell lines) have been used for establishing CAM tumors for 3-5 days. We tested different conditions to optimize the growth of the tumors.
Results
We were able to optimize the conditions for the growth of all solid tumors as well as the hematopoietic malignancies. All tested cell lines grow well on CAM and are mitotically active. solid tumors show angiogenic activity.
Conclusion

CAM assay is well suited for the investigation of different tumors. To get the best results, the conditions must be well tested beforehand. The CAM assay can be seen as a reliable in vivo method to study hematopoietic malignancies and the interaction between tumor cells and immune cells, thereby helps to reduce the number of animals used in research.

P4.04

Regulation of T cell functions by cytoskeletal proteins in the melanoma tumor microenvironment

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Background: The clinical course of melanoma patients has been revolutionized in recent years by targeted therapy (TT) and immune checkpoint inhibitors (ICIs). However, 50% of melanoma patients fail to respond to monotherapy and around 30% of responders relapse after initial response to therapy. A tumor microenvironment (TME) with dysregulated T cell populations facilitates progression as well as relapse of disease and determines a patient’s ability to respond to therapy. Tight regulation of actin dynamics is reported to be important for cellular trafficking, activation, as well as response to disease. As there is limited understanding of how T cells remodel their cytoskeleton in response to tumor cells, this study aims to investigate the role of actin-binding proteins in modulating T cell functions in the TME.

Methods: Through extensive analysis of single-cell RNA sequencing (scRNAseq) data obtained from melanoma patient samples, we evaluated the transcriptomic expression and distribution of genes for actin-binding proteins across various T cell subsets in the TME. To further analyze the distribution of these proteins on single-cell level and across T cells in tumor and healthy tissues, we performed immunofluorescence assays and high-content imaging analysis.

Results: ScRNAseq revealed a pattern of increased expression of certain actin-binding protein genes in T cells present in the TME when compared to cutaneous T cells from healthy individuals. The immunofluorescence staining of the tumor tissue samples showed a similar pattern of significant upregulation in actin-binding proteins within T cells present in TME suggesting a significant role of actin-binding proteins in modulating T cell functions under inflammatory conditions.

Outlook: Through further in-vitro functional analysis on patient samples as well as genetically modified cell lines, we aim to map out the effects of downstream actin remodeling events on governing T cell functions in the melanoma TME.

P4.06

Investigating the impact of eosinophils on lung cancer progression

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Until recently, eosinophils were mainly recognized for their role in defending the body against helminth infections and their detrimental effects in allergic diseases. However, recent studies have revealed an additional immunoregulatory role. They have been found in the tumor microenvironment (TME) of various solid tumors, where they directly impact malignant cells and orchestrate immune cell behavior in the TME, resulting in altered tumor development.
The prognostic value of increased tumor-infiltrating eosinophils appears to depend on the tissue microenvironment. In detail, eosinophils were described to be anti-tumorigenic in e.g. colon, skin and breast cancer, but were considered pro-tumorigenic in e.g. cervical cancer. The role of tumor-infiltrating eosinophils in lung cancer remains unclear, but blood eosinophilia of checkpoint inhibitor-treated lung cancer patients positively correlates with better outcome. Thus, we hypothesize that eosinophils could play an important role in development of lung cancer.
To investigate this hypothesis, we injected LLC cells (Lewis lung carcinoma cell line) subcutaneously into both wild type (WT) and eosinophil-deficient ΔdblGATA-1 mice, to detect alterations in tumor development. Subsequently, we performed tumor single cell suspensions to analyze infiltrating immune cell populations (lymphoid and myeloid) by flow cytometry, and collected tumor tissue for the detection of soluble mediators by RT-qPCR. Additionally, we assessed eosinophil effector functions in response to supernatants from specimens from human primary lung cancer and lung cancer cell lines, focusing on e.g. migration and degranulation. Immunohistochemistry was used to identify eosinophils in lung cancer and healthy lung tissue.
Altered tumor growth of LLC cells was detected in ΔdblGATA-1 mice as compared to WT controls. Furthermore, increased eosinophil migration towards lung cancer cell, but not healthy lung epithelial cell supernatants was detected.
Collectively, these results suggest that eosinophils contribute to the development of lung cancer, underscoring the need for further investigation in this area. (Supported by FWF P33325)

P4.07

Influence of age on the tumor microenvironment in a cohort of non-small cell lung cancer

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Background: 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 responses to treatment. Additionally, age is suggested to be an important factor in ICB response. It was reported that the survival advantage of ICB in comparison to conventional therapy was only modest in patients aged over 75. To evaluate the influence of age on the immune environment (IE) in lung cancer, a thorough characterization was performed in patients with untreated NSCLC.
Methods: To characterize the immune environment, flow cytometry and multiplex immunohistochemistry were used. Additionally, TCR sequencing and RNA sequencing were performed. The findings were validated in public datasets.
Results: Higher infiltration of T cells in older patients was found, which could be mainly attributed to CD4 T cells. Validation in the TCGA-LUAD cohort additionally revealed an upregulation of regulatory T cells. No difference was found regarding T cell clonality and Tumor Mutational Burden (TMB)
Conclusion: Higher regulatory T-cell infiltration is associated with treatment failure of ICB. Further research on the biochemical mechanisms of regulatory T cells in ICB response in elderly lung cancer patients is warranted.

P4.08

The involvement of Immunogenic Cell Death in Extracorporeal Photopheresis

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Immunogenic cell death (ICD) gained attention for its ability to reactivate dysfunctional antitumor immune system response. ICD is a type of regulated cell death, but not in a tolerogenic way: during ICD the recognition of antigens of dying cells is induced by the expression of specific danger signals. Indirect evidence supports the hypothesis that Extracorporeal Photopheresis (ECP) triggers ICD. ECP is a photoimmunotherapy providing both immunity against cancer cells and suppression of immune reactions in transplant patients. ECP is providing effective treatment for cutaneous T cell lymphoma (CTCL) with minimal side effects. However, the molecular mechanism behind ECP remains unclear. We hypothesize that ICD is directly responsible for the favourable outcome of ECP in CTCL, which has never been directly investigated in primary human ECP-treated samples.
The induction of ICD danger signals was investigated in primary CTCL patient samples that received ECP treatment. Furthermore, we established a human in vitro model for ECP applied to healthy lymphocytes. ECP-treated patient cells and in vitro treated lymphocytes were incubated for up to 72 hours without additional immunostimulation. ICD-related danger signals were investigated using Mulitplex FACS analysis (Calreticulin) and qPCR (e.g.: HMGB1, CXCL10, etc.).
ECP-treated CTCL patient cells demonstrated increased ICD danger signal expression compared to pre-treatment on both mRNA as well as protein level. Remarkably, the upregulation of ICD signals was primarily seen on tumor cells. In vitro ECP-treated human lymphocytes illustrated a comparable upregulation of ICD markers.
Proving the induction of ICD in ECP grants a new perspective for elucidating the mode of action of ECP and further insights in ICD-mediated cancer immunotherapy. With further experiments based on the functionality of ICD induction in ECP-treated patients, we will decipher the role of ICD in ECP. This will also pave the way towards better understanding of the pathogenesis of the disease.

P4.09

FcgR requirements and costimulatory capacity of Urelumab, Utomilumab and Varlilumab

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Background: Targeting costimulatory receptors of the TNFR superfamily with agonistic antibodies is a promising approach in cancer immuno therapy. It is known that their efficacy strongly depends on FcγR cross-linking.

Methods: Here, we made use of a Jurkat-based reporter platform to analyze the influence of individual FcγRs on the costimulatory activity of the 41BB agonists, Urelumab and Utomilumab, and the CD27 agonist, Varlilumab.
Results: We found, that Urelumab (IgG4) can active 41BB-NFκB signaling without FcγR cross-linking, but presence of the FcγRs (CD32A, CD32B, CD64) augments the agonistic activity of Urelumab. The human IgG2 antibody Utomilumab exerts agonistic function only when crosslinked via CD32A and CD32B. The human IgG1 antibody Varlilumab showed strong agonistic activity with all FcγRs tested. In addition, we analyzed the costimulatory effects of Urelumab, Utomilumab and Varlilumab in primary human PBMCs. Interestingly, we observed a very weak capacity of Varlilumab to enhance cytokine production and proliferation of CD4 and CD8 T cells.
Conclusion: Collectively, our data underscore the importance to perform studies in reductionist systems as well as in primary PBMC samples to get a comprehensive understanding of the activity of costimulation agonists.

P4.10

Myeloperoxidase creates an immunosuppressive tumor microenvironment in non-small cell lung cancer by altering T cell function

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Background: 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 H2O2, 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.

P4.11

TPC-CS nanoparticles as a novel drug delivery system for dendritic cell mediated anti-tumor immunity

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Nanomedicines support an efficient delivery of antigens and adjuvants, further maximizing the immune response against the tumor via dendritic cells (DCs) and holding great potential as complementary therapies to well-established practices such as surgery, chemotherapy and radiotherapy. DCs are key players in anti-tumor immunity as they are exceptionally specialized in capturing, processing and presenting antigens on MHC I or II, thereby activating CD8+ or CD4+ T cell responses, respectively.
However, the immunosuppressive tumor microenvironment (TME) that occurs in many solid tumors affects DC activation, compromising an efficient DC-T cell crosstalk and subsequent T cell responses, enabling tumor growth. Thus, we hypothesize that specific nanoparticles (NPs) have the potential to (re)activate DCs that have been previously tolerogenized by the TME.
To assess this, we developed an in vitro platform of human tumor-associated tolerogenic DCs (tolDCs) and used it to demonstrate that light sensitive tetraphenyl chlorin chitosan (TPC-CS) NPs have the potential to restore DC activation.
Using non-small cell lung cancer as model disease, we have observed that the supernatants (SNs) isolated from single cancer cell line cultures render DCs tolerogenic, with increased expression of immunosuppressive markers such as PD-L1, ILT3, ILT4 and IDO, and reduced secretion of TNFα, even upon stimulation with inflammatory mediators. Moreover, this phenotype is also reflected at the functional level, with increased polarization of CD4+ Treg cells (CD25+FoxP3+CTLA4+ICOS+PD1+CD127-), thus representing the phenotype of immune cells in the TME of cancer patients. Using this platform, we show that TPC-CS NPs hold great potential in preventing polarization into tumor-associated tolDCs in the TME in a light dose dependent manner, either through direct photochemical internalization of NPs in DCs; or indirectly, through photodynamic therapy of cancer cells and subsequent conditioning of DCs with tumor-antigen enriched SNs, leading to increased expression of CD86 and MHC II.

P4.13

Functional characterization of tumor-infiltrating B-cells in NSCLC

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The presence of tumor-infiltrating B-cells and intratumoral immunoglobins correlates with survival of patients presenting several types of solid tumors, including patients with non small cell lung cancer (NSCLC). However, both anti- and pro-tumorigenic functions have been described for B-cells present in the tumor microenvironment (TME). B cells are also a major component of tertiary lymphoid structures (TLS). These ectopic lymphoid organs develop in non-lymphoid tissues at sites of chronic inflammation (like tumors) and display a similar structural organization to that of secondary lymphoid organs (SLO). Their presence can exacerbate the local immune response, as antigen-specific T- and B-cells can undergo terminal differentiation into effector cells within them. The presence of TLSs correlates with better patient outcome in NSCLC and other solid tumor cancer types. The specific phenotype and function of B cells present in a tumor might ultimately depend on the specific cellular

and signaling context within the TME. Identifying and characterizing pro- and anti-tumorigenic B-cell phenotypes could be key to modulating B-cell responses in the TME towards anti-tumor immunity. To address this, we combined high dimensional protein and RNA expression data of human B cells present in lung, blood and tumor samples to determine B cell subpopulations that are enriched in the TME of NSCLC patients.

P4.14

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 ex vivo expanded γδT 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.

P5.01

Targeting HDAC1 catalytic activity in T cells protects against experimental autoimmune encephalomyelitis

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Background: Histone deacetylases are key epigenetic regulators that control T cell-mediated immunity. A T cell-specific deletion of Hdac1 (HDAC1 cKO) protects mice against experimental autoimmune encephalomyelitis (EAE). However, it remains elusive whether inhibition of HDAC1 enzymatic activity and thus mimicking HDAC1 inhibitor treatment is sufficient to block EAE induction.
Methods: In order to address this question, we generated a novel mouse strain that expresses catalytically inactive HDAC1 (HDAC1 Off) from the Rosa26 locus in HDAC1 cKO CD4 + T cells to mimic selective inhibition of HDAC1 enzymatic activity in vivo. Mice expressing wildtype HDAC1 in HDAC1 cKO CD4 + T cells (HDAC1 On) were generated as corresponding controls.
Results: In contrast to HDAC1 On mice, HDAC1 Off mice did not develop EAE, and this correlated with diminished leukocyte CNS infiltration. HDAC1 Off CD4 + T cells in the CNS displayed a severe reduction of IFNγ, IL-17A and TNFα proinflammatory cytokine expression, and in vivo activated HDAC1 Off CD4 + T cells downregulated gene sets associated with T cell activation, cytokine expression and cell migration. This indicates impaired effector functions of HDAC1 Off CD4 + T cells.
Conclusion: Taken together, our study demonstrates that the inhibition of the catalytic activity of HDAC1 in T cells is sufficient to achieve a clinical benefit in EAE disease development. This raises the exciting translational perspective that targeting HDAC1 enzymatic activity is a promising therapeutic strategy in treating human T cell- mediated autoimmune diseases.

P5.02

The soluble cytoplasmic tail of CD45 regulates dendritic cell via TLR4 signalling

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The soluble cytoplasmic tail of the prototypic receptor-like protein tyrosine phosphatase (PTP) CD45 (ct-CD45), is cleaved and released into the human plasma by activated phagocytes. Released ct-CD45 was found to inhibit T cell proliferation and cytokine production via engagement of Toll-like receptor 4 (TLR4). In this study, we analyzed the impact of the ct-CD45/TLR4 pathway on the function of monocyte-derived dendritic cells (DCs). We could demonstrate that activation of DCs by ct-CD45 upregulated the expression of certain cell surface markers (e.g. CD71 and CD86) and induced IL10

production via TLR4. Co-culture of ct-CD45 stimulated-DCs with T cells prevented the induction of IL10 production in T cells. Treatment of mature DCs with ct-CD45 modulated the cytokine profile in co-cultured T cells. While IFN production was strongly inhibited, the release of IL4 was increased in T cells upon stimulation with ct-CD45-treated mature DCs. In contrast, ct-CD45 stimulated DCs had no effect on IL2 production of T cells. In addition, the T cell proliferation-stimulatory capacity of DCs was upregulated by ct-CD45 treatment and this upregulation was further enhanced by ct-CD45 in combination with LPS.
In summary, we could demonstrate that ct-CD45 acts as an immunoregulatory factor not only on human T cells but also on DCs via non-canonical TLR4 activation.

P5.03

Neutrophil proteome atlas in first trimester pregnancy

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Objectives: Pregnancy is an immunological challenge for mother and fetus, and the establishment of immune tolerance at the feto-maternal interface is essential for normal pregnancy development. In pregnancy, neutrophils impact maternal tolerance through the induction of regulatory T-cells and their depletion led to abnormal development of the feto-maternal unit in mice. Further than that, there is very little information available on the role of neutrophils infiltrating the feto-maternal unit. Therefore, our study aims to elucidate neutrophil subpopulations, functions and interactions in decidua basalis (DB) of first trimester pregnancies.
Methods: First, we evaluated immune infiltration in DB in comparison to decidua parietalis (DP) using immunohistochemistry and flow cytometry. Further, we screened for 360 surface proteins, comparing matched DB, DP and blood immune cells from first trimester elective abortion samples. Neutrophil function in DB
was measured by NETosis, ROS production and degranulation.
Results: Preliminary data suggest abundant neutrophil infiltration in DB, whereas there is very low infiltration of neutrophils in DP. We identified 58 differentially expressed surface proteins on neutrophils infiltrating DB in comparison to blood or DP neutrophils. Aditionally, 30 proteins were further validated on higher number of samples (N=9). Neutrophils in tissue exert different phenotype in comparison to blood and cluster into three distinct subtypes. We could confirm that tissue supernatants stimulate neutrophils into activated state, which resulted in more ROS production and NET formation.
Conclusion: Our study comprehends a detailed picture of the immune landscape and specific surface protein expression pattern at the feto-maternal interface in the first trimester pregnancy, with the focus on neutrophils. Further, it reveals the role of neutrophils in maintaining immune tolerance at this critical state. To our knowledge, this is the first study that focuses on the specific subpopulation of neutrophils infiltrating DB as well as their function and interaction with other cell populations.

P5.07

Extracellular vesicles of the probiotic bacteria E. coli O83 activate innate immunity and prevent allergy in mice

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Escherichia coli A0 34/86 (E. coli O83; serotype O83:K24:H31) is commercially available as a live oral vaccine due to its probiotic properties. When administered orally, it reduces allergic sensitisation but not allergic asthma. We have shown that intranasal administration of E. coli O83 reduces allergic airway inflammation induced by ovalbumin (OVA) in mice by targeting the airways directly.
Outer membrane vesicles (OMVs) released by Gram-negative (G-) bacteria have been shown to carry biomolecules to recipient cells and efficiently modulate host immunity. Here, we investigate whether intranasally administered E. coli O83 OMVs (EcO83-OMVs) can reduce allergic airway inflammation in mice. Our work demonstrates that EcO83-OMVs are nanosized spherical structures containing cargo derived from the parent bacterium. They contain LPS and proteins. We have identified 136 proteins that are enriched in EcO83-OMVs compared to the whole bacterium.
Intranasal administration of EcO83-OMVs in OVA-challenged mice reduced airway hyperresponsiveness, airway eosinophilia, and allergen-specific Th2 cytokines in re-stimulated lung, spleen and bronchial lymph node cells. Stimulation of human embryonic kidney cells transfected with TLR2, TLR4, TLR5, NOD1 or NOD2 resulted in the production of cytokines, indicating that these receptors play a role in the immunomodulatory effects of EcO83-OMVs. EcO83-OMVs induced the production of pro- and anti-inflammatory cytokines in splenocytes and bone marrow-derived dendritic cells. We demonstrate for the first time that intranasally administered OMVs from probiotic G- bacteria have an anti-allergic effect.
These results show that non-replicative OMVs can serve as an effective tool to modulate immune responses in the host, in a safer manner than using live bacteria.

P5.08

Decongestant effect of treatment with Sorbitol-containing Carragelose® nasal spray.

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Background: The randomized double blinded cross-over trial was conducted to evaluate a decongestant effect of Sorbitol-containing Carragelose® nasal spray in comparison to saline nasal spray.
Methods: Grass pollen allergic subjects (ITT: 41, discontinued: 2, completed: 39), were exposed to 6 hours continuous allergen challenge in an environmental exposure chamber. After developing high allergic nasal symptoms, like blocked nose, runny nose, sneezing, and itching, subjects were treated at 1:45 hours with one actuation per nostril of either Sorbitol-containing Carragelose® nasal spray or saline nasal spray. Besides recording nasal symptoms every 15 minutes, objective nasal anterior airflow was first measured 30 minutes after treatment and then every 30 minutes during allergy challenge. Nasal secretion was assessed by determining tissue weight every 30 minutes during allergy challenge.
Results:Treatment with Sorbitol-containing Carragelose® nasal spray led to significantly higher mean anterior nasal airflow compared to saline treated subjects (p=0.039) at 6 hours of allergen challenge. In total, 23 (60%) of the Sorbitol-containing Carragelose® nasal spray treated subjects had an

increased anterior nasal airflow whereas in the control group only 13 subjects (34%) had a benefit (p=0.024).
After treatment with Sorbitol-containing Carragelose® nasal spray, mean nasal secretion was significantly reduced by 1 g (25%) in average during the 4 hours of grass pollen challenge (p=0.003). The course of nasal secretion revealed a continuous decline reaching significance at the end of allergen challenge compared to control group (p=0.004). Subjective total nasal symptom score did not show significant differences.
Conclusion: Application of Sorbitol-containing Carragelose® nasal spray is beneficial for patients suffering from blocked nose.

P5.09

Carragelose® nasal spray is effective in reducing allergic nasal symptoms

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Background: The randomized open-label cross-over trial was conducted to evaluate the effectiveness of a Carragelose®-containing nasal spray as treatment of allergic rhinitis.
Methods: Allergic subjects (N=42) were randomly assigned to either of the three investigation groups: one actuation Carragelose®-containing nasal spray per nostril; one actuation saline nasal spray per nostril (comparator); untreated. Subjects applied the respective nasal spray five to ten minutes ahead of a continuous 3-hours grass pollen challenge. After a wash-out period of 7 days allergic subjects changed to the next investigation block. The nasal allergy symptoms runny nose, itching, nasal congestion and sneezing were recorded every 15 minutes on a 4-point scale during the challenge period. Single symptoms were summarized to the reported total nasal symptom score (TNSS).
Results: During the three hours of grass pollen challenge all subjects suffered from increased nasal allergic symptoms. Subjects receiving a single prophylactic treatment with either nasal spray experienced lower TNSS during the whole challenge period. In the Carragelose®-treated subjects a significant reduction (Δ0.596;p=0.028) of TNSS was recorded compared to untreated subjects at 180 minutes. 50% of the Carragelose®-treated subjects showed a TNSS of 8 or lower compared to 8.5 or lower in the untreated subjects. The 25% most affected subjects experienced a TNSS of 9 or above in the Carragelose®-treated subjects and 10 or above in the untreated group. “Runny nose” was the most reduced single symptom (Δ 0.262; p=0.013) followed by nasal congestion (Δ 0.17; p=0.076). Treatment with comparator nasal spray did not show a significant reduction in TNSS but resulted in a significant reduced nasal congestion (Δ 0.309; p=0.010) compared to untreated subjects at 180 minutes.
Conclusion: Prophylactic treatment with Carragelose®-containing nasal spray is effective in reducing allergic nasal symptoms and can be a valuable treatment option for mild allergic symptoms.

P6.01

Recombinant VP1 capsid protein target of Human Boca 1 (HBoV1)-specific antibodies

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Background: Besides rhinovirus (RV) and respiratory syncytial virus (RSV), Human Bocavirus 1 (HBoV1) has been suggested as important virus, which may trigger acute exacerbations of chronic respiratory diseases, such as asthma and chronic obstructive pulmonary disease (COPD). However, there is currently no serological test available which could allow detecting antibody responses against different HBoV1 antigens and the diagnosis of HBoV1 infections is mainly performed by the direct detection of the virus by PCR-based methods. However, there are no serological tests which confirm by measurements of HBoV1-specific antibodies that infection has occurred
Methods: The HBoV1 capsid proteins VP1 and VP2 were expressed in Escherichia coli as C-terminally hexahistidine-tagged recombinant proteins and purified by Nickel-affinity chromatography. The purity and identity of targeted proteins were analyzed by SDS-PAGE and by Western-blotting using a monoclonal anti-His-tag antibody, respectively. Synthetic peptides of approximately 30 amino acids length spanning the complete proteins were produced by solid phase synthesis, purified by HPLC and characterized by mass spectrometry. Enzyme-linked immunosorbent assay (ELISA) as well as microarray technology were used to study the occurrence of VP1- and VP2- as well as specific antibodies in serum samples from adult individuals.
Results: Recombinant proteins were expressed and purified as soluble proteins. VP1- and VP2-specific IgG responses were found in almost all tested serum samples, VP1-specific IgG antibody levels were higher than those measured specific for VP2. Furthermore, compared to rhinovirus (RV)-derived VP1 and respiratory syncytial virus (RSV)-derived G protein, the major targets of RV and RSV-specific humoral immunity VP1-specific IgG responses were significantly lower. IgG reactivity to certain VP1-derived peptides were also detected but it was lower than IgG reactivity to complete HBoV1-derived VP1.
Conclusion: Recombinant HBoV1-derived VP1 can be used to investigate if HBoV1 infections induce increases of HBoV1-specific antibody responses and if such increases of HBoV1-specific antibodies may be related with exacerbations of chronic respiratory diseases.

P6.03

SARS-CoV-2 virions hijack host cellular proteins to evade innate immunity

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Virions of enveloped viruses often contain not only viral, but also host cellular proteins, which are incorporated into the mature virus particles before or during budding from infected cells. The functional roles of virion-associated cellular proteins in virus-host interactions, particularly in the context of the host immune response, are poorly understood. Here, we aimed to characterize the

cellular protein content of virions of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), using mass spectrometry analysis of purified virions produced by a human cell line. We show that SARS-CoV-2 virions hijack a subset of cellular proteins to evade humoral innate immune responses. Blockage of the biological function of the host-derived proteins restored the sensitivity of SARS-CoV-2 to innate immunity. In the future, we will investigate the biological impact of SARS-CoV-2 humoral immune evasion on cellular immune responses, with particular emphasis on human blood monocytes and monocyte-derived macrophages. 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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P6.04

Human epidermal Langerhans cells are activated and migrate in response to tick feeding and transmission of tick-borne pathogens

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Langerhans cells (LCs) provide immunity and tolerance at the skin barrier in response to outside stimuli and pathogens. Recently, we found reduced epidermal LCs numbers after tick attachment on human skin, indicating an active role in the immune response following tick bites.
Here, we investigated the migration and polarization patterns of human LCs in response to clinical and experimental tick bite and infection with B. burgdorferi as common tick-borne pathogen using flow cytometry, migration assays, multi-color imaging and single-cell RNA sequencing.
We observed a sharp decrease in epidermal LCs after tick feeding on the skin of healthy human donors and in an experimental tick bite model using tick saliva injection. LCs did not migrate to supernatant and we could not detect increased cell death. However, LCs were increased in deeper skin layers and in proximity to dermal lymph vessels. LCs over-expressed the migration marker CXCR4 as well as the lymph node homing molecule CCR7, indicating their capability for lymphatic migration. In line with this, LCs stimulated with tick saliva 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 LCs expressing CCR7, CXCR4 and activation marker genes. Interestingly, in our single cell sequencing data these LCs exhibited a tolerogenic phenotype, indicated by the increased expression of IRF4, IDO1, IL4I1 compared to LCs from healthy skin of the same individuals.
Collectively, our results indicate that tick-feeding on human skin modulates the cutaneous immune repertoire by the induction of LC emigration to the lymphatic system. In addition, we detected a tolerogenic phenotype of LCs in cutaneous Lyme disease, potentially modulating the adaptive immune response to tick-borne pathogens.

P6.05

Immune programs in human dendritic cells upon immunization with Chlamydia trachomatis

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Background: 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 to be understood. From studies in mice, we know that immunization with live chlamydia leads to priming of a protective immune response by activation of effector T cells, whereas immunization with UV-inactivated chlamydia produces responses of tolerance by activation of regulatory T cells. We aim to assess human dendritic cell (DC) subsets for their protective or tolerogenic potential against Ct.
Methods: We are using monocyte derived DCs (moDCs) and established an in vitro chlamydia infection model with cervix samples from donors undergoing hysterectomy. To investigate immune mechanisms in Ct-immunized DC, we performed flow cytometric analysis as well as bulk RNA-Seq of moDCs.
Results: MoDCs stimulated with Ct/UV-Ct show differential transcriptional immune programs of pathways regulating cytokine response and apoptosis. Further, we could show that moDCs stimulated with live and UV-inactivated Ct massively activate T cells, suggesting that no antigen-dependent T cell priming occurred but the T cell activation happens in response to global DC activation. When assessing Ct infection in the in vitro chlamydia infection model with tissue-derived immune cells, we observed CD14+CD11c+ and CD14-CD11c+ antigen-presenting cells as the main immune cell subsets taking up Ct. In a next step, we will further analyze the activation profile of tissue derived DCs upon Ct uptake and investigate their capacity to stimulate T cells.
Conclusions: Our results suggest that moDCs stimulated with live and UV-Ct exhibit transcriptional immune regulatory programs causing T cell activation independently of the stimuli. By investigating Ct infection in tissue-derived immune cells in vitro, we can assess the first steps of human cervical chlamydia infection to better understand antigen uptake and presentation.

P6.07

Infection and innate sensing of SARS-CoV-2 virus by circulating monocytes

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The innate immune system is considered as the first line defense against viral infections, such as SARS-CoV-2. Monocytes, as important cellular components of the innate immune system, play an inevitable role in the antiviral immune response, however, the mechanism how these cells sense SARS-CoV-2 is not yet clearly understood. Because Toll-like-receptors (TLRs) are important cell surface and intracellular sensors of monocytes and other innate immune cells, we conducted experiments to investigate the TLR-mediated activation of circulating monocytes by SARS-CoV-2. As virus uptake or infection of target cells is also a prerequisite to stimulate intracellular TLRs, we also studied the entry and subsequent stimulatory effect of SARS-CoV-2 on intracellular TLRs expressed in monocytes.
Our results yielded three key findings: Firstly, we observed that circulating monocytes of healthy donors undergo activation in the presence of SARS-CoV-2 through a mechanism independent of TLRs expressed on the cell surface. Secondly, we found that the virus is capable to enter and infect monocytes through a mechanism that remains unidentified at this stage. Lastly, our results suggest

the involvement of intracellular TLRs that sense single-stranded RNA in the innate immune response of monocytes to SARS-CoV-2.
Funding: This work is supported by the Austrian Science Fund (FWF) grant P 34253-B.

P6.08

Two-year follow-up of immune responses to primary and booster vaccination with COVID-19 mRNA vaccines in cancer patients under different treatment schedules

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Background: Patients with cancer are at increased risk for severe COVID-19. Furthermore Covid-19 mRNA primary vaccination showed an impaired immune response in this group. Though additional doses and shorter booster intervals after primary vaccination were recommended, the effect of breakthrough infections on antibody levels to the ancient virus and its variants is less well established in cancer patients.
Methods: We investigated immune responses to primary and booster vaccinations in patients with different types of cancer using monovalent SARS-CoV-2 mRNA vaccines. Patients with solid tumors of the lung or breast (SoTu, n=63), multiple myeloma (MM, n=70) and healthy controls (n=66) were included.
We measured S1-specific antibody levels to the initial virus hu-1 before and after each vaccination up to the fourth dose. Moreover, Omicron BA.4/5 and Omicron XBB 1.5 RBD-specific binding and inhibitory antibodies were analyzed before and after booster vaccinations. In the subgroup of multiple myeloma patients, we further investigated memory B cell responses and cellular responses before and after booster doses according to their treatment regimes.Additionally, breakthrough infections were recorded.
Results: Although the majority of SoTu and MM patients seroconverted after primary COVID-19 vaccination, 10% of SoTu and 18% of MM showed early antibody waning and turned seronegative already before six months after primary vaccination. After the third vaccine dose antibody concentrations increased in all cancer patients. In a minority of participants (independent of treatment) breakthrough infections occurred during the Omicron wave, all with mild course. Following breakthrough infection, antibody levels to the ancient virus hu-1 increased until the next booster dose. Controls showed a cross-inhibitory immunity to Omicron variant BA.4/5 while in the immunocompromised groups Omicron variant BA.4/5 and Omicron XBB 1.5 binding and inhibitory antibodies were diminished after the third and fourth dose. In a part of the Multiple Myeloma subgroup no difference of antibody concentration between patients and controls was observed, while in MM patients with a progressed disease stage and anti-CD38 therapy significantly lower antibody levels and possible delayed memory responses were observed
Conclusion: In dependence of the tumor entity and the treatment schedules cancer patients showed earlier antibody waning to mRNA COVID-19 vaccination, possibly associated with reduced long-term protection. This indicates that personalized/targeted vaccination strategies/schedules are necessary

in patients with different immunosuppressive conditions. In general, though, they may benefit from repeated booster applications with variant adapted vaccines

P6.10

The Impact of Obesity on Vaccination to SARS-CoV-2 in C57BL/6 Mice

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Background: Obesity is a severe health problem, and its prevalence is increasing worldwide. Previous studies have shown that obesity alters the immune response to certain vaccines and impairs the production and maintenance of antigen-specific antibodies. However, the effect of obesity on the Covid-19 vaccine response is not clear yet.
Methods: We fed male C57BL/6 mice a high-fat diet (HFD) or standard diet (STD) for 18 weeks. After ten weeks, we intramuscularly vaccinated the mice with 3 different doses (1 µg/mouse, 0,5 µg/mouse, and 0,20 µg /mouse) of Spikevax (mRNA-1273) vaccine, and they received a second dose four weeks later. Seven weeks after the second dose, we administered a booster mRNA-1273 dose. We measured the levels of spike-specific antibodies in bronchoalveolar lavage (BAL) and sera of HFD and STD-fed mice. Spleen cells were cultured and stimulated with peptides containing immunodominant sequence domains of the spike protein to measure the spike-specific cytokine response. We also conducted the FACS analysis on the spleen and the lung to determine T-cell and B-cell subpopulations in vaccinated and control mice.
Results: We found that spike-specific antibodies were induced in mRNA-1273-treated mice. However, the spike-specific IgG and IgG1 levels in the BAL and sera of HFD-fed mice were lower after receiving the booster vaccine than in STD-fed mice. Interestingly, FACS analysis revealed a higher percentage of B cells in lymphoid organs and a lower T cell percentage in the spleen and lung of vaccinated obese mice than in vaccinated lean mice. Moreover, when we gated on T cell subpopulations, we observed a significant increase in effector CD4+ and CD8+ T cells in the spleen of vaccinated obese compared to the respective lean animals.
Conclusion: Our study suggests that obese mice exhibit a decreased spike-specific immune response to mRNA-1273 compared to lean animals, although we observed a higher percentage of B cells in vaccinated obese compared to lean mice. We suggest that the function of B cells in obese mice is altered, and they do not produce appropriate levels of spike-specific antibodies comparable to those of lean mice.

P6.11

Pneumococcal CRM197 conjugate vaccines simultaneously induce diphtheria- and pneumococcal-specific immune responses

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With age the burden of infectious diseases increases partially due to a decline of the immune system. Among the elderly Streptococcus pneumoniae, which causes pneumococcal disease, is a very common pathogen. In addition, cases of diphtheria caused by Corynebacterium diphtheriae are rising in all European countries. Therefore, vaccination against Streptococcus pneumoniae as well as regular booster vaccinations against diphtheria are recommended for older adults in many countries. Unfortunately, the effectiveness of currently licensed vaccines varies considerably among the elderly.

The serum concentrations of diphtheria-specific antibodies are often below protective levels in older individuals. Conjugates of pneumococcal polysaccharides with the non-toxic mutant of diphtheria toxin CRM197 are used as pneumococcal vaccines. They induce polysaccharide-specific antibodies and provide protection against pneumococcal disease. Despite this, the potential of these pneumococcal conjugate vaccines to boost diphtheria-specific memory responses remains to be clarified. In this study, diphtheria- and pneumococcal-specific immune responses induced by the pneumococcal conjugate vaccine 13 (PCV13) were compared among elderly (≥65 years), pre-elderly (50-65 years) and adult (25-50 years) participants of the VITAL (Vaccines and Infectious disease in the Ageing Population) longitudinal intervention study. All participants of this study received the PCV13 vaccine for the first time. We show that vaccination with PCV13 efficiently induces both pneumococcal- and diphtheria-specific antibodies as well as diphtheria-specific T cells in all three age groups. Taken together, our results suggest that PCV13 vaccination might offer simultaneous protection against pneumococcal disease and diphtheria.

P6.12

Targetting of dendritic cells by poly(oxazoline)-coated nanoparticles

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Poly(2-oxazolines) have been gaining interest over the last years as nanoparticle (NPs) stealth coatings due to their reduced protein adsorption and potential decrease in phagocyte capture, being suggested as an alternative to poly(ethylene glycol) (PEG). However, in vitro tests using human serum and phagocytes show that nanoparticles coated with poly(2-methyl 2-oxazoline) (PMOXA) trigger complement system activation, and consequently show increased macrophage and monocytes capture, compared to uncoated and to PEG-coated homologous NPs. Given this interesting behaviour we decided to determine the uptake of these particle by dendritic cells (DCs), specialized antigen presenting cells triggering immune response, and if they were able to trigger their maturation.
Organically modified silica nanoparticles coated with PMOXA, poly(2-ethyl 2-oxazoline) (PEOXA) and PEG, with sizes of approximately 100 nm and almost neutral zeta potential, were synthesized and conjugated with rhodamine B. For the cellular assays, monocytes were isolated from fresh buffy coats and differentiated with 50 ng/mL of GM-CSF and 20 ng/mL of IL-4 over 5 days. At the 5th day, nanoparticles were pre-incubated with 60% (v/v) human serum at 37 °C for 20 min, and then diluted 10X when incubated with cells, making for a final serum concentration of 6% (v/v). After 24 h of incubation, cells were collected, incubated with antibodies for CD86 and HLA-DR and analysed with flow cytometry.
We show that PEOXA coated NPs were captured most effectively, followed by PEG and PMOXA. These strong difference between PEOXA and PMOXA coated NPs uptake are unexpected when considering that the difference between their monomer units consists of just one extra methyl group in PEOXA compared to PMOXA. We also observed minor adjuvant activities of our NPs, with PMOXA-coated particles inducing a small upregulation in CD86 expression, with no effect on MHC II levels. Although the mechanism of uptake and the reason for these dramatic changes between nanoparticles and cellular models are still to be investigated, PEOXA-coated particles might provide a very attractive tool for DC-specific and more effective antigen delivery in nanovaccine formulations.

P6.13

Insufficient seroprotection against diphtheria in Austria in light of the notified cases in 2022

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An increase of diphtheria cases were notified in several European countries including Austria since 2022. So far, cases occurred migrant communities and the question arose on the seroprotection of Austrian residents to avoid transmission.
Thus, we analyzed retrospectively seroprotection rates and antibody waning against diphtheria toxoid (DT) across all age groups from samples tested at the Austrian reference laboratory for diphtheria, tetanus and pertussis serology at the ISPTM between March 1, 2010 and January 31, 2022. Additionally, we compared results with tetanus toxoid (TT) antibody concentrations, if available, as usually combined diphtheria and tetanus vaccines are used. We assessed anti-DT and -TT antibody concentrations with commercial ELISA according to the manufacturer´s instructions. Antibody concentrations ≥0.1IU/mL are considered as seroprotective.
We detected an overall prevalence of seroprotection against DT of 63.96% (95%CI: 62.82-65.09; 6,554/10,247) and against TT of 95.99% (95%CI: 95.43-96.48; 7,712/8,034) between 2018 and 2022. DT seroprotection differed between age groups ($p<0.0001$) with lowest seroprotection prevalence in ≥60 year-olds (51.39%; 95%CI: 47.75-55.01) and with the highest prevalence of seroprotection in the ≥15-<60 year-olds (66.43%; 95% CI: 65.19-67.65). Accordingly, the geometric mean antibody concentration (GMC) against DT was lowest in the ≥60 year-olds (0.9 IU/mL; 95%CI 0.08-0.10). In contrast, the GMC against TT was 7.9-fold higher compared to that for DT. From 89 individuals, antibody concentration results from multiple time points between 2010 and 2022 were available to determine antibody waning. The annual percentage change of TT antibody concentrations was higher (6.9%) than for DT (2.9%).
The observed low seroprotection against DT is most likely due to missed booster vaccinations and a lower immunogenicity of the reduced DT antigen content in booster vaccine formulations. Therefore, public health concepts to raise awareness for regular booster vaccinations against diphtheria combined with tetanus and pertussis need to be implemented to close gaps in seroprotection.

P7.02

Bhlhe40 regulation of Langerhans cell fate and function

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Langerhans cells (LC) play a key role in the maintenance of tolerance and are primarily located within the epidermis. LCs are established prenatally and require TGF-b family signaling for their differentiation. LCs can be replenished postnatally by peripheral blood monocytes; however, these precursors can give rise to inflammatory monocyte-derived dendritic cells (moDCs). Several key regulators of LC versus moDC fate have been identified, yet many of the molecular mechanisms remain poorly understood.
Here we identified the transcription factor Bhlhe40 (Basic Helix-Loop-Helix Family Member E40) as a potential player in LC versus moDC fate. We observed that Bhlhe40 is induced during the early stages of LC differentiation and is likely regulated by the TGF-b/ALK5 signaling cascade. Using a lentiviral-mediated knockdown, we have shown that Bhlhe40 is required for the differentiation of CD34+ hematopoietic precursors into LCs yet is dispensable for other dendritic cell lineages. In addition, we have shown that a deficiency of Bhlhe40 leads to abnormal expression of LC activation and migration markers.
Bhlhe40 has been identified as a key regulator of immunity during infection, autoimmunity, and inflammation in various immune cell populations, yet the role of Bhlhe40 in myeloid cell populations remains poorly understood. We suspect that Bhlhe40 is a key component of LC differentiation, therefore playing a decisive role in anti-inflammatory LC versus pro-inflammatory moDC differentiation from monocytes.

P7.03

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 poorly 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 bacterial infection. Our results demonstrate that lncRNA-124 limits effector CD8+ T cell expansion and differentiation into memory cells. We also show that lncRNA-124 is evolutionarily conserved and the human ortholog of lncRNA-124 is expressed in human memory T cells. In conclusion, lncRNA-124 is a novel long noncoding RNA that regulates CD8+ T cell expansion and effector-to-memory differentiation following bacterial infection in vivo.

P7.05

The adaptor protein TRAT1 modulates Th17 and Treg effector functions

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Background: T Cell Receptor Associated Transmembrane Adaptor 1 (TRAT1) is an adaptor protein with multiple intracellular signal domains which is associated with the TCR. Yet, the role of TRAT1 in CD4+ effector and regulatory T-cell responses is only incompletely understood.
Objectives: We here aimed to define the contribution of TRAT1 to the function of effector T cells (Teff) and regulatory T cells (Treg).
Methods: Expression of TRAT1 was measured in resting and anti-CD/anti-CD28 activated Teff and Treg specimen by intracellular flow cytometry. For functional testing we performed CRISPR/Cas9-mediated knockout of TRAT1 or retroviral overexpression in primary human CD4+ T cells. Subsequently, bulk RNAseq from TRAT1-KO CD4+ T cells and functional testing in 2D and 3D cell culture models was performed.
Results: Expression analysis using qPCR and flow cytometry showed low expression levels in Treg compared to Teff, which further induced TRAT1 expression following anti-CD3/anti-CD28 activation. RNAseq analyses revealed gene signatures of hyper-activated PI3K signaling in TRAT1-KO T cells which was supported by increased proliferation, surface marker expression and phosphorylation of STAT transcription factors. In contrast, a marked down-regulation of IL-17 was observed, which was mechanistically related to the hyperactivation of STAT6. Retroviral overexpression of TRAT1 into Treg led to enhanced suppressive capacity in both iTreg and tTreg was associated with the up-regulation of the TGFb-associated functional markers LAP and GARP. Activation of tTreg in the presence of IL-2 led to increased TRAT1 expression in these cells. Accordingly, TRAT1-KO tTreg showed less suppressive capacity in response to IL-2. TRAT1-overexpressing tTreg also showed superior function in a murine intestinal organoid based 3D cell culture xeno-GvHD model.
Conclusion: Our data support crucial roles for TRAT1 signaling in the function of Th17 and regulatory human CD4+ T cells.

P7.07

Identification of highly suppressive regulatory T cells in old age

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The Immune system is an exquisitely regulated system that defends our body against foreign antigens while tolerating self-antigens. 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. It is well known that T lymphocytes (CD4+ as well as CD8+ cells) are affected by aging processes showing a more pro-inflammatory phenotype and a less reactive status. While some molecules such as CD28 are described to be downregulated on senescent T cells, other inhibitory molecules like PD-1 and CTLA-4 are found to be up-regulated in age-related exhaustion of T cells. On the other hand, well-functioning Treg cells express some markers described to be related to aged T cells. Our study aims to define differences between CD4+ Treg cells from young and old donors. While the expression of Treg-related markers stays stable over age, we found that Treg cells from older donors tend to express higher levels of the CD28 TCR co-receptor. We analysed some of the inhibitory molecules on Treg and found that CD28high-Treg cells express more PD-1 than CD28low-Tregs and this higher expression increases with age. In addition, CD28hi-Treg are mostly CD45RO+ indicating an effector Treg phenotype. In order to investigate the functional role of CD28 levels on Treg suppressive activity, we sorted CD4+ CD25+ CD127low Treg cells according to their CD28 expression and co-cultured them with proliferating PBMCs. Regardless of age, CD28high

expressing Treg suppress proliferating PBMCs more than their CD28low counterparts. Our results propose high CD28 expression by Treg in old age as a new tool to identify highly suppressive Treg which would partially explain the restrained immune response in the elderly.

P7.08

Analysis of blood immune cell composition and activation in eosinophilic esophagitis

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Eosinophilic esophagitis (EoE) is a chronic, inflammatory, antigen-driven disease of the esophagus. EoE is thought to be a ´tissue-centered´ disease is routinely diagnosed via biopsy. Moreover, disease exacerbations and treatment response are similarly investigated in biopsies. In our study we aimed to comprehensively analyze peripheral blood mononuclear cells (PBMC) composition and activation using surface marker measurements with multicolor flow cytometry in both blood and tissue of EoE patients and controls simultaneously.
Control, reflux, active EoE and patients in remission were recruited from the Department of Internal Medicine (Medical University of Graz). Proximal and distal mucosal biopsies were immediately processed into single cell suspensions. Similarly, PBMC were isolated from blood for further analysis by flow cytometry within two hours of blood draw. We additionally performed indirect and direct co-cultures of PBMCs with primary esophageal epithelial cells. Finally, we utilized multicolor flow cytometry to assess immune cell activation and composition in a mouse model of inducible EoE, where IL-33 is overexpressed in esophagus under doxycycline exposure.
Our results indicate that specific PBMC populations are enriched and alter their surface expression of activation markers in tissue of active EoE disease. In particular, we observed upregulation of CD38 on CD4+ T cells and on myeloid cells in active EoE biopsies. Moreover, we observed significant upregulation of PD-1 on CD4+, CD8+ NK T cells as well as B cells, and more so after corticosteroid treatment. With co-culture experiments we demonstrated that direct cell contact is needed for PD-1 upregulation on CD4+ T cells. Finally, we validated our findings of PD-1 and CD38 upregulation in an inducible mouse model of EoE.
Herein we show significant alterations in PBMC activation profile in patients with active EoE disease in comparison to healthy controls, patients in remission and reflux patients, which could have potential implications for patient therapy prescription.

P7.12

Sjögren´s Syndrome in Id3KO mouse model is associated with T cell senescence and mitochondrial dysfunction

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Primary Sjögren’s Syndrome (pSS) is a systemic autoimmune disorder characterized by lymphocytic infiltrations in exocrine glands. T-cells are considered major players in the pathogenesis of pSS. Previously we reported that peripheral lymphopenia in pSS mostly affects naive CD4+T-cells which show signs of immune cell aging. Id3-deficient mice (Id3KO) develop a Sjögren's-like syndrome and are used as an animal model for pSS.
Immune cells were isolated from peripheral blood, lymph nodes and spleens of Id3KO and wild type (WT) mice. Flow cytometric analysis was performed to identify and further characterise CD4+ and CD8+T-cell subsets (Naïve, Central memory, Effector memory, Double negative) at single-cell level: protein p53 and senescence-associated β-galactosidase activity (SA-β-GAL) were assessed as markers of cellular senescence. Staining with mitochondrion-selective fluorescent dyes MitoTracker® Green, MitoTracker® Deep Red, MitoSox Red was performed to assess mitochondrial mass, membrane potential (MtMP) and superoxide production, respectively. Agilent Seahorse XFe96 Analyzer was used to measure the oxygen consumption rate in isolated total CD4+T-cells.
We found a significant decrease in the naïve cell compartment in CD4+ and CD8+T-cells of Id3KO mice in all studied tissues compared to WT mice. Id3KO T-cells showed enhanced expression of senescence marker p53 and higher SA-β-GAL activity. These T-cells also exhibited a remarkable decrease of mitochondrial mass and MtMP. Despite having reduced mitochondrial mass and MtMP, we observed that Id3-deficient T-cells produced a higher amount of mitochondrial superoxide. Naïve CD4+T-cells were most affected by this increase. In line with our findings, basal respiration was significantly decreased in Id3KO CD4+T-cells.
These data imply pre-mature T-cells aging in the Id3KO mouse model as previously found in pSS patients. This senescent phenotype is linked with mitochondrial dysfunction associated with accumulation of mitochondrial superoxide, which could be involved in the pathology of pSS.

P7.13

B/T cell interaction fuels intestinal inflammation during autoimmunity

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Inflammatory bowel disease (IBD) is a group of immune-mediated disorders of the intestine and is one of the most prevalent gastrointestinal diseases today with increasing incidence. Dysregulated B cell responses and imbalanced intestinal IgG production in IBD patients correlate with disease severity; however, the role of B cells and antibody-secreting cells (ASC) in IBD pathology remained unknown.
A large genome-wide association study among IBD patients associated mutations in the Wiskott-Aldrich Syndrome (WAS) protein to IBD risk. WAS is a severe immunodeficiency, accompanied by systemic autoimmune manifestations, including IBD. WAS is caused by loss-of-function in the WAS protein (WASp) or in the WASp interacting protein (Wipf1). We here describe Wipf1-/- mice as novel mouse model of spontaneous, chronic colitis with characteristic immunological features of human IBD and dissected the contribution of B cells and ASC to IBD pathogenesis.
We provide evidence for a detrimental role of activated B cells during the onset of autoimmune intestinal inflammation. We identified CD86 expression on activated B cells as a crucial factor exacerbating pro-inflammatory cytokine production of intestinal CD4 T cells. In addition, B cells aberrantly differentiated into IgG ASCs during IBD. Mechanistically, we here describe that LPS stimulation led to a state of heightened metabolic activity of Wipf1-/- B cells, mediated by efficient activation of the MAPK/Erk and mTOR/Akt/4E-BP1 pathways. These findings provide a likely cause for aberrant IgG production in colonic tissue of Wipf1-/- mice, as well as for the systemic, microbe-dependent auto-antibodies in sera of Wipf1-/- mice. Together, elevated B cell-mediated pro-inflammatory cytokine secretion and B cell-derived inflammatory antibody production contributed to exacerbated intestinal inflammation.

P7.14

Dietary supplementation with spermidine increases autophagic flux and ameliorates ROS production of T-cells of patients with spondyloarthritis

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Background: The Th17/Treg axis is considered to play a major role in the disease pathogenesis of spondyloarthritis (SpA). The polyamine pathway was recently described as an important modulator of the Th17/Treg balance and the dietary intake of the polyamine spermidine was reported to have anti-inflammatory properties in various mouse models.
Objective: Here, we performed an open-label, pilot study to investigate the impact of dietary spermidine on immune cell functionality on patients suffering from spondyloarthritis (SpA).
Methods: In this pilot study, patients suffering from SpA (n=14) with active disease (BASDAI ≥4) received 6 mg/d spermidine as a food supplement in addition to their regular medication for 3 months. At baseline and at the 3 months visit we performed clinical examination, standard laboratory measurements, immune cell phenotyping and analyzed intracellular ROS (CellROX staining) and autophagic flux (CytoID staining) in T-cell subsets via flow cytometry. The spermidine rich wheat germ extract CelVio Complex® used in this study and known as ingredient in the supplement spermidineLIFE® was provided by TLL The Longevity Labs GmbH
Results: In this interim analysis, patients reported a significant decrease in the disease activity score BASDAI following spermidine intake. On the other hand, the functional index BASFI as well as CRP and ESR remained unchanged.
Immune cell phenotyping revealed an increase in Tregs in absolute numbers as well as in frequency. The total counts of lymphocytes and CD4+ T-cells, however, were stable over time. Furthermore, we observed a distinct decline in levels of cell stress (presence of cellular ROS) in T-cells following spermidine intake. This phenomenon was present in CD45RA+ naïve, CD45RO+ memory and CD28- senescent CD4+ and CD8+ T-cells subsets. This finding went hand in hand with an increase in the autophagic flux of these cells following spermidine intake that could similarly be noted in all T-cell subsets. Interestingly, the induction of autophagy showed good correlation with the reduction of cell stress in our patient cohort.
Conclusion: Our data suggest that dietary spermidine enhances autophagy in T-cells, further leading to amelioration of cell stress and a potential induction of a regulatory immune response in patients suffering from a chronic inflammatory condition.

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