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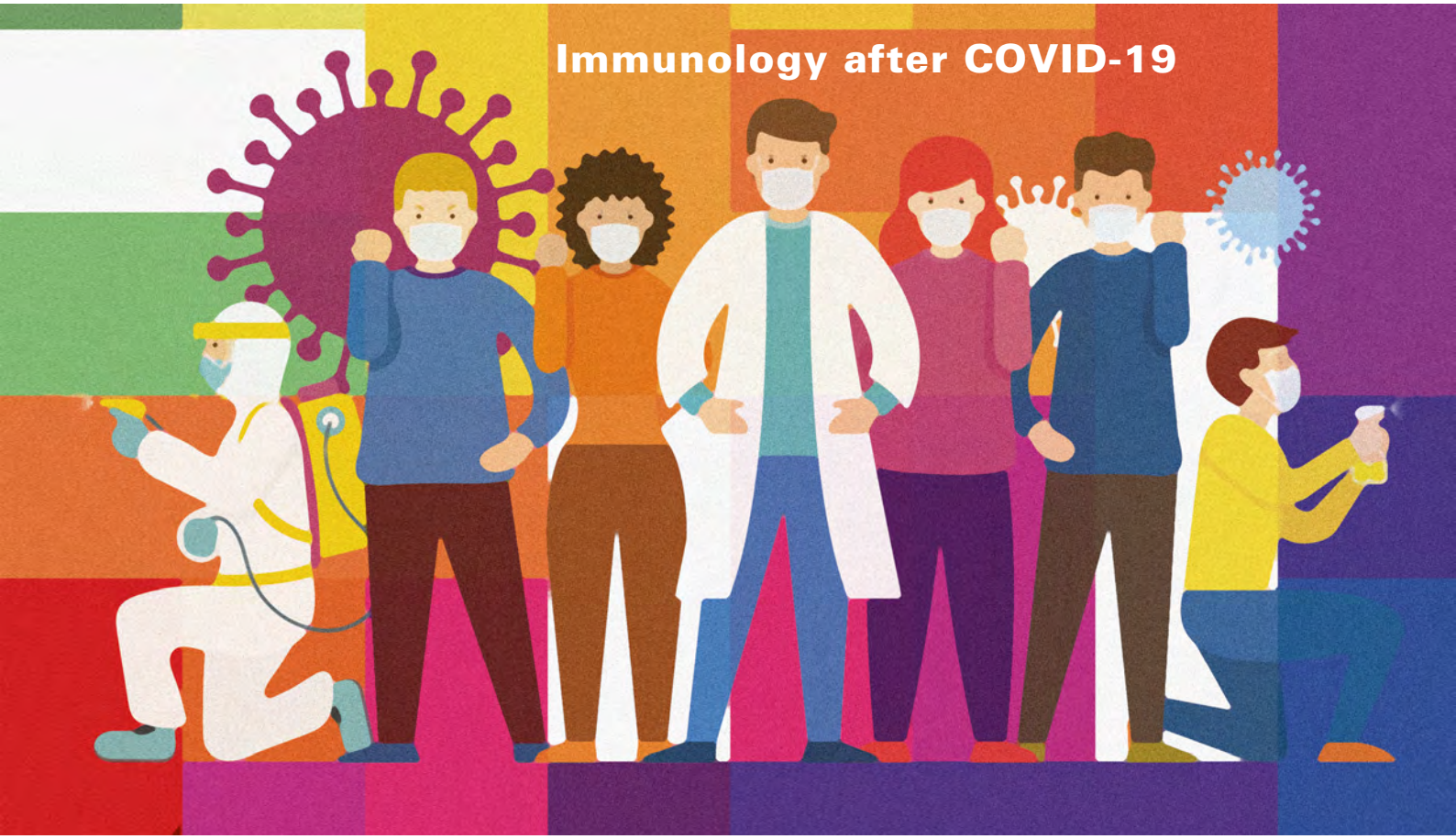

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POSTER

P1

Benralizumab for corticosteroid-refractory DRESS in two COVID-19 patients

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Drug rash with eosinophilia and systemic symptoms (DRESS) is a rare severe drug hypersensitivity reaction. Treatment of DRESS currently consists of systemic corticosteroids. Here, we report benralizumab (Fasenra®) as a treatment for corticosteroid-refractory DRESS occurring in two severely ill COVID-19 patients. Both patients received high-dose intravenous corticosteroids for 4-6 days, but cutaneous symptoms, eosinophilia and signs of related organ damage were deteriorating. Based on its successful use in PDGFRA-independent hypereosinophilia, we decided to treat our patients with benralizumab. The patients showed clinical improvement and a rapid substantial drop in eosinophils. Targeted high-throughput serum proteomics prior vs. after treatment revealed a significant reduction mostly in eosinophil- and T cell response-related proteins (a.e. IL-5, CD8, TNF and PD-L1), thus pointing towards an impact of benralizumab on the (drug-directed) T cell response in DRESS.

P2

Systemic hyperinflammation as a driver of maculopapular drug exanthema in severely ill COVID-19 patients?

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Coronavirus disease 2019 (COVID-19) has been associated with cutaneous findings, some being the result of drug hypersensitivity reactions. Here, we utilize imaging mass cytometry (IMC) to characterize the cutaneous immune response in maculopapular drug rashes (MDR), including those associated with COVID-19 infection (COVID MDR). For comparison, skin from healthy controls and patients with drug rash with eosinophilia and systemic symptoms (DRESS) was analyzed. Results demonstrated that COVID MDR are characterized by a more prominent infiltration of cytotoxic CD8+ T cells and highly activated, phenotypically shifted monocyte/macrophage (Mo/Mac) clusters in comparison to MDR and DRESS. RNA sequencing transcriptome of the affected skin also demonstrated a more robust cytotoxic response in lesional COVID MDR skin. Serum proteomic profiling of COVID MDR patients revealed up-regulation of various inflammatory mediators (IL-4, IL-5, IL-8, IL-18, IL-6, TNF, and IFN-γ), eosinophil and Mo/Mac -attracting chemokines MCP-2, MCP-3, MCP-4 and CCL11. Analyses of cytokine networks demonstrated a relatively milder cytokine storm in DRESS compared to COVID MDR, while MDR did not exhibit such features. Our results suggest that a massive systemic cytokine storm promotes activation of Mo/Mac and cytotoxic CD8+ T cells, which impacts MDR development in severely ill COVID-19 patients.

P3

Major differences in sensitization patterns of atopic dermatitis patients in Tanzania vs. Switzerland.

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Atopic dermatitis (AD) bears many pheno- and endotypical facets, with ethnicity being one of the factors influencing these. More data is becoming available to show stunning differences between African, Asian and Caucasian descent. One explanation is the genetic background, but there are also environmental, socioeconomical and individual factors that play a role. Very little is known about environmental factors and sensitizations potentially influencing disease phenotype and development in African as compared to European/American AD patients.

In this study, we explore sensitization patterns of AD patients and healthy controls (HC) in Tanzania and Switzerland. We thereby sought to get shed light on potential environment-associated factors influencing AD development and/or pathogenesis in these distinct environments. We collected serum of AD patients and healthy age- and sex-matched individuals both in Moshi, Tanzania, and Zurich, Switzerland (n = 10 for each group). We measured total IgE and specific IgE using an in vitro multiplex allergy test (ALEX). Overall IgE levels were significantly higher in Tanzanian as compared to Swiss AD patients. Analyses of specific IgEs revealed major differences in the sensitization patterns between AD patients, the most important ones in two categories. Swiss but not Tanzanian AD patients showed a strong sensitization against Malassezia antigens (s1, s11, s5, s9). In contrast, sensitization against tropomyosin was a major feature in Tanzanian AD patients. This was evidenced by increased IgE levels agains house dust mites (der p), cockroaches (per a7) and seafood (hom g, lit s, pen m1). Taken together, our study shows major differences in the sensitization patterns of European (Swiss) vs. African (Tanzanian) AD patients. Most interestingly, Malassezia spp. exists in sub-Saharan climate, but seems not responsible for sensitization in that enviroinment. On the other hand, seafood antigens lead to sensitization in Tanzanian population without taking part in daily diet. The functional relevance of these differences and their impact on AD pathogenesis remains to be elucidated.

P4

Icarapin (Api m10) sensitization in honey bee venom allergic patients: Potential cause of increased immunotherapy failure?

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Sensitization to Api m10 is associated with low effectiveness of honeybee venom immunotherapy (bVIT). This retrospective double-center study aimed to assess the severity of sting reactions in patients with Api m10 sensitization and the outcomes of bVIT. Patients were included if sIgE to Api m10 concentration (C) was >0.35 kU/L measured by ImmunoCAP.

72 patients with elevated sIgE to Api m 10 were included. Mean sIgE Apim10 (C) was 4.03 kU/l (IQR: 3.1). Mean age was 47 years (IQR: 20.8), and 39 were males. 8 patients had anaphylaxis (A) grade (G) I, 10 G II, 27 G III, and 22 G IV (*classification* of H. L. *Mueller)* and 5 patients had (A) after wasp sting. 45 patients were undergoing or starting bVIT and 8 patients experienced (A) after field sting during bVIT (G I: n = 2, G II: n = 4, G III: n = 2). 1 patient with G III received bVIT with Pharmalgen® (P) (ALK-Abello,) and 1 patient with grade II reaction bVIT with Venomil® (V) (Bencard Allergie GmbH). All other patients received bVIT with Alk-Alutard SQ® (Alu).

Patients with Api m10 sensitizations appear to be at higher risk for treatment failure under bVIT. Of 45 patients undergoing bVIT, 8 had anaphylaxis G I-III after a field sting. Although (P) and (V) have been shown to contain high Api m10 (C), there were also treatment failures with these products. No information on the Api m10 (C) of (Alu) is available. To further assess the efficacy of bVIT in Api m10 sensitized patients and to determine whether an increase of the dose is necessary, additional analyses including Api m10 IgG4 levels are required.

P5

Interleukin-2 signals converge in a lymphoid–dendritic cell pathway that promotes anticancer immunity

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Dendritic cells (DC) are considered indispensable in orchestrating T cell responses to intracellular pathogens and tumors. Although tumor-infiltrating DCs have correlated with effective anticancer immunity and improved responsiveness to anti-PD-1 checkpoint immunotherapy, the upstream drivers of DC expansion and intratumoral accumulation remain uncertain.

We report that administering interleukin-2 (IL-2) in both humans (i.e., in a clinical trial) and mice caused the pronounced expansion of type-1 and type-2 DCs, including migratory and cross-presenting DC subsets. However, neither their precursors nor mature DCs expressed functional IL-2

receptors. In mechanistic studies, IL-2 signals stimulated innate lymphoid cells, NK cells, and T cells to synthesize FLT3L, CSF2, and TNF. These cytokines redundantly caused DC expansion and activation, which resulted in improved antigen processing and T cell activation. In transplantable and inducible mouse melanoma models, treatment with IL-2 but not anti-PD-1 increased intratumoral DC infiltration and correlated with favorable antitumor responses. Moreover, melanoma patients with high intratumoral IL- 2 gene signatures correlated with DC infiltration and prolonged survival.

Our study revealed the complementary difference and unappreciated advantage of IL-2 immunotherapy compared to treatment with anti-PD-1. IL-2 immunotherapy expanded tumor-infiltrating DCs and favored the conversion of poorly immunogenic into immunogenic tumors. Such insights could help to overcome primary and secondary tumor resistance to immune checkpoint inhibitors.

P7

Characterization of dysfunctional CD8 T cell priming during chronic hepatitis B virus infection

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HBV as a carcinogenic virus is one of the most important etiological factors of liver cancer. The tumorigenic environment of chronic HBV is characterized by persistent low-grade inflammation, cirrhosis, and dysfunctional CD8 T cells. We have recently uncovered that IL2 rescues dysfunctional CD8 T cells in mouse models of HBV and may therefore constitute a breakthrough therapy.

To further our understanding of intrahepatic T cell priming and design of IL2 immunotherapy HBV-specific CD8 T cells derived from HBV infection models were subjected to single-cell RNA sequencing. Preliminary analysis revealed that the fate of CD8 T cells diverges from day 3 after priming. On days 5 and 7 effector and IL2 treated cells cluster together in UMAP plots and away from dysfunctional cells. On day 28 however, IL2 treated cells cluster with dysfunctional cells indicating that IL2 does not durably rescue the dysfunctional phenotype in presence of continuous antigen stimulation. Nevertheless, a small population with stem-like phenotype was uncovered at day 28 in the IL2 treated group which was absent in the dysfunctional population. This stem-like population might be valuable since it has been shown to be a source of terminally exhausted cells which mediate control of chronic infection in different models.

Elucidating the mechanism of dysfunctional CD8 T cell priming has a substantial impact on immunotherapeutic strategies in HBV. Furthermore, dysfunctional or exhausted CD8 T cells are a hallmark of chronic infections and cancer. Consequently, the insights generated here are broadly applicable.

P8

Immunological gene signatures in B cell follicle reticular cells are highly conserved across organs and species

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Aim: Secondary lymphoid organs (SLO) are strategically positioned to survey bodily surfaces and to support the generation of cellular and humoral immunity. The movement and interaction of antigens, antigen presenting cells, B and T lymphocytes within SLOs is coordinated by fibroblastic reticular cells (FRCs) that form dedicated microenvironments and provide essential niche molecules such as the chemokine CXCL13. High-resolution transcriptomic analysis of *Cxcl13*-expressing cells in mouse models has previously enabled the molecular characterization of heterogenous B cell-interacting reticular cells (BRC) in lymph nodes. However, it remains unknown to what extent the molecular identity of niche-forming BRCs is conserved across SLOs.

Methods: Here, we employed single cell RNA-sequencing of *Cxcl13*-expressing cells from murine lymph node, spleen and Peyer's patch to compare the molecular identity of BRCs across SLOs.

Results: While developmental genes dominated organ-specific gene signatures, we found conserved gene signatures reflecting crucial immunomodulatory functions. The highest conservation was observed in follicular dendritic cells, a BRC subset specialized in the capture and presentation of antigen. Moreover, immunomodulatory gene signatures were preserved in BRCs from human lymph nodes and palatine tonsils.

Conclusion: Taken together, our results demonstrate a strong conservation of immunomodulatory functions in B cell niche-forming cells highlighting the important role of BRC-defined microenvironments in steering efficient immune responses in SLOs across species.

P9

Human Herpesvirus 8 directed Adaptive Immune Responses mounted in a pre-clinical in vivo Model

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Development of Primary Effusion Lymphoma (PEL) is causally linked to infection with Human Herpesvirus 8 (HHV8) and is also associated with the Epstein-Barr Virus (EBV), which is found in 90% of PEL cells. PELs usually affect patients with compromised T cell immunity. Yet, T cell responses against HHV8 are still poorly explored, mainly due to the lack of in vivo models as well as large cohort studies. Humanized NSG mice (huNSG), reconstituted with human haematopoietic stem cells, were recently found to be susceptible to HHV8 if coinfected with EBV, to maintain HHV8 over weeks and to establish PEL-like tumors. In this study, we investigated the adaptive immune response in HHV8 and EBV co-infected huNSG mice compared to EBV only infected animals.

We found higher $CD3^+$ $CD8^+$ T cell expansion upon HHV8 infection compared to EBV single infected animals and also an increase in central and effector memory T cells, indicating priming and expansion of virus reactive T cells. Screening of the T cells from co-infected animals for IFN-γ secretion upon challenge with HHV8 peptide pools showed reactivity against at least three HHV8 proteins. In addition, HHV8-specific IgM antibodies were detected, including three IgM specificities that are also found in seropositive humans.

In conclusion, we show the potential of this mouse model to raise HHV8 specific adaptive immune responses. In a next step, we aim to investigate the protective role of HHV8-specific T cells by adoptive transfer of HHV8-specific T cell receptor-transduced autologous T cells into our huNSG mouse model.

P10

PPAR-gamma promotes proliferation of pathogenic Th2 cells through regulation of IL-2 signaling

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Recently, a subset of allergen-specific Th2 cells has been identified and termed "pathogenic" Th2 (pTh2) cells, based on their crucial role in mediating type-2-mediated immunopathology and their expression of the ligand-activated transcription factor peroxisome proliferator activated receptor gamma (PPAR-g). The functional role of PPAR-g for pTh2 cells, however, remains incompletely understood.

Here, we analyzed the effect of PPAR-g inhibition on basic T cell functions, such as IL-2- or T cell receptor (TCR)-induced proliferation in pTh2 cells.

pTh2 cells were isolated from peripheral blood. Transcriptomic analysis of T cell clones treated with a chemical inhibitor (GW9962) of PPAR-g was performed and further analysis was done by flow cytometry.

Pathway analysis revealed that the IL-2 signaling pathway is affected by PPAR-g-inhibition. To assess the impact of PPAR-g inhibition on IL-2 signaling, we systematically measured the effect on phosphorylation of signal transducer and activator of transcription (STAT) molecules. Cells treated with GW9662 showed a significantly reduced phosphorylation of STAT3 and STAT5, while phosphorylation of STAT6 remained unaffected.

Together, our findings suggest that PPAR-g is a positive regulator of the IL-2 signaling pathway in pTh2 cells. Since IL-2 is crucial for T cell proliferation and survival, PPAR-g might provide a selective advantage for pTh2 over conventional Th cells in conditions of limited IL-2 availability

in tissue. These findings further highlight the potential of PPAR-g as a therapeutic target in type 2 immunopathology.

P11

The role of Regulator of G-protein Signaling (Rgs)-1 in CD8+ TRM-cell mediated intestinal immunity

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The gene encoding regulator of G-protein signaling 1 (*Rgs1*) is consistently one of the most up-regulated genes in tissue-resident T_{RM} cells and there is a striking genetic association of RGS1 SNPs with altered incidence of T cell-mediated autoimmune disorders in humans (e.g. celiac disease, multiple sclerosis). The precise functions of Rgs1 in the differentiation of T cells, however, remain ill-defined.

We used an adoptive co-transfer of congenic $Rgs1^{-/-}$, and $Rgs1^{+/+}$ OT-I CD8 T cells into recipient mice and infection with *L. monocytogenes*-OVA or LCMV-OVA to monitor the impact of Rgs1 on the generation of CD8 T_{RM} cells. These experiments revealed the critical requirement of Rgs1 for the accumulation of CD8 T_{RM} cells, and for the efficient immunoprotection from systemic dissemination of the pathogen upon re-infection. We now generated a Rgs1-tdTomato reporter mouse, using the P2A system, and confirmed the strong Rgs1 expression in intestinal resident unconventional and conventional T-cell subsets during homeostasis, and the rapid induction of Rgs1 expression in antigen-specific T cells following local infection with a pathogen. This reporter mouse further allows us to specifically compare the functional capacities of Rgs1 expressing, vs. non-expressing cell subsets. Ongoing experiments focus on the precise Rgs1 regulated mechanisms on MPEC vs. SLEC differentiation upon initial antigen contact, but also on Rgs1 mediated effects in regulating T cell function that affect the generation of T_{RM} cells, but also on the impact of Rgs1 expression on regulating T cell activation.

P12

Two cases of hereditary alpha-tryptasemia.

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Aim: Hereditary alpha-tryptasemia (HAT) is a recently described autosomal dominant genetic condition in patients having inherited extra copies of the alpha tryptase gene *TPSAB1*, leading to elevated tryptase levels. Patients might be asymptomatic or develop a syndrome involving multiple organ systems and characterized by symptoms sometimes similar to those of systemic mastocytosis.

Methods: The diagnosis of HAT can be challenging and requires a careful analysis of the *TPSAB1* and *TPSB2* copy number variation (CNV). Droplet digital PCR (ddPCR) is the most reliable technology for the detection of CNV in the *TPSAB1* gene. The total copy number of *TPSAB1* and *TPSB2* for normal individuals is four; individuals with a duplication of the alpha tryptase coding *TPSAB1* gene have a total copy number of five or more.

Results: We report two patients with HAT confirmed by ddPCR.

Conclusions: HAT might be more common than previously considered, as recently reported in an unselected British birth cohort where 5% had raised *TPSAB1* copy numbers. The availability of a genetic test for HAT will help to identify a particular population of patients among those with elevated basal tryptase levels of hitherto unknown cause. The clinical significance of HAT, in particular whether these patients require a close follow-up and specific treatment due to an increased risk for severe anaphylaxis or SM, remains to be studied. Which patient qualifies for *TPSAB1* CNV testing is currently a matter of debate, as HAT has also been described in patients with basal tryptase levels <11.4 µg/l.

P13

Rheumatoid factor has no impact on tryptase measurement

D. Spoerl[1], P. Roux-Lombard[1] ([1]Geneva CH)

Aim: Tryptase is frequently requested to confirm immediate type hypersensitivity reactions and when systemic mastocytosis is suspected. Rheumatoid factor and anti-heterophilic antibodies and have been reported to interfere with the Thermo immunoCAP assay. Since 2015, this assay uses Fab fragments as a conjugate reagent to reduce the impact of anti-heterophilic antibodies. Here, we wanted to evaluate whether the presence of a rheumatoid factor still has an effect on tryptase measurements.

Methods: We retrospectively analyzed 192 patient samples that had been tested for tryptase and IgM rheumatoid factor during the last two years.

Results: 32 patients had a positive IgM rheumatoid factor, 15 patients had tryptase values above normal limits. No correlation was found between these two parameters, indicating no association and likely no interference of rheumatoid factor on tryptase measurement (Spearmann r 0.06, 95%CI -0.09 to 0.21; p value 0.4).

Discussion: The use of Fab fragments in the immunoCAP assay did not only reduce the interference of anti-heterophilic antibodies, but also that of IgM rheumatoid factor. This is not surprising as rheumatoid factor targets the Fc region of IgG antibodies.

P14

IL-33 mediated stromal-myeloid cell crosstalk controls intestinal helminth infestation

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Interleukin-33 (IL-33) is a nuclear cytokine of the interleukin-1 family and is released upon cell damage thereby acting as an alarmin. IL-33 plays an essential role in promoting host-protective immune responses against helminth parasites at different mucosal surfaces including the intestine. However, early events after gastrointestinal nematode infection are poorly understood and the cellular source of IL-33 in the gut remains ill-defined. Here we show that Cxcl13-Cre-positive fibroblastic stromal cells (FSCs) of the lamina propria are the main source of IL-33 in the small intestine. Within the first days of nematode infection, lamina propria FSCs showed strongly increased IL-33 expression. Ablation of IL-33 in Cxcl-13-Cre-positive FSCs resulted in an increased infestation with the gastrointestinal nematode *Heligmosomoides polygyrus bakeri*. We found that myeloid cells were recruited to the site of worm invasion early after infection and that the upregulation of a distinct set of inflammatory cytokines was dependent on FSC-derived IL-33. Collectively, these data unveil that IL-33-mediated crosstalk between stromal and myeloid cells is a critical event during the initial immune response against gastrointestinal nematodes.

P15

NFAT5 induction by the tumor microenvironment enforces CD8 T cell exhaustion

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The tremendous success of immunotherapy, including adoptive T cell transfer and CAR T cells, proved that T cells are a powerful tool to fight against cancer. However, cancer immunotherapy still has its limitations. The immunosuppressive tumor microenvironment (TME) strongly inhibits CD8 T cells, leading to poorly functional, "exhausted", CD8 T cells. Several transcription factors, such as TOX, have been shown to regulate this mechanism.

Aim: In this study, we examine the role of NFAT5 in T cell exhaustion.

Methods: We investigated the role of NFAT5 by using a T cell specific KO of NFAT5 and we generated a reporter mouse model to decipher the mechanisms regulating NFAT5 expression.

Results: We show, in both murine and human tumors, that NFAT5 is highly expressed in tumor infiltrating CD8 T cells (TILs). NFAT5 KO TILs showed lower PD-1 and higher IFNγ expression compared to their WT counterparts resulting in an increased tumor control without losing CD8 T cell fitness. Surprisingly, NFAT5 KO CD8 T cells had no advantage in controlling chronic LCMV infection, suggesting a tumor specific induction of NFAT5. To describe concrete mechanisms linking NFAT5 with altered TIL effector functions, we investigated how the TME is regulating NFAT5 expression. We found TCR triggering and osmolar variation

within the TME to be responsible for the induction and activity of NFAT5 in CD8 TILs.

Conclusions: Altogether, this study demonstrates that NFAT5 is an important regulator of tumor induced T cell exhaustion and may be a potential target to increase the efficiency of immunotherapy.

P16

Attenuated immune control of Epstein-Barr virus in humanized mice is associated with the multiple sclerosis risk factor HLA-DR15

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Our aim is to study the synergistic effect between Epstein-Barr virus (EBV) and the main genetic risk factor of Multiple Sclerosis (MS), HLA-DRB1: 15*01 (HLA-DR15), in the context of this autoimmune disease, and investigate changes in virus and autoantigen specific T cell responses.

With our model of NSG mice engrafted with human immune system components (huNSG), we can study EBV infection *in vivo*. Through HLA typing of the donor cells, the genetic make-up of our huNSG can be influenced to study the infection in the context of HLA-DR15. The generation of T cell clones derived from huNSG allows to assess T cell functionality and cross-reactivity towards MS autoantigens.

We show that animals reconstituted with HLA-DR15$^+$ donors have a hyperreactive T cell compartment at steady state and upon EBV infection. Interestingly, these animals display elevated blood EBV viral titers. CD4$^+$ T cell clones reactive against HLA-DR15 transfected BLCLS (Bare lymphocyte syndrome patient-derived lymphoblastoid cell line) were derived from EBV infected animals. HLA-DR15 restricted T cell clones showed reactivity against peptide pools spanning the MBP autoantigen. Those clones showed a tendency towards higher cross-reactivity trough cytokine production and killing upon co-culturing with allogeneic HLA-DR transfected BLCLs compared to HLA-DR4-restricted CD4$^+$ T cell clones.

The MS-associated genetic risk factor HLA-DR15 could predispose individuals to an inefficient immune control of EBV-infected B cells and possibly support the priming of cross-reactive and MS autoantigen specific T cells.

P17

Association of atypical memory B cells defined by CD21-CD27- with clinical disease activity in patients with systemic lupus erythematosus

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Aim. Determining disease activity (DA) in systemic lupus erythematosus (SLE) patients remains a challenge given the lack of reliable biomarkers. The altered distribution of B cells represents great potential for assessing DA, but previous studies are limited by inconsistent definitions of B subsets. We performed a systematic study of peripheral B cells in 93 SLE patients to identify an accurate biomarker of DA.

Methods. B cells were studied in two separate cohorts of patients included in the Swiss SLE Cohort Study. In cohort A, cryopreserved PBMCs from 30 SLE and 30 age-, sex- and ethnicity-matched healthy controls (HC) were analyzed by mass cytometry. In cohort B, fresh blood from 63 other SLE, 14 Sjögren (pSS), 14 Sarcoidosis (Sarc) and 39 age-matched HC were analyzed by flow cytometry.

Results. In cohort A, using high-dimensional analysis and unsupervised clustering, we identified 6 B subsets, which were confirmed by manual gating. B subsets lacking the expression of CD21 and CD27 were increased in SLE compared to HC. They exhibited phenotype resembling atypical memory B cells (aMBC): CD11c^{hi}CXCR5$^-$. We confirmed the increase in aMBC in SLE from cohort B, compared to HC, pSS and Sarc. In both cohorts, aMBC were correlated with DA using two validated scales. Compared to anti-dsDNA and complement, aMBC showed better correlation with DA.

Conclusion. aMBC were significantly increased in SLE and the increase was strongly correlated with DA. aMBC frequency could be a novel biomarker for assessing DA. Longitudinal study is needed to define correlation with response to treatment.

P18

Harnessing the attenuated yellow fever virus 17D strain for Epstein Barr virus specific vaccine development

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Epstein Barr virus (EBV) infects more than 95% of the human adult population. Infection occurs asymptomatic in most of the cases. However, it is also linked to cancer, especially lymphomas. Immunotherapy can result on a better outcome for patients affected with EBV-associated malignancies. Nevertheless, the available individualized immunotherapies, like adoptive T cell transfer, are expensive and time-consuming. An alternative to overcome these limitations is the development of an EBV specific vaccine. In this project, we assess immunogenicity and prophylactic capacity of a yellow fever 17D viral vector encoding the Epstein Barr nuclear antigen 1 (YFV17D-EBNA1) against EBV-associated tumours. Infection of human peripheral blood mononuclear cells (PBMCs) with YFV17D-EBNA1, followed by EBNA-1 peptide restimulation, increased IFN gamma secretion, indicating successful EBNA-1-specifc T cells activation and expansion. *In vivo*, humanised NSG mice were vaccinated with YFV17D-EBNA1 and subsequently challenged with an autologous lymphoblastoid cell line (LCL). This prophylactic vaccination led to reduction in tumour metastasis to the lymph nodes; however, there was no difference in primary tumour growth rate. Our data suggests that the YFV17D-EBNA1 vaccine is able to expand already primed EBV-specific immune responses *in vitro*, and prevents tumour cell migration *in vivo*. Higher vaccine doses or prime-boost vaccination could improve the treatment effect against the primary tumour. The development of an effective vaccine represents an essential tool to control EBV-associated diseases.

P19

Different adjuvant treatments have diverse effects on systemic inflammation in Epidermal Necrolysis patients

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Aim: Stevens-Johnson Syndrome (SJS) and toxic epidermal necrolysis (TEN) are rare life-threatening cutaneous adverse reactions. There is no consensus on the use of adjuvant treatments in SJS/TEN. This is the first study to explore the effects of intravenous immunoglobulins (IVIG), cyclosporine A (CSA) and best supportive care (BSC) on the systemic immune response.

Methods: 16 patients with SJS/TEN received high-dose IVIG (n = 8), CSA (n = 4) or BSC only (n = 4). Serial serum samples were obtained prior-, 5-7 days and 21 days after treatment. High-throughput proteomics assay (OLINK) and ELISA were performed on serum samples to measure inflammation-associated proteins. Nanostring was performed on RNA extracted from skin biopsies collected prior treatment.

Results: SJS/TEN patients showed increased levels of Th1-associated chemokines and decreased levels of regulatory proteins. Few proteins were expressed differently between SJS/TEN severity grades (CD8, TGFb, IL-33, CX3CL1, CCL11, IL-17A, GDNF, ITM2A and IRAK1). The serum overexpression of Th1-associated chemokines correlated with their upregulation in mRNA of lesional skin. Serial measurements showed diverse dynamics between the 3 treatment groups, without any difference in clinical outcome. Both IVIG- and CSA-treated patients showed a decrease in Th1-associated proteins at day 5-7, while BSC patients showed an increase of regulatory and TNF-associated proteins. In all 3 groups, Th1-associated proteins were decreased at day 21.

Conclusion: BSC only, CSA and IVIG have diverse effects on the systemic inflammatory response in SJS/TEN.

P20

The symbiotic system in the tumor microenvironment that support tumor progression

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Cancer-associated fibroblasts (CAFs) and tumor-associated macrophages (TAMs) are two important stromal cells in the tumor microenvironment, which have been reported to communicate with each other for promoting tumor outgrowth and immune evasion. However, it remains unknown whether these two cell types have metabolic interaction and whether this metabolic crosstalk can favor tumorigenesis. In this study, we found that tumor interstitial fluid contained higher levels of glutamine compared with that in serum in both murine tumor model and hepatocellular carcinoma patients. Targeting glutaminolysis could skew TAMs to obtain M1-like phenotype, creating a more immunosupportive tumor microenvironment. We further revealed that cancer cell-derived palmitic acids promote CAF's ability to synthesize glutamine by stimulating an IL-6/STAT3 autocrine pathway. As a result of this cancer-CAF interaction, CAFs increase glutamine production to support M2-skewed phenotype in TAMs. Our findings demonstrated the triple crosstalk among cancer cells, CAFs and TAMs, that plays a pivotal role on maintaining the suppressive activity of TAMs for promoting tumor progression.

P21

What is first: Pillow, Bird or Egg ?

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We present the case of a 25-year-old woman living in an urban environment, who suffered from respiratory symptoms and excema since the age of ten years, after exposure to birds (she owned a canary bird and few budgerigars) and secondarily from allergic symptoms after the ingestion of eggs and chicken meat.

Furthermore, she slept in a bed with a pillow made out of natural materials (with bird feathers).

Skin samples were tested positive to egg yolk, chicken, goose and duck; Serum-specific IgEs for chicken egg white (ImmunoCAP 1.62 kU / l), α-livetin / serum albumin (ISAC, 0.4 ISU-E) and conalbumin / ovotransferrin (ISAC, 0.8 ISU-E) were determined.

The perennial rhinitic and asthmatic symptoms improved after replacing the pillows made out of natural materials by pillows made out of artificial materials.

The bird-egg syndrome is a rare clinical entity, mainly seen in adults. This syndrome displays a cross- sensitization to egg yolk and bird allergens (feathers, serum, droppings, and meat).

The bird-egg syndrome should be suspected in patients with perennial rhinitis, asthmatic symptoms, a bird meat allergy and intolerance to egg yolk. Meat consumption of poultry should be avoided. Advice against bird and animal husbandry make sense. Taking a detailed medical history facilitates clarification and diagnosis.

P22

Genome editing at the NCF1 gene and pseudogene loci primes for homologous directed chromosomal rearrangement

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Aim: Safety and efficacy of gene therapy relying on genome editing depend on the genetic context of the targeted region. The interaction of genetic regions that exhibit a high degree of sequence homology, while being localised on the same chromosome, may significantly contribute to post-editing chromosomal rearrangements. We targeted a two-nucleotide deletion in the neutrophil cytosolic factor 1 (*NCF1*) gene responsible for the p47^{phox}-deficient chronic granulomatous disease (CGD), as model for an editing target located in a region rich in repetitive DNA sequences.

Methods: Our strategy aimed at inhibiting rearrangements between the on-target *NCF1* locus and the two highly homologous *NCF1* pseudogenes, localised on the same chromosome. For a clear assessment of copy number variation, we developed a ddPCR-based method, allowing for reliable quantification and comparison between tested samples.

Results: We successfully employed diverse CRISPR/Cas-based editing strategies at the *NCF1* gene and pseudogene loci in a p47^{phox} CGD model cell line and in healthy human hematopoietic stem cells. However, genetic manipulation of *NCF1* loci primed for deletion and duplication of chromosomal regions between *NCF1* and the unprocessed *NCF1* pseudogenes.

Conclusions: These findings emphasize the need for careful evaluation of the genetic context and the editing outcomes of genome editing prior to application of editors in the clinical setting.

P23

Assessing the cellular and molecular landscape of murine Coronavirus infection in the lung

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The beta-coronavirus SARS-CoV-2 induces a respiratory illness called coronavirus disease 2019 (COVID-19) causing millions of deaths worldwide. Severe disease progression in patients is correlated with an impaired or defective type I interferon response, whereas patients with mild disease reveal an intact interferon response. Since a potent type I interferon response is critical for early viral clearance we used the murine beta-coronavirus-A59 (M-CoV-A59) to dissect the mechanism by which impaired type I interferon signalling lead to severe disease. By genetically impairing sensing of type I interferon (IFN) on LysM-Cre-expressing myeloid cells, we could observe less efficient viral clearance, more cell death and higher mortality in mice. Transcriptomic analysis revealed that reducing the ability of LysM-Cre-expressing cells to respond to type I IFN impaired the maturation of an antiviral state in myeloid cells abrogating downstream antiviral pathways. Collectively our data show that consistent with the impaired upregulation of downstream antiviral pathways, a lack of early viral control by the innate immune system results in reduced containment of the virus and more viral spread to peripheral organs.

P24

Photochemical internalization (PCI): a novel vaccination method for induction of cytotoxic CD8 T-cell responses

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Background: Cancer is a public health matter and a leading cause of death, but cancer vaccines could stimulate anti-tumor immune response, especially CD8 cytotoxic T lymphocytes (CTLs). However, a major problem of cancer vaccines is the inefficacious delivery of antigens to the MHC class I pathway of antigen presentation. Here, photochemical internalization (PCI) may bypass the problem by co-delivery of antigens and photosensitizer to APCs, resulting in cytosolic antigen release for presentation with MHC class I.

Aim: To develop PCI-based vaccination as a method for the stimulation of CTLs and to study treatment-associated innate immune reactions in the skin.

Methods: Mice received intradermal injections of antigen ± photosensitizer. Light was administered at 18h, and at various time points thereafter, skin and spleens were analyzed. CD8 T-cell responses were measured in the spleen by flow cytometry and ELISA, while skin was analyzed by histology and fluorescence microscopy.

Results: PCI improved proliferation of antigen-specific CD8 T cells and cytokine production. Skin histology and microscopy revealed light- and photosensitizer-dose-dependent innate inflammatory responses including acanthosis, edema, and infiltration of immune cells.

Conclusions: The results demonstrate the PCI can facilitate proliferation and activation of antigen-specific CTLs. Early innate immune responses may be an important part of the mechanism of action of PCI-based vaccines, and further studies will focus on how these innate immune responses translate into effective anti-tumor CTL responses.

P25

Autocrine IL-9/IL-9Rα signaling induces a pathogenic phenotype in Th2 cells

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IL-9 is a pleiotropic cytokine, for which an overarching role in humans remains elusive. IL-9 and its receptor, IL-9Rα, are specifically expressed by pathogenic T_H2 cells (pT_H2) in the skin, suggesting an important function of autocrine IL-9 signals in cutaneous immunity and allergy. Yet, the regulation of IL-9Rα expression on pT_H2 cells and the autocrine functions of IL-9 remain incompletely understood.

Here, we confirmed that IL-9Rα is strongly enriched in CRTh2+ memory T_H2 cells. As previous data showed that these cells express the transcription factor PPAR-g, we hypothesized that PPAR-g controls IL-9Rα expression. Indeed, we found that PPAR-g inhibition downregulates the expression of IL-9Rα at the RNA and protein level.

To decipher the autocrine function of IL-9 on T_H cells, we isolated human T_H cells from acute atopic contact dermatitis biopsies, expressing high levels of IL-9Rα. Transcriptional profiling showed that approx. 800 genes are differentially expressed in response to IL-9. Pathway analysis revealed that upregulated genes are associated with conventional T_H2 immune response. Strikingly, we observed a strong induction of genes specifically associated with the pathogenic T_H2 phenotype, such as *IL9*, *IL17RB* and *HPGDS*.

In summary, we discovered that PPAR-g regulates IL-9Rα expression and that autocrine IL-9 signals promote pathogenic features of T_H2 cells. Together, our data provide an explanation for the consistently observed coexpression of *PPARG*, *IL9*, and *IL9R* and suggest that T_H2 cells might induce their pathogenic phenotype through autocrine IL-9 signaling.

P26

Deciphering the function of bone morphogenetic proteins in regulating stromal cell-immune cell interactions

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Bone morphogenetic proteins (BMPs) are multifunctional cytokines belonging to the transforming growth factor β (TGFβ) superfamily of differentiation and growth factors and play an essential role as morphogens during embryonic development. Importantly, BMPs act on different levels as inducible tissue cytokines during development and homeostasis and their functions during fibrotic or inflammatory processes remained unknown. Here, we examined the expression of BMPs and their antagonists in secondary lymphoid organs and found specific expression of *Bmp4* in fibroblastic reticular cells (FRCs) and lymphatic endothelial cells (LECs) of murine lymph nodes. To manipulate the function of BMP4 *in vivo*, we have immunized mice with recombinant human BMP4 and generated antibodies specifically binding to both human and murine BMP4. Screening of a comprehensive anti-BMP4 antibody library identified distinct clones with strong capacities to neutralize BMP4 and cross-reactivity to BMP2. Selected antibody clones will be used to assess the expression pattern of BMP2/4 proteins *in situ* and their ability to neutralize BMP2/4 activity *in vivo*. Finally, we aim to use a reverse genetics mouse model to elucidate the function of BMP4 in distinct fibroblastic stromal cell subsets.

P27

The role of IgE glycosylation patterns on its biological activity

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Aim: Immunoglobulin E (IgE) is crucial in allergen-mediated allergic reactions. We have previously reported that high concentrations of the anti-IgE antibody omalizumab lead to active desensitization of basophils and that an engineered omalizumab-resistant IgE-Fc glycovariant may be used to replace the IgE-repertoire and inhibit allergen-mediated activation of human basophils ex vivo when co-applied with omalizumab. Here, we characterize additional omalizumab-resistant IgE-Fc glycovariants, evaluate their ability to inhibit allergen-mediated basophil activation and investigate potential underlying mechanisms involving inhibitory carbohydrate receptors.

Methods: IgE-Fc glycovariants were characterized using ELISA and surface plasmon resonance measurements. The selected variants were tested in a human basophil activation test alone or in combination with omalizumab measured by flow cytometry. The gene- and surface expression of potential inhibitory-receptors were analyzed by gene array and flow cytometry.

Results / Conclusion: IgE-Fc glycovariants retained FcεRI binding and some revealed resistance to omalizumab. The selected IgE-Fc glycovariants diminished basophil activation in a competition independent manner either alone or in combination with omalizumab representing an interesting treatment approach. Our data suggest that the IgE glycosylation pattern on IgE affects its biological activity. Assessment of expression status reveals a potential role of inhibitory carbohydrate receptors in allergen-induced activation of human basophils and proposes for further investigations.

P28

A call to the arms: How T cells are primed during primary infection with the Epstein Barr virus (EBV)

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Background and aim: The Epstein-Barr Virus (EBV) lists among the most successful human tropic pathogens. While most people acquire EBV during their childhood and undergo a silent asymptomatic infection, some others may experience a symptomatic primary infection as adolescents or young adults. This symptomatic primary infection, also referred to as Infectious Mononucleosis (IM), is marked by one of the greatest expansions and activations of $CD8^+$ T cells observed upon viral infection in humans. We aim to identify the cell type(s) that are involved in T cell priming and activation upon acute primary EBV infection.

Methods: We developed a humanised mouse model to assess the importance of EBV infected B cells (LCLs) in T cell priming. To this end we knocked out co-stimulatory molecules in LCLs by CRISPR/Cas9 and injected these as autologous tumours subcutaneously into humanized NSG mice. The influence of knock-out vs. wildtype LCL tumours on $CD8^+$ T cell expansion and activation was analysed.

Results: When autologous LCLs were subcutaneously injected into humanized mice, T cells were primed and activated with kinetics similar to that observed during infection with EBV. Knockout of the co-stimulatory molecule CD48 on LCLs prior to injection increased the expansion rate of $CD8^+$ T cells. In addition, a higher fraction of $CD8^+$ T cells was positive for activation markers 2B4 and granzyme B when mice were challenged with CD48 knockout LCLs.

Conclusions: We propose that EBV infected B cells play a role in T cell priming and activation by a pathway dependent on the CD48-2B4 axis.

P29

Characterization of a new genetically-engineered mouse model of bladder cancer

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Bladder cancer (BC) is a common malignancy presenting poor prognosis for advanced stages. Although immune checkpoint blockade has revolutionized the field of immunotherapy, it is still poorly effective. In muscle-invasive BC (MIBC), only 15-20% of patients respond to anti-PDL1 treatment.

Aim: In this study we aim to better understand the immune mechanisms in non-responsive patients and improve their outcome by taking advantage of a new mouse model of MIBC.

Method: We have already characterized a genetically-engineered mouse model of MIBC through the deletions of *Tp53* and *Pten* genes in the bladder, reflecting the portion of patients unresponsive to anti-PDL1 treatment. To make our model more immunogenic, as observed in human MIBC, we added a T cell antigen (SIY) and the luciferase transgene to better follow tumor growth (Luc-SIY transgene).

Results: Using bioluminescence imaging, we first confirmed tumor development. Then, upon analysis of the tumor immune microenvironment, we did not find differences compared to tumors lacking Luc-SIY

transgene. To understand this lack of immunogenicity, we transferred isolated bladder tumor cells derived from the various strains into WT, immunodeficient or Luc-SIY$^+$ animals. We concluded that central tolerance limited the antigen-specific T cell response.

Conclusions: By catheterizing our isolated bladder tumor cells into WT recipient mice, we aim to bypass these tolerance limitations and reach the desired tumor immunogenicity with the hope to further optimize immunotherapy strategies.

P30

A novel mouse model to study fibroblastic stromal cells in pancreatic cancer

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Pancreatic ductal adenocarcinoma (PDAC) is characterized by a desmoplastic tumor microenvironment, which consists of tumor cells, immune cells and a substantial proportion of fibroblasts. Single-cell RNA sequencing approaches have allowed the identification and molecular description of different cancer-associated fibroblast populations. However, their individual roles in promoting or restraining malignant progression, as well as their cellular origins are still incompletely understood. We developed a novel mouse model to study the role of fibroblasts in tumors of the pancreas by driving the expression of the viral oncogene SV40 large T antigen in pancreatic ductal cells. These mice develop tumors in the pancreas at high penetrance 16 weeks after induction of ductal transformation. Similar to PDAC, SV40-induced tumors in mice are characterized by a ductal morphology and a strong fibroblastic reaction. Interestingly, intratumoral T and B cells locate mostly in the tumor margin where they were found in close contact with podoplanin expressing fibroblasts. In contrast, the tumor parenchyma appeared almost devoid of lymphocytes and exhibited a dense network of smooth muscle-actin expressing fibroblasts. Overall, this autochtonous tumor model closely resembles human PDAC pathology and is thus well suited to study different fibroblast populations in pancreatic cancer. Using high dimensional transcriptomic and flow cytometric analysis, we are aiming to dissect the fibroblastic landscape in pancreatic cancer and to elucidate their origin and function during tumor progression.

P31

Speculative role of IgD on immediate hypersensitivity reactions.

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Aim: If the functions of IgE antibodies in immediate hypersensitivity are well known, the biological role of IgD remains contentious. There is cumulating evidence of ligation of soluble IgD on human basophils and mast cells that could inhibit IgE-activated degranulation (1). Tryptase is a marker of mast cell degranulation and is thus mainly used to confirm immediate hypersensitivity reactions.

This study aimed to investigate the correlation between IgD and tryptase, suggesting a possible biological role for IgD on tryptase degranulation.

Methods: We retrospectively analyzed all patients that had been tested both for tryptase and IgD at University Hospital of Geneva between 2007 and 2019. 30 patients were found. Total IgD were measured by nephelometry using BN ProsPec System (Siemens, Marburg, Germany). Total tryptase levels were determined by ImmunoCAP (Thermo Fisher Scientific, Uppsala, Sweden) according to the manufacturer's instructions.

Results: The results showed that tryptase levels were inversely proportional (slope -0.38, p = 0.03) to IgD concentration.

Conclusions: Our preliminary results support very recently published studies showing that binding of IgD to basophils and mast cells could inhibit IgE-mediated degranulation (1). These observations suggest a protective role of IgD in immediate hypersensitivity reactions. As our study was only conducted on a small sample of patients, it would be interesting to confirm these results on a larger scale and to investigate the mechanism underlying these observations.

1. Nguyen TG, Int Rev Immunol 2021.

P32

Acute Respiratory Distress Syndrome due to Gadolinium-based contrast agents: a review of the literature

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Purpose: Acute respiratory distress syndrome (ARDS) is a rare complication associated to the injection of Gadolinium-based contrast agents (GBCA). After describing the clinical case of a 60-year old woman who developed ARDS after injection of gadobutrol, we performed a comprehensive review of the literature to deeper characterize ARDS due to GBCA.

Methods: A systemic review of the medical literature was carried out with the keywords "ARDS Gadolinium" on PubMed, Google Scholar and Google.ch. Relevant articles in English, French or German on ARDS due to GBCA were considered.

Results: Thirteen cases of ARDS due to GBCA have been published so far. The reaction starts between 0 to 120 min after GBCA injection. GBCA associated ARDS was reported only after the injection of gadobutrol. PaO2/FiO2 ratio was described as severely compromised in 38% of the cases. Intubation was required in 46% of the patients and hemodynamic support in 69%. Treatment with glucocorticoids (GCS) was administered in ten cases (77%). Outcome was favorable in 54% of the patient who were discharged home within ten days. One death (8%) was reported.

Discussion: ARDS due to GCBA is a very rare and potentially severe adverse event. The pathogenesis remains elusive but involves increased vascular permeability linked to GBCA toxicity. In addition to hemodynamic and ventilatory support, GCS are often administrated. In the absence of studies, it is recommended to avoid any injection of GBCA in patients with a history of ARDS due to GBCA.

P33

IL-33/ST2 signaling differentially contributes to intestinal tumorigenesis

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Introduction: Colorectal cancer (CRC) is one of the leading causes of cancer-related deaths, with chronic inflammation in the gut microenvironment being a prominent catalyst. Interleukin-33 (IL-33) signaling plays a controversial role in CRC and its exact mode of action in the tumor environment is unclear.

Aims: 1. To characterize tumor-infiltrating lymphocytes that depend on the IL-33/IL-33 receptor (ST2) axis and 2. To address whether IL-33 may also play an ST2-independent role in CRC.

Design: CRC was induced by treating wild-type, *St2*$^{-/-}$ and *Il33*$^{-/-}$ mice with azoxymethane (AOM) and dextran sodium sulfate (DSS). Tumor growth was followed longitudinally using miniature endoscopy. CRC lesions were analyzed after 10 weeks by flow cytometry analysis of infiltrating cells and using bulk RNA sequencing of total CRC tissue.

Results: ST2 expression induces an activated and migratory phenotype in tumor-infiltrating regulatory T cells (Tregs), which favors their accumulation into the tumor microenvironment and promotes CRC. *St2* ablation diminishes Treg numbers, which correlates with increased effector CD8$^+$ T cell frequency and impaired CRC development. IL-33 curtails IL-17 production by ST2 Tregs and inhibits their differentiation to Th17. *St2*$^{-/-}$ and *Il33*$^{-/-}$ mice show a different phenotype, which suggests an additional, ST2-independent function of IL-33 in CRC.

Conclusion: These data indicate a multifaceted role of IL-33/ST2 signaling during intestinal tumorigenesis. Blockade of soluble IL-33 or ST2 may enable modulation of CRC immune infiltrates.

P34

Self-glycolipids-reactive T cells in Guillain-Barré syndrome

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Guillain-Barré syndrome (GBS) is a rare disabling disease that affects the peripheral nervous system (PNS). It is considered an autoimmune disor-

der in which pathogenic cross-reactive auto-antibodies (auto-Abs) induced by microbial infections cause nerve damage by targeting PNS self-glycolipids through a mechanism of molecular mimicry. However, auto-Abs are detected in a minority of GBS patients, thus suggesting that alternative immune-mediated mechanisms may be involved.

The aim of this study is to explore the hypothesis that T cells directed against PNS-glycolipids presented on monomorphic CD1 molecules may play a role in GBS. To this end, we are optimizing an experimental approach based on the combination of *in vitro* T cell screenings and *ex vivo* tetramer staining to screen for the presence of human CD1-restricted lipid-reactive T cells. This workflow could potentially be used in virtually all donors. Here we show that by using CRISPR/Cas9 technology and lentivirus-mediated overexpression system we were able to generate "universal CD1-expressing APCs" that lack HLA-class I and II and constitutively express CD80, CD86 and 4-1 BBL co-stimulatory molecules. The use of these cells as APCs in *in vitro* screening assays of total T cells from the blood successfully led to the identification and isolation of CD1-restricted lipid-reactive T cell clones.

Overall, we have established a tool that would allow us to study self-lipid reactive T cell responses in GBS patients, thus shedding light on the disease immune-pathology, with potential biomedical implications.

P35

The impact of size on particle drainage dynamics and antibody response

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Vaccine-induced immune response can be greatly enhanced by mimicking pathogens properties. The size and the repetitive geometric shape of virus-like particles (VLPs) influence their immunogenicity by facilitating drainage to secondary lymphoid organs and interaction with and activation of B cells and other innate humoral immune components. VLPs derived from the plant Bromovirus cowpea chlorotic mottle virus (CCMV) are *T = 3* icosahedral particles. They can be easily expressed in an *E. coli* host system and package ssRNA during expression. We have engineered CCMV-VLPs by incorporating the universal tetanus toxin (TT) epitope at the N-terminus. The modified $CCMV_{TT}$-VLPs properly form icosahedral particles *T = 3*, with a diameter of 45nm analogous to the parental VLPs. Interestingly, incorporating TT epitope at the C-terminus of CCMV-VLPs results in the formation of Rod-shaped VLPs, ~1µm in length and ~45nm in width. In this study, we have investigated the draining kinetics and immunogenicity of both engineered forms as potential B cell immunogens. Our results reveal that icosahedral $CCMV_{TT}$-VLPs are more efficient in draining to secondary lymphoid organs to charge antigen-presenting cells as well as B-cells. Furthermore, icosahedral, compared to Rod-shaped, $CCMV_{TT}$-VLPs led to more than 100-fold increased systemic IgG and IgA responses accompanied by prominent formation of splenic germinal centers. Up to our knowledge, this is the first study investigating the draining kinetics and immunogenicity of one and the same VLP monomer forming nano-sized icosahedra or micrometer-sized rods.

P36

Glycan-specific IgG anti-IgE autoantibodies contribute to protectivity against allergic diseases.

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Allergic diseases have become a severe problem worldwide and are mainly driven by the sensitization of effector cells with IgE. Cross-linking of IgE molecules with an allergen leads to the release of mediators, which cause the symptoms of allergic reactions. Previous studies have shown IgE-specific IgG antibodies in atopic as well as in healthy human donors which may exert regulatory functions[3]. Yet, the mechanisms by which these anti-IgE antibodies are induced are still not known. In this study, we investigated whether immunization with IgE-allergen immune complexes not only results in antigen-specific responses but also in antibody responses against IgE itself.

To investigate this, we immunized mice with the major cat allergen Fel d 1 in complex with a Fel d 1-specific monoclonal IgE. A single immunization with IgE-allergen complexes induces IgE-specific IgG autoantibodies. Interestingly most of these antibodies were glycan specific. We then analyzed theirs *in vitro* and *in vivo* impact on anaphylaxis. The induced anti-IgE autoantibodies capture IgE resulting in reduced FcεRI sensitization and increased serum clearance. Moreover, they protected mice from IgE sensitization and challenge with Fel d 1 and other allergens. Passive immunization with anti-IgE IgG antibodies purified from serum confirmed, that IgG antibodies are responsible for protection. Currently, we use Phage Display technology to characterize the binding specificity and variable gene repertoire. This work will provide first evidence on the role of glycan-specific IgG anti-IgE autoantibodies in IgE regulation.

P37

Extracellular vesicles cause neutrophil dysfunction leading to reduced resistance to secondary bacterial infection during malaria

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Malaria is a life-threatening disease caused by the infections with the parasites from the Plasmodium species. Malaria is associated with severe immune dysregulation, resulting in increased susceptibility to bacterial infections. Several studies have shown that neutrophils are impaired in their reactive oxygen species production as well as their migratory capacity.

Extracellular vesicles (EVs), including exosomes and microvesicles, are small membrane vesicles derived from multivesicular bodies contain a subset of proteins, lipids and nucleic acids that are derived from the parent cell. Recent works demonstrated that EVs play important roles in intercellular communication, both locally and systemically, as they transfer their contents, including proteins, lipids and RNAs, between cells. EVs are involved in numerous physiological processes, and vesicles from both non-immune and immune cells have important roles in immune regulation. Here we investigated the role of EVs in the neutrophil dysfunction. We demonstrate that EVs released during malaria infection strongly inhibit neutrophil functions, including migration, reactive oxygen species production resulting in reduced ability to kill bacteria. The dysfunction is caused by miR451a, a microRNA that is transferred from infected red blood cells to neutrophils. By using bulk and single cell RNA sequencing, we showed that miR451a target key nodal genes in the regulation of neutrophil activation resulting in their paralysis.

P38

Dominant inheritance of life-threatening multi-organ autoimmunity associated with a novel monoallelic missense mutation in DNA ligase 4

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Aim: Homozygous and compound heterozygous mutations in *LIG4* encoding DNA-ligase 4 cause an immunodeficiency syndrome presenting typically early in childhood with life-threatening and/or opportunistic infections, skeletal malformation, radiosensitivity and malignancy. Human haploinsufficiency has not been described so far. LIG4 is pivotal during DNA repair *via* nonhomologous end-joining (NHEJ) as it performs the final DNA-break sealing. DNA repair is not only required to assure genome integrity after genotoxic assaults but also physiologically during the generation of the B and T cell receptors after V(D)J recombination.

Methods: Whole exome sequencing, immune cell & autoantibody profiling, genotoxic *in vitro* assays, DNA duplex ligation assay, molecular dynamic simulations.

Results: Heterozygous *LIG4* mutated T cells displayed reduced DNA repair capacity and increased susceptibility to genotoxic stress. We demonstrate alterations in T_{reg} phenotype, altered autoantibody profiles, while changes in the T and B cell receptor repertoires were minimal. The

identified *LIG4* missense variant affected the *in vitro* DNA ligation capacity. Using molecular dynamics simulation, we show that the mutated LIG4 exhibited reduced DNA binding strength.

Conclusions: For the first time, we report a monoallelic *LIG4* haploinsufficiency in patients with life-threatening autoimmunity associated with reduced DNA binding capacity. This widens the phenotypic spectrum of LIG4-dependent immunodeficiency and has important diagnostic, therapeutic and prognostic implications.

P39

Real-time pollen and spore monitoring in Switzerland

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Aim: MeteoSwiss is responsible for the operation of the national pollen monitoring network in Switzerland. While this network delivers data useful for allergy sufferers, their doctors and researchers, the manual counting process results in data being only available at a daily resolution after a delay of up to nine days. Following a cost-benefit analysis, MeteoSwiss decided to automatize the national pollen monitoring network: up to 18 stations shall be equipped by the end of 2021.

Methods: The Swisens Poleno, a device providing in-flight imaging of airborne particles by digital holography has been chosen to equip the future network. We give a technical overview on the system and show how machine learning algorithms have been trained to identify and count pollen and spore taxa relevant for respiratory allergies.

Results: Validation against manual reference countings and certified instruments at the Swiss Institute of Metrology METAS showed good counting and identification performance. Following those tests, data was made available to the public in spring 2021 in a pilot phase.

Conclusions: The operational automatic network shall provide timely information to the end-users on the bioaerosol concentrations (pollen and fungal spores). This data will in addition greatly improve the quality of pollen forecasts, in particular numerical weather models will use real-time pollen data as input. The high temporal resolution of the new measurements will open new venues in research: it is now possible to study the interplay between weather, biological processes and the allergic response.

P40

Functional Characterization of Non-Coding Regulatory Drivers in Chronic Lymphocytic Leukemia

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Cancer cells accumulate somatic mutations in both coding and non-coding genome. Patients with chronic lymphocytic leukemia (CLL) present an excess of somatic mutations in local modules of coordinated non-coding regulatory elements (Cis Regulatory Domains, CRDs). The functional characterization of the effect of CRDs on their target genes is challenging since CLL cells die in culture.

Aim: To prove that somatic mutations in CRDs can be drivers in CLL development. For such, the non-coding genetic variations present in different EBV-transformed human lymphoblastoid cell lines (LCLs) were correlated with cancer progression phenotypes.

Methods: Three cancer-like phenotypes (cell migration, proliferation, and apoptosis) of 87 genetically different LCLs were correlated with variations in RNA_{seq} data by genome-wide association study (GWAS) and eQTL (expression quantitative trait loci) analysis.

Results: The GWAS analysis for the cancer-like phenotypes on LCLs found 37 suggestive associations: 1 for proliferation, 36 for apoptosis, and 20 of these associations included non-coding SNPs. Moreover, one of the strongest and significant GWAS hits was associated to apoptosis, being an eQTL for the *GALNT15* gene.

Conclusions: Data showed a trend for the associations between non-coding mutations in LCLs holding cancer-like phenotypes with genes that could be potential drivers in CLL. The promising association found with *GALNT15*, a member of *GALNT*s gene family identified as actors in neoplastic contexts support our hypothesis. The same approach could be used in the context of autoimmune diseases.

P41

Heterozygous hypomorphic mutations in Rag1/Rag2 alter the adaptive immune responses following infection and the susceptibility to immune dysregulation

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Aim: Patients harboring homozygous or compound heterozygous hypomorphic mutations in RAG1/2 suffer from delayed-onset combined immunodeficiency with granulomas and/or autoimmunity (CID-G/AI). The B and T cell receptor repertoire requires the RAG1/2 endonucleases, to initiate somatic V(D)J recombination. We present three patients suffering from CID with multiple complications carrying double heterozygous missense mutations in *RAG1/RAG2*. It has not been studied, which impact heterozygous hypomorphic mutations in either *RAG* gene or double heterozygosity have on the immune system.

Methods: We analyzed two hypomorphic $Rag1^{mut}$ and $Rag2^{mut}$ murine models, creating an *in vivo* RAG activity gradient.

Results: $Rag2^{mut/mut}$, $Rag2^{mut/+}$ and $Rag1^{mut/+}$ mice had only subtle changes in lymphocyte development and demonstrated a conserved adaptive immune response to LCMV infection. In contrast, double heterozygous *Rag* mutant mice ($Rag1^{mut/+}Rag2^{mut/+}$) displayed a significant block in lymphocyte development at RAG dependent steps. High-throughput T cell receptor sequencing of double heterozygous *Rag* mutant thymocytes revealed a higher abundance of productive sequences with an augmented oligoclonality. Following LCMV infection, fewer LCMV-specific $CD8^+$ T cells of reduced affinity expanded in double heterozygous *Rag* mutant mice. Interestingly, IgG autoantibodies were augmented in double heterozygous *Rag* mutant mice *post*-infection.

Conclusion: This study for the first time documents an additive-immunodeficiency and immune-dysregulation caused by double heterozygous *RAG1/RAG2* hypomorphic mutations.

P42

Neuro-psychiatric manifestations in patients with Systemic Lupus Erythematosus: A systematic review and Results from the Swiss Lupus cohort Study

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Aim: Systemic lupus erythematosus (SLE) is a systemic autoimmune disease associated with neuro-psychiatric (NP) manifestations. Frequency and patterns of neuro-psychiatric systemic lupus erythematosus (NPSLE) vary substantially between patients. We conducted a systematic review (SR) of the literature and examined prevalence and characteristics of NPSLE in the Swiss SLE cohort study (SSCS).

Methods: The SR search was performed between January 1999 and January 2020. We included prospective/ cross-sectional studies focussing on NPSLE. We secured study characteristics, cohort compositions and frequencies of NP manifestations, assessed heterogeneity across reports and investigated sources of variation using meta-regression models. Regarding the SSCS, we reviewed all patients included and classified NP manifestations.

Results: The SR searches identified 530 studies. We included 22 studies in our meta-analysis, the mean frequency of NPSLE ranged from 10.6% to 96.4%. The frequency of NPSLE in the SSCS was 28.1%. Severe events including cerebrovascular insults, seizures and psychosis appeared in 7.1%, 5.3% and 6.5% respectively. There was a linear relationship between duration of SLE and cumulative incidence of NPSLE.

Conclusions: The spectrum of NPSLE is very broad. The diagnostic work-up and rates of reported manifestations varied substantially across studies. We call for concerted efforts and consensus regarding definitions of NPSLE that will facilitate accurate diagnosis and attribution to SLE, particularly with a view to timely intervention and patient outcomes.

P43

Helping the killers: innovative cancer immunotherapy harnessing quasi-universal tumor antigen-specific CD4 T cells

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While cancer immunotherapy has mainly focused on exploiting CD8 T cells given their role in the direct elimination of tumor cells, increasing evidence highlights the crucial roles played by CD4 T cells in anti-tumor immunity. However, their very low frequency, the lack of robust algorithms to predict peptide binding to MHC class II molecules and the high polymorphism of MHC class II molecules render the study and use of circulating tumor antigen-specific CD4 T cells challenging. In this regard, the HLA-DRB3*02: 02 gene encoding an HLA allele that is expressed by half of the Caucasian population, offers a way to identify CD4 T cell-defined tumor antigens with broad cancer patient coverage.

We aim to identify, isolate and functionally characterize "quasi-universal" human tumor antigen-specific HLA-DRB3*02: 02-restricted CD4 T cells in cancer patients. Using an algorithm we recently developed in house, tumor-associated antigenic peptides binding to this allele are identified. We have generated a large collection of HLA-DRB3*02: 02-restricted CD4 T cell clones of different tumor-antigen specificities. We will perform *in vitro* co-cultures of CD4 T cell clones with tumor cells to measure cytokine secretion, their tumor cell killing and their phenotypic profile. We will sequence and clone the TCR of the most promising candidates for adoptive cell transfer therapy. Lastly, we will directly evaluate the presence of these cells *ex-vivo* and longitudinally monitor them in patients. Together, these results should contribute valuable targets for coordinated CD4 and CD8 T cell-based immunotherapy of cancer.

P44

Molecular profiling of tumor-specific cytolytic CD4 T cells in human cancer

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Aim: Increasing evidence shows that antigen-specific CD4 T cells are key players in the anti-tumor response. We recently reported on the frequent existence of fully active, tumor-specific cytolytic CD4 T cells in cancer patients[a]. The aim of this work is to characterize the molecular determinants involved in the cytotoxic process of the CD4 T cell-mediated responses, to identify highly potent clones that can be potentially used in cancer immunotherapy.

Methods: We are using the combinatorial peptide-MHCII-multimer technology to isolate tumor-specific CD4 T cells from patients. Helper and cytolytic tumor-specific CD4 T cells are profiled by an integrated phenotypic and functional characterization, down to the single cell level, through a novel high-throughput nanobiochip consisting of massive arrays of picowells and machine learning.

Results: We demonstrated a direct, contact- and partial granzyme B-dependent cytotoxic activity against tumors, with delayed kinetics compared to classical cytotoxic lymphocytes. We discovered that this cytotoxic activity was in part dependent on the receptor SLAMF7. By pharmacologically and genetically manipulating primary CD4 T cells we are investigating the impact of cytotoxic gene targeting on killing efficiency and kinetics, and synapse formation.

Conclusion: Overall, we believe that targeting "best-of-class" cytolytic CD4 T cells might prove synergistic with other cancer immunotherapies.

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P45

Drug Allergy Diagnosis from the distance: effect of pretest-time on test results

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Part of drug allergy diagnosis relies on *in vitro* tests like the cytokine-based lymphocyte transformation/activation test (Cyto-LTT). As the test relies on culturing live cells from patients, the duration between blood draw until cell culture (pretest time) may affect the cells' vitality and test results. While the pretest time from the local centres in Bern are <4h, samples from other centres (Lucerne, Geneva, St. Gallen, Ticino) take 18-24 hours until cellular analysis due to sample logistics. Here, we summarise the quality control data of Cyto-LTT in our cellular diagnostics specialised laboratory. We grouped samples into 4 groups from from different drug allergy centres located 110-277 km away from our laboratory in Bern. Samples from the allergy outpatient clinics in Bern served as a control group for same day diagnostics and short distance transportation.

We compared the levels of 5 cytokines (IL-5, IL-13, IFNγ, GranzymeB & Granulysin) secreted by activated cells after 7 days of cell culture by two stimuli: tetanus toxoid, a positive control for recall antigen response (5 µg/ml; n = 456), and amoxicillin (20-500 µg/ml; n = 148), from routine diagnostics between Feb 2018 to Dec 2019. No consistent differences in the test results for both tetanus toxoid and amoxicillin were observed based on distance or logistics. Minor differences were observed in single cytokine determinations. In conclusion, pretest times <24hrs did not significantly affect Cyto-LTT results, facilitating such cell-based tests for blood sent from throughout Switzerland.

P46

Perforin-independent cytotoxicity enhanced by cytokine complexes

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The *in vivo* biological activity of cytokines can be significantly increased by complexing with anti-cytokine monoclonal antibodies. Some cytokine complexes (cx), like interleukin-2 (IL-2) cx, can selectively stimulate effector cells such as Natural Killer cells and cytotoxic T lymphocytes. Controlling the activation of these cells and promoting Perforin (Prf)-independent killing mechanisms via IL-2cx, might be beneficial for patients with inborn errors of perforin-dependent cytotoxicity. Due to these mostly monogenic defects, infections cannot be cleared and affected patients may develop life threatening haemophagocytic lymphohistiocytosis (HLH).

To investigate the impact of IL-2cx on cytotoxic effector cell functions and HLH disease severity, we infected established PrfKO mouse model with Lymphocytic Choriomeningitis virus and compared the survival rate of IL-2cx treated vs untreated mice and analyzed lymphocytes by flow cytometry. As a preliminary finding, we observed altered maturation on NK cells and increased FasL expression. For functional assays, we have developed a high-content imaging workflow using mouse splenocytes incubated with target cells known to stimulate cytotoxicity. We are using several cell lines to test whether IL-2cx can modulate perforin- or death receptor-dependent NK cell cytotoxicity.

Thus, we have established a pipeline to test the biological activity of cytokine complexes and investigate the hypothesis of whether skewing the cytotoxic pathway away from Prf-dependency towards alternative killing mechanisms will improve immune homeostasis in HLH.

P47

Ligelizumab achieves sustained symptom control up to 1 year in the majority of patients with chronic spontaneous urticaria

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Background: Ligelizumab achieved greater control of symptoms versus omalizumab and placebo in patients with chronic spontaneous urticaria (CSU) inadequately controlled with standard of care including H1-antihistamines up to Week 20 in the core study. Here, we report the efficacy and safety of ligelizumab 240mg up to 1 year in an open-label, single-arm extension study) in patients who completed the core study and presented with active disease.

Methods: After washout of last dose in the core study and evidence of relapse, patients entering the extension study received ligelizumab

240mg q4w for 52 weeks; further monitoring for a 48-week follow-up is ongoing. Disease activity was assessed with the 7-day Urticaria Activity Score (UAS7).

Results: From the core study, 70.6% of patients entered the extension study, with 88.9% completing 1 year of open-label treatment. Complete symptom control (UAS = 0) was achieved in 35.4% of patients after the first dose of ligelizumab (Week 4). Complete responses were sustained and over 50% of patients achieved UAS7 = 0 at the end of Week 52. Throughout the one-year treatment period, 75.8% of patients (95% confidence interval [69.9%, 81.3%]) cumulatively experienced complete symptom control at least once by the end of Week 52 based on the Kaplan-Meier method. No new or unexpected safety signals were observed after 1-year of treatment in the extension study.

Conclusion: A high rate of sustained and complete symptom control was achieved with ligelizumab 240mg q4w in patients with CSU inadequately controlled with standard of care including H1-antihistamines.

P48

Mitophagy insults ferroptosis induction to modulate CD8+ T cell differentiation

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CD8+ T cell metabolism is dynamically regulated in the time course of an infection. At the peak of the response, effector T cells utilize aerobic glycolysis for proliferation and function. In contrast, memory T cells rely on oxidative metabolism. In addition, autophagy is known to be required for memory formation for unknown reasons. Here we found that mitophagy, a specialized form of autophagy degrading mitochondria, is upregulated during memory development in response to IL-15 signaling. Deletion of either Parkin or NIX, two key proteins controlling mitophagy, leads to severe impairment of memory T cells formation. We show that NIX-deficient memory T cells display an impairment in maintenance. Mechanistically, NIX-deficient T cells have increased lipid peroxidation and undergo ferroptosis during memory cells development. Our findings indicate that the mitophagy machinery is necessary during memory T cells formation and deletion of key components involved in this machinery, such as NIX or Parkin, further leads to ferroptosis of differentiating memory T cells.

P49

Lung-derived extra-adrenal glucocorticoids contribute to immune regulation in infectious and allergic diseases

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Aim: The lungs represent an important contact zone between our environment and the body. Constant exposure to potentially harmful particles and pathogens requires a tightly controlled immune system along the respiratory tree. We have previously shown that the extra-adrenal synthesis of glucocorticoids (GCs) in the lung epithelium is mainly mediated by 11-beta-hydroxysteroid dehydrogenase 1 (Hsd11b1). Moreover, GC synthesis in the lung is inducible upon immune cell stimulation. However, (patho) physiological relevance of this local GC synthesis has not yet been elucidated. We are currently investigating the role of lung-derived GCs in a mouse model of Hsd11b1 deficiency in the pathogenesis of influenza A infection and house dust mite-induced allergy.

Methods: The lungs of mice infected with influenza A or chronically exposed to house dust mite extract were examined for extra-adrenal GC synthesis, gene expression and histological changes. Immune cell infiltration was analyzed using high-dimensional fluorescent antibody panels for flow cytometry.

Results: Our data indicate a critical contribution of extra-adrenal GCs in the regulation of these lung-specific immune responses, similar to that in other well-studied extra-adrenal organs, such as the gut or skin. Thus, we find exacerbated inflammatory responses in Hsd11b1-deficient mice upon chronic exposure to house dust mite allergen.

Conclusions: Our findings encourage further research to determine the relevance of GCs in the lung under allergic and infectious inflammatory conditions and potentially open new targets for disease treatment.

P50

CD4 T cells with identical TCR clonotypes show functional plasticity in a mouse model of relapsing-remitting colitis

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Chronic inflammatory diseases, like inflammatory bowel diseases are often characterized by a relapsing-remitting course. We established a mouse model of a reversible, CD4 T cell- induced colitis, where treatment with an anti-CD4 mAb leads to rapid remission, followed by a spontaneous relapse of colitis.

We now compared by scRNASeq the transcriptome of distinct CD4 TCR clonotypes in this model of reversible colitis.

Indeed, a large fraction of the immunodominant CD4 T cell TCR clonotypes detected in the blood during active colitis are also found in the colon during remission and relapsing disease. Our initial transcriptomic analysis reveals a unique gene expression profile of colonic CD4 T cells during remission with a distinct up-regulation of genes involved in the maintenance of a tissue residency, such as CD69 and Rgs1, and, intriguingly, also of genes encoding proteins that actively attenuate inflammatory responses, such as NR4A1, a potent repressor of AP-1 functions. During relapsing disease, the CD4 T cells regain their transcriptional signature seen during initial colitis induction.

These findings thus demonstrate a high functional plasticity of CD4 T cells with identical TCR clonotypes during distinct phase of reversible colitis. Identifying the differentially expressed genes and their regulation may lead to more specific therapies to delay, or even prevent flares of active disease. Furthermore, the identification of the distinct signature of CD4 T cells that appear early in circulation mice may further allow to predict more accurately an imminent clinical relapse.

P51

Ligelizumab Achieves High Rate of Complete Response in Patients with Moderate to Severe Chronic Spontaneous Urticaria

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Background: Ligelizumab, has demonstrated greater control of symptoms vs. omalizumab and placebo in adult patients (pts) with chronic spontaneous urticaria (CSU).

Method: Data from a randomised, double-blind study of ligelizumab (24, 72 or 240 mg every 4 weeks [q4w] or 120 mg single dose) vs. omalizumab 300 mg q4w or placebo in adult pts with moderate to severe CSU (weekly urticaria activity score [UAS7]≥16), was analysed. UAS7 values were assigned to five score ranges: severe activity, moderate activity, mild activity, low activity, and urticaria-free. The percentage of pts with baseline (BL) moderate to severe CSU activity achieving complete control or low-activity/well-controlled at Wks 4 and 12 in the ligelizumab and omalizumab arms are reported here.

Results: At Wk 4, 70.0% and 48.1% of pts with moderate CSU at BL and treated with ligelizumab 72 and 240 mg achieved UAS7≤6, respectively, vs. 34.4% with omalizumab. Among pts with severe CSU activity at BL, 42.9% and 41.0% achieved UAS7≤6 with ligelizumab 72 and 240 mg, respectively, vs. 28.0% with omalizumab. At Wk 4, 35.0% and 25.9% of pts with moderate CSU at BL and treated with ligelizumab 72 and 240 mg achieved UAS7 = 0, respectively, vs. 12.5% with omalizumab. Among pts with severe CSU activity at BL, 28.6% and 32.1% achieved UAS7 = 0 with ligelizumab 72 and 240 mg, respectively, vs. 22.0% with omalizumab.

Conclusion: From Wk 4 onwards, a markedly higher percentage of patients who had moderate disease activity at BL or severe disease activity at BL had a complete response with ligelizumab treatment vs omalizumab.

P52

Healthy and patient ILC2s are differently affected by in vitro culture conditions

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Aim: Group 2 innate lymphoid cells (ILC2s) have emerged as key players in immunological processes such as type 2 inflammatory diseases including allergy and asthma. Due to their low number in the circulation, their *in vitro* expansion is needed to study their functions. Our aim was to assess how different culture conditions affect human ILC2 proliferation, phenotype and functions.

Methods: We cultured fresh peripheral blood ILC2s isolated from healthy donors (HDs) and allergic patients, combining OP9 cells with IL-2, IL-7 and a cytokine cocktail composed of IL-25, IL-33 and TSLP. Moreover, we tested the impact of adding phytohaemagglutinin (PHA).

Results: Among all these culture conditions, the cytokine cocktail induced the highest proliferation of HD ILC2s, while the patient ILC2s grew better with the PHA. We observed that the presence of OP9 cells did not boost the proliferation but impaired the activation marker expression of patients ILC2s. Furthermore, we noticed that the culture conditions differently affected the expression of activation marker in c-Kit^{high} and c-Kit^{low} ILC2s in both HDs and patients. Finally, we demonstrated that ILC2s expanded with IL-2 and IL-7 were the most prone to secrete IL-5 and IL-13 upon IL-33 re-stimulation. In contrast, the expansion of patient ILC2s with OP9 cells restrained their capacity to secrete type 2 cytokines.

Conclusions: This work highlights that culture conditions distinctly impacted the healthy or patient ILC2 behavior, with consequences that need to be considered for the study of ILC2s in type 2 diseases.

P53

Treatment with ligelizumab achieves over forty percent higher complete response rate in CSU patients originally treated with omalizumab

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Background: Ligelizumab, a humanized monoclonal anti-IgE antibody, binds to IgE with stronger affinity than omalizumab. Here, we report efficacy and safety of ligelizumab 240 mg up to 1 year in an open-label, single-arm extension study in patients originally treated with omalizumab in the core study and presented with active disease (Urticaria Activity Score [UAS7] ≥12) after omalizumab cessation.

Method: In the core Phase 2b trial, adult patients with moderate to severe CSU ([UAS7] ≥16) were randomised to receive ligelizumab 24, 72 or 240 mg, omalizumab 300 mg, ligelizumab 120 mg (single dose) or placebo every 4 weeks for five injections. Following a 16 weeks wash out, eligible patients (UAS7≥12) entered a 1-year open-label, single-arm (ligelizumab 240 mg q4w) extension study.

Results: The mean absolute change in UAS7 from baseline to Week 12 in patients treated with omalizumab 300 mg in the core study was -17.65 whereas retreatment with ligelizumab 240mg, showed a -20.88 change. The percentage of patients achieving a complete response (UAS7 = 0) at week 12 with omalizumab in the core study was 30.2 %, increasing to 43.4% upon retreatment with ligelizumab when assessed after 12 weeks. The percentage of patients achieving a complete response (UAS7 = 0) at week 20 with omalizumab in the core study was 32.1%, and 56.6% upon retreatment with ligelizumab when assessed again after 52 weeks.

Conclusion: CSU patients initially treated with omalizumab experienced upon retreatment with 240 mg ligelizumab over forty percent higher rate of complete responses after 12 weeks of treatment.

P54

Characterization of cytotoxic immunological synapses of NK cells with multiplexed imaging

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Inborn errors of immunity (IEI) manifest with defective host immune defense and regulation. In this study, we focus on inborn errors altering the cytotoxicity of Natural Killer (NK) cells, which result in hemophagocytic lymphohistiocytosis (HLH), a potentially fatal hyperinflammatory disease. Currently, the need for a high-sample capacity, comprehensive functional characterization of patients' specific cytotoxic effector potential is unmet. For this, we have established an experimental workflow that allows high-content imaging in 384-well format and automated quantification of multiple morphological features and subcellular distribution of signalling and immune cell-specific markers. Specifically, NK-cell specific cytotoxicity events following incubation with K562, a cell line inducing cytotoxicity, are analysed using Iterative Indirect Immunofluorescence Imaging (4i), allowing highly multiplexed image acquisition. Next, we apply a customized sequential set of computer vision algorithms to filter out imaging artifacts and obtain reliable quantifications of features pertaining to NK cytotoxic effector functionality at single-cell level. As a preliminary finding, we show that impaired cytotoxicity correlates with altered vimentin polarization and deficient pERK activation in NK cells of several IEI patients. Thus, we established an innovative workflow that provides a large array of immune related phenotypic parameters and responses, allowing unique insights into the mechanisms underlying NK-cell cytotoxicity and paving the way for drug screening in HLH.

P55

CD40 agonist targeted to Fibroblast activation protein a synergizes with radiotherapy in murine HPV-positive head and neck tumors

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CD40 agonistic antibodies enhance antigen-presenting cell activation and tumor-specific T cell priming. However, dose-limiting side and sink effects hamper their efficacy. These limitations can be overcome by a tumor targeted CD40 agonistic bispecific antibody FAP-CD40. In this study, we assessed the safety and therapeutic efficacy of a novel fibroblast activation protein (FAP)-targeted CD40 agonist in combination with local hypofractionated radiation in a preclinical HPV$^+$ HNSCC model.

We established orthotopic tumors and treated tumor-bearing mice with local hypofractionated radiotherapy (2x6Gy) alone or in combination with systemic administration of the FAP-CD40 bispecific antibody. Following up the mice, we evaluated the changes in the tumor microenvironment by immunofluorescence, FACS and NanoString RNA analysis.

While the suboptimal radiotherapy regimen chosen failed to control tumors in the treated mice, the FAP-CD40 administered in monotherapy transiently controlled tumor growth in 40% of mice and the combined therapy induced durable complete responses in more than 70% of the tumor-bearing mice. Moreover, radioimmunotherapy led to long-term tumor free survival and strong memory protective against secondary tumor challenge. This notable clinical efficacy was associated with remodeling of the TME and activation of the CD8$^+$ T-cell-cDC axis that required FAP expression in the tumor stroma.

Our study provides proof of concept, as well as mechanistic insights of the therapeutic efficacy of FAP-CD40 combined with local radiotherapy. It represents a promising option to fully leverage the CD40 pathway for cancer immunotherapy.

P56

Dual anti-viral and immunomodulatory activity of the CXCR4 inhibitor Balixafortide (POL6326) in preclinical in vitro and in vivo SARS-CoV2 infection models

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Balixafortide (POL6326) is a potent, selective inhibitor of the chemokine receptor CXCR4 currently in PhIII for treatment of advanced metastatic HER2-negative breast cancer patients in combination with eribulin (NCT03786094). Clinical proof-of-concept has been demonstrated in a PhI single arm dose-escalation trial (NCT01837095).

Recently, CXCR4-positive lung "bystander" T cells and neutrophils of Covid-19 patients were correlated with disease progression and fatal outcome[1, 2]. Consequently, CXCR4 inhibition has been suggested to have favourable effects on the prevention and treatment of acute respiratory distress syndrome and associated cytokine storm, lung fibrosis and unbalanced angiogenesis in the SARS-CoV-2 infected lung.

In vitro, balixafortide displayed a dose-dependent cell-protective effect in a SARS-CoV-2 induced cytopathic effect assay (CPE) in an EC50 range of 10 µM. Head-to-head comparison with remdesivir and time-of-virus addition studies suggest a different mode of action of balixafortide.

The activity of Balixafortide on SARS-CoV-2 infection was investigated in vivo in the free choice diet-induced obese hamster, a model that also develops nonalcoholic steatohepatitis and heart failure with preserved ejection fraction[3]. Balixafortide was subcutaneously administered 20mg/kg twice daily for 10 days after SARS-CoV-2 infection.

Upon balixafortide treatment, significantly less infectious SARS-CoV-2 particles were found on day 4 post infection, and a lower total viral load at day 10. Balixafortide statistically reduced CXCL10 gene expression (-40% compared to vehicle) at day 4. Elevated CXCL10 in conjunction with IL-6 is a key feature in COVID-19 disease generating a vicious circle resulting in a cytokine storm. In addition, balixafortide treatment led to a marked reduction (-40% compared to vehicle) of ISG15 gene expression in the lung on day 10. Elevated free ISG15 is associated with immune pathology in viral infection. There was no loss in body weight compared to vehicle control and no balixafortide-related mortality suggesting that balixafortide is well-tolerated.

The data suggest a favourable dual activity of balixafortide based on the reduction of both, viral load and inflammatory driving factors of COVID-19 disease.

(1) Pathogenic neutrophilia drives acute respiratory distress syndrome in severe COVID-19 patients. Eddins et al., bioRxiv, 2021
(2) Distinctive features of SARS-CoV-2-specific T cells predict recovery from severe COVID-19. Neidleman et al., medRxiv, 2021
(3) Elafibranor improves diet-induced nonalcoholic steatohepatitis associated with heart failure with preserved ejection fraction in Golden Syrian hamsters. Briand et al., Metabolism 2021 Jan 11;117: 154707. doi: 10.1016/j.metabol.2021.154707

P57

Construction and characterization of a panel of immortalized Natural Killer cell lines expressing allelic variants for FCGR3A

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Several allelic variants of the *FCGR3A* gene exist, where the high-affinity single-nucleotide polymorphism (SNP) V158F associates with the effectiveness of monoclonal antibody therapy. As to SNP L48H/R, H exhibits stronger binding to IgG1, IgG3, and IgG2 than R. The NK92 cell line resembles activated natural killer (NK) cells but lacks the expression of CD16 (*FCGR3A*).

Aim: To obtain a panel of NK92 cell lines expressing CD16 with the most common combinations of the SNPs L48H/R and V158F; and to test for differences in antibody-dependent cellular cytotoxicity (ADCC).

Methods: The different expression vectors were obtained by cloning *FCGR3A* into pVITRO and site-directed mutagenesis. NK92 cells were transfected by electroporation followed by geneticin selection. The CD16 expression was analysed by flow cytometry using three different anti-CD16 monoclonal antibodies, and ADCC tested by non-radioactive TDA release assays using anti-CD20 antibody and CD20-expressing Daudi cells as targets.

Results: We generated and characterized four *FCGR3A* combinations of SNPs on NK92 cell lines capable of ADCC. The NK92 transfectants exhibited no differences in terms of lytic activity triggered by anti-CD20 antibody engagement by CD16, regardless of the genetic variants involved. Albeit, dose-response curves showed that ADCC depended on anti-CD20 concentrations present in the assay.

Conclusions: We developed a tool for future ADCC studies. The four NK92 transfectants, accounting for more than 70% of the genotypes present in the population, showed no differences in ADCC.

P58

KSHV lytic gene expression drives increase in tumorigenesis in EBV co-infection in vivo

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Primary effusion lymphoma (PEL) is a B cell lymphoma associated with KSHV, and about 90% of patients show co-infection with EBV. Recent advances in our laboratory established a model for persistent KSHV infection by EBV co-infection of NOD-scid $\gamma c^{-/-}$ mice reconstituted with human immune system components (huNSG). This model revealed that dual-infection increases tumorigenesis and EBV lytic gene expression. Sequencing of dual-infected B cells and PEL cell lines displayed upregulation of KSHV lytic genes, leading to the hypothesis that KSHV lytic genes interact with EBV and host factors to drive this increased tumorigenesis.

Our study focuses on the replication and transcription activator RTA, that induces the expression of KSHV lytic genes and the switch to lytic phase, and examined its role in vitro and in vivo using a KSHV RTA Stop mutant virus. RNA Sequencing was performed using dual-infected human B cells in order to examine the impact of RTA on host and EBV gene expression and reveal involved cellular and viral pathways and processes.

We could reveal that the absence of KSHV lytic gene expression does not impact KSHV persistence, however it does decrease growth potential of in vitro generated dual infected B cell lines (EKCLs) and decreases tumorigenesis in dual-infected huNSG mice.

Our study is the first to examine the effect of KSHV lytic gene expression in a dual-infection setting close to the naturally occurring infection and PEL development and can extend our knowledge on the interaction of KSHV with host and EBV genes, revealing new treatment targets.

P59

The role of PPARβ/δ in orchestrating metabolic switch during memory CD8+ T cells differentiation

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The formation of antigen specific $CD8^+$ memory T cells is one of the most important features of the adaptative immune system and allows the establishment of the long-term protection against second infections. Although emerging evidence suggests that metabolic reprogramming is crucial for memory T cell formation, the underlying mechanisms that control metabolic reprogramming needed for memory T cell formation remain unclear. Our work focuses on understanding the role and the mechanism of the metabolic fitness changes that $CD8^+$ T cells undergo during their response to a viral infection. We find that the nuclear receptor PPARβ/δ was involved in the metabolic reprogramming that occurs in the transition from effector to memory $CD8^+$ T cells. Our work indicates that PPARβ/δ is involved in the downregulation of aerobic glycolysis and the promotion of fatty acid oxidation to facilitate the metabolic reprogramming required for the establishment of a metabolic profile favorable for memory T cells longevity in acute LCMV infection and progenitor exhausted population in chronic viral infection. We are also investigating the upstream signals necessary to trigger the PPARβ/δ pathway and this metabolic switch.

P61

Novel mosaic VLPs CuMVtt-Ara h 2 for peanut allergy treatment

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Rising allergy diagnosis rates are propelling the intensive search for novel treatments and therapies. One of the most common food allergies worldwide is the allergy to peanut, a known cause of food induced anaphylaxis, the life-threatening immune reaction. Over 50,000 cases of anaphylaxis and approximately 100 deaths due to allergy to peanuts are reported just in the U.S. every year.

In our lab we seek for an effective and safe immunotherapy against peanut allergy by development a vaccine candidates based on virus like particles (VLPs) technology platform, derived from Cucumber Mosaic Virus (CuMV). CuMV VLPs are comprised of multiple copies of viral structural proteins which assemble into nanoparticles upon expression in heterologous system. Furthermore, they can be engineered to incorporate the target epitope on its surface. For our research we use an immunologically optimized CuMV-derived VLPs incorporated with Tetanus Toxoid - T-cell stimulatory epitope that are genetically fused with major peanut allergen Ara h 2 (termed CuMVtt-Ara h 2).

The immunizations of naive mice show that the novel CuMVtt-Ara h 2 vaccine is able to induce specific anti-Ara h 2 IgG antibodies. Further tests indicate its long-term protective effects against anaphylaxis in peanut-sensitized mice, both after intravenous challenge with whole peanut extract and after local skin prick test. Moreover, the passive transfer of IgG antibodies, purified from serum of mice vaccinated with CuMVtt-Ara h 2, also protects against anaphylaxis, what indicates the major role of IgG antibodies in vaccine efficacy.

P62

Immunodeficiency and lymphoma in patient with Jacobsen syndrome

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Purpose: Here we report the case of a 46-year-old male adult patient with dysmorphic features, mental impairment, who lived for many years with a wrong diagnosis. He presented a clinical phenotype of late-onset combined immunodeficiency with high-grade B cell lymphoma.

Methods: We describe the clinical phenotype. The immunological evaluation included immunoglobulin levels, vaccine responses, number and function of T, NK and B cell subsets and genetics with an Array Comparative Genomic Hybridation.

The patient had a history of severe intellectual disability, dysmorphic features, chronic thrombocytopenia and recurrent upper and lower respiratory tract infections. At the age of 46 he presented a high-grade B cell lymphoma. After completion of the chemotherapy, the patient suffered from recurring upper and lower respiratory tract infections. PET-CT scan confirmed a successful recovery from the lymphoma, but showed residual bronchiectasis, pulmonary infiltrates and ground glass opacities. We found thrombocytopenia, hypogammaglobinemia, lymphopenia with low B-cell, CD4+ and CD4+gamma-delta T-cells. There was no response to pneumococcal polysaccharide vaccination. IRT 0.6 g/kg monthly was initiated. A terminal deletion of chromosome 11q compatible with Jacobsen syndrome was found.

Conclusions: Although there are no reports available on the association between Jacobsen syndrome and neoplasia in the literature, 11q deletions may play a role in malignant transformation of hematopoietic malignancies including B cell malignancies.

P63

Allergy testing with SARS-Cov2 mRNA vaccines

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Background: The newly approved mRNA-based vaccines BNT162b2 mRNA (Pfizer-BioNTech) and mRNA-1273 (Moderna) may be associated rarely with anaphylaxis. Many uncertainties persist concerning the allergic tests that can be proposed to evaluate the risk of anaphylaxis before vaccination.

Methods: We report two patients with history of anaphylaxis respectively to paclitaxel and macrogol 3350, who were referred to our allergy clinic to evaluate the risk related to SARS-Cov2 mRNA vaccines. Skin testing included both Pfizer-BioNTech and Moderna vaccines, polysorbate-80 1% PEG-2000 1%, and trometamol 1%.

Results: The first case is an 81-year-old woman who was hospitalized in 2018 for an anaphylactic shock attributed to paclitaxel. The second case is a 55-year-old patient who developed generalized pruritus, urticaria, dyspnea and dysphonia minutes after starting a preparation of macrogol 3350 for a colonoscopy. Skin prick testing was negative in both patients. Intradermal testing (IDT) was positive for both mRNA-based vaccines and polysorbate-80 but not for PEG-2000 and TRIS. Basophil activation tests (BAT) were performed in the first case and were positive for both mRNA vaccines, paclitaxel and to a lesser extent polysorbate-80 and 20.

Conclusion: The only shared component between the Pfizer-BioNTech and Moderna vaccines, paclitaxel, polysorbate and macrogol 3350 is PEG, although with different molecular weights. IDT and BAT with mRNA-based vaccines can be useful to evaluate patients with a history of severe anaphylaxis associated with laxatives or parental drugs containing PEG or polysorbate.

P64

Innate lymphoid cells (ILCs) in chronic myeloid leukemia (CML): do ILC2s influence the progress to acute leukemia and/or the resistance to therapy?

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Aim: Innate lymphoid cells (ILCs) are emerging as key players in tumor development and clearance, including hematologic malignancies. However, their role in chronic myeloid leukemia has not been characterized yet. Our aim is to assess the crosstalk between ILCs and CML cells to understand whether ILCs can influence their progress to the blast phase and/or the establishment of mechanisms of resistance to tyrosine kinase inhibitors.

Methods: We are using 16-color flow cytometry panels and cytokine quantification assays to characterize the ILC frequency, phenotype and functions in CML patients' samples. ILCs are FACS sorted and cocultured with CML cells to test whether they can modify CML cell proliferation, differentiation, apoptosis.

Results: Our preliminary results showed an enrichment in a subpopulation of ILCs (ILC2) in treatment-naïve CML patients, coupled with increased serum concentrations of IL-18 and VEGF, factors known as possible drivers of ILC2 proliferation and function. We also found that the CML cell line K562 constitutively secretes VEGF and it will be therefore used as in vitro model.

Conclusions: Our preliminary data suggest an involvement of ILC2s in the CML pathogenesis, as we already demonstrated for acute promyelocytic leukemia, with potential implications in the clinics.

P65

Quantitative pan-immunoglobulin titers against the receptor binding domain of the SARS-CoV-2 spike protein following infection with SARS-CoV-2

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Aim: We aimed to determine the distribution of WHO-standardized quantitative measurements of pan-immunoglobulin titers against the receptor binding domain of the SARS-CoV-2 spike protein following SARS-CoV-2 infection.

Design & Methods: In a retrospective analysis we analyzed anonymized routine data of patient samples with SARS-CoV-2 infection, as evidenced with a pan-immunoglobulin assay directed against nucleocapsid-antigen measured with an electrochemiluminescence immunoassay (ECLIA; Roche Diagnostics, Switzerland). Pan-immunoglobulin titers of antibodies directed against the receptor binding domain of the SARS-CoV-2 spike protein were measured with the same technology. Results are given as binding antibody units (BAU) per mL. Cut-off for positivity is >0.8 BAU/mL.

Results: A total of 1436 samples originating from patients (635 males; 801 females) aged 52 (median, interquartile range, IQR, [38,64]) years were included. There was no correlation between age and antibody titers. Females (111, IQR [27,>257] BAU/mL) had significantly lower median antibody titers than males (147, IQR [36,>257] BAU/mL; p = 0.03). The antibody levels at the 2.5, 5, 10, 25, 68th percentiles were 1,3,6,27, and >257 BAU/ml, in females. The antibody levels at the 2.5, 5, 10, 25, 62nd percentiles were 0.4, 2, 9, 36, and >257 BAU/ml in males.

Conclusions: Among patients with evidence of past SARS-CoV-2 infection, one third exhibits antibody titers above the upper quantification limit (i.e. 257 BAU/mL). Fifteen percent of female and 12% of male patients have antibody titers of 10 BAU/mL or lower.

P66

Disturbed Mitochondrial Dynamics Rewire the Epigenetic Program for CD8+ TIL Exhaustion

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Cancer immunotherapy, including checkpoint blockade and adoptive transfer of tumor-reactive T cells, represents a paradigm shift in the treatment of malignancies in recent years, and yields remarkable responses by reawakening anti-tumor immunity in established tumors. Nevertheless, a significant portion of patients are refractory to cancer immunotherapies, which may be in part due to the persistent impairment of anti-tumor effector functions in T cells, a phenomenon referred to as T cell exhaustion. However, it remains elusive how T cells engage epigenetic reprogramming to orchestrate exhausted state. Here, we examined the mitochondrial fitness in CD8+ TILs. We found that tumor-infiltrating T cells with accumulation of damaged mitochondria display more severe exhausted phenotypes, including decreased proliferation capacity, reduced cytokine production and up-regulation of co-inhibitory receptors. Importantly, we found that the accumulation of dysfunctional mitochondria is controlled by the affinity of TCR-pMHCI interaction, and also supported by the PD-1 expression. Moreover, the combination of glucose deprivation, hypoxia and TCR signaling *in vitro* can drastically weaken T cell immunity. Ultimately, supplementation with nicotinamide riboside enhances T cell mitochondrial fitness and improved responsiveness to anti-PD-1 treatment. Taken together, our study suggests that mitochondrial fitness is pivotal for orchestrating T cell-mediated anti-tumor immunity and the accumulation of dysfunctional mitochondria could instruct epigenetic reprogramming for T cell exhaustion.

P67

Candida albicans and the host: the secrets of adaptation

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All surfaces of the human body, including the skin, the oro-gastrointestinal tract and the female reproductive tract are densely colonized with microbes. Being usually harmless for the host, these microorganisms can have pathogenic potential and cause disease under certain conditions. *C. albicans* is a good example of a commensal fungus that can cause infections if host defences are breached. Th17 immunity is implicated in controlling *C. albicans*However, how fungal commensalism is maintained, and fungal overgrowth prevented during homeostasis is not fully understood. This project explores the hypothesis that the fungus itself promotes commensalism by undergoing adaptation to the host. Re-isolation of *C. albicans* from colonized mice revealed that the fungus acquires enhanced capacity to metabolize alternative carbon sources, such as those that are abundant in mucosal tissue. This adaptation process was found to be conserved across different fungal strains and in immunologically different hosts. In IL-17-deficient mice commensal strains of *C. albicans* acquire an invasive phenotype that disrupts mucosal tissue homeostasis and triggers inflammation, while systemic dissemination of the fungus was prevented in a neutrophil- and monocyte-dependent manner. Together, these data indicate that *C. albicans* adaptation in immunocompetent hosts is supported by the fungus' own metabolic program, while in absence of a functional IL-17 pathway, the fungus adopts pathogenic traits associated with the induction of tissue damages and barrier disruption

P68

The mitochondrial pyruvate carrier regulates antitumor function and memory T cell differentiation

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The mitochondrial pyruvate carrier (MPC) is a key metabolic transporter at the crossroad of glucose fermentation versus oxidation, but its role in T cell function remains unexplored. In tumor-bearing mice, MPC KO CD8 T cells formed more central memory T cells, but failed to control tumor growth. We found that the nutrient depletion in a tumor microenvironment metabolically suppressed MPC KO T cell function, due to the inhibition of mTOR signaling and epigenetic exhaustion. WT CD8 T cells can partially overcome this metabolic suppression by metabolizing lactate, while MPC KO T cells cannot. Interestingly, transient pharmacological MPC inhibition during in vitro CD8 T cell expansion allows adoptive cell transfer of MPC-proficient T cells with enhanced memory differentiation and, therefore, a superior anti-tumor function. Mechanistically, altered metabolic fluxes following MPC inhibition led to an increase in acetyl-CoA levels due to glutamine anaplerosis. This was accompanied by elevated glutamine-derived acetyl deposition on histones, associated with an epigenetic activation of memory gene expression, orchestrated by the transcription factor RUNX1. Thus, this study shows that mitochondrial pyruvate import is crucial for preventing total loss of effector CD8 T cell function in a nutrient-deprived environment. However, nutrient-proficient conditions allow for metabolic flexibility in MPC-inhibited T cells, inducing long-lasting central memory T cell differentiation, and thereby improving their anti-tumoral therapeutic potential upon adoptive cell transfer.

P69

Hidden ingredients in food- the surprise comes while running

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A 55-years old female patient has been known to have celiac disease since 2002 proven by intestinal biopsy. She is referred for evaluation of allergic reactions related to food. The symptoms are primarily cutaneous such as urticaria and angioedema, with associated rhinorrhea, tachycardia and fatigue. Regardless of all reactions, she was previously active in sports. Further allergological history includes mild allergic rhinoconjunctivitis allergica, but not other allergic diseases.

Five episodes were described from 2017 to 2018 with different foods such as French fries Duchess, rosemary chicken (frozen products), Smarties, and French and Italian dressings.

Serologically, a sensitization to wheat (32.10 kU/L, CAP 4), Tri a19 (2.6 kU/L, CAP 2) and gliadin (59.20 kU/L, CAP 5) could be proven, which corresponds to a gluten allergy. The episodes each occurred in connection with exercise and gluten consumption. On the first glance, these products seem not to contain any wheat, but after detailed analysis of content, they do.

We therefore diagnosed, in addition to the already known celiac disease, a Wheat Dependent Exercise Induced Anaphylaxis (WDEIA) because of the positivity of Tri a 19. Wheat can be found in traces in many products not suspicious at first glance. It is therefore useful to have a second look to avoid unpleasant surprises.

P70

Characteristics of dermatological patients with blood eosinophilia: a retrospective analysis of 453 patients

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Skin diseases associated with blood and tissue eosinophilia are common. Eosinophil infiltration in the skin is observed in allergic, autoimmune bullous, infectious, hematologic diseases and tumors. So far, data on dermatological patients' characteristics presenting with various degrees of blood eosinophilia are scarce.

We analyzed demographical, clinical and laboratory parameters of 453 patients with blood eosinophilia (BEC) referred to the Department of Dermatology, Inselspital, University Hospital of Bern, from 2014 to 2018. Pruritus was present in 88.1% of patients. The morphological spectrum of cutaneous lesions was broad with an eczematous pattern being the most frequent one. Using semantic map analysis, three distinct hubs were identified: Low blood eosinophilia (0.5-0.99 G/L) correlated with localized skin lesions, history of atopy and final diagnoses of infectious disease or eczema. Medium blood eosinophilia (1.0 – 1.49 G/L) was linked to pruritus, generalized skin lesions and autoimmune bullous disease. High blood eosinophilia (≥1.5 G/L) was associated with the final diagnoses hypereosinophilic syndrome and drug hypersensitivity reaction. A history of neoplasia showed significant correlation with BEC and was most frequent in patients with hypereosinophilia.

To our knowledge, this is the first study analyzing characteristics of dermatologic patients with various degrees of BEC. Our data emphasize the requirement of a profound diagnostic work-up in dermatological patients with BEC to provide optimal patient care including eosinophil-targeted therapies.

P71

Challenging and progressive inflammatory infundibulitis

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Aim(s) or purpose: Pituitary stalk lesions may arise from the stalk itself, the pituitary gland, or the hypothalamus. The management of inflammatory pituitary stalk thickening is challenging. We demonstrate that a conservative approach with corticosteroids in combination with close clinical and radiological monitoring can be effective in reducing pituitary stalk thickening, prevent progressive pituitary hormonal deficiencies and spare patients from risky surgical biopsies.

Design & methods: A 39-year-old man presented with a history of fatigue, polydipsia, polyuria and loss of libido. Biochemical testing revealed hypernatremia and low urinary sodium, with inability to concentrate urine and low ADH levels. MRI revealed a 6 x 5 mm pituitary stalk lesion suggestive of inflammatory infundibulitis, causing central diabetes insipidus.

Results: Treatment with oral desmopressin was initiated and the patient's thirst and water balance normalized. Four years later he presented with poor beard growth and libido due to a suppressed gonadotropin-gonadal axis. We suspected an inflammatory origin. Three pulses of methylprednisolone (1g/day) were administered. Beard growth, libido and gonadotropin-gonadal axis normalized. Pituitary stalk size returned to 7 x 3 mm. To this day, ten years after the initial diagnosis, he shows no progression of pituitary swelling and no new signs of pituitary hormone deficiencies.

Conclusions: Corticosteroids in combination with close clinical and radiological monitoring can be effective and safe in reducing pituitary stalk thickening.

P72

Frequency and utility of repeat antinuclear antibody (ANA) testing

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Aim: Testing for antinuclear antibodies (ANA) is an important part of the diagnostic workup for systemic autoimmune rheumatic diseases (SARD). Although there is no clear evidence that repeated ANA tests have clinical value, patients often undergo serial testing. The aim of this study was to evaluate the frequency and utility of repeat ANA testing.

Methods: This retrospective study included all ANA requests sent to a tertiary hospital immunology laboratory between January 2018 and December 2020. A repeat ANA was defined as a test requested within 2 years of a previous test. Clinical information was retrieved from medical records.

Results: In 2020, a total of 3'663 ANA tests were performed. Among these, 565 (15.4%) were repeat tests, generating a total cost of 28'250 CHF to the test payers. Of all repeats, 66.4% were first time repeats, while 16.1% and 17.5% were second and third or more repeats, respectively. Frequency ranged from 1 to 6 repeats per patient per year. Overall, 92.1% of all repeats showed no significant change compared to the previous ANA. Of all 565 repeats, 215 (38.1%) were positive, with 24 (4.2%) being previously negative. Of these, only 1 (4.2%) was associated with a new diagnosis of SARD after the repeat testing.

Conclusions: ANA tests are frequently repeated resulting in substantial costs. The vast majority of repeated test results do not change in comparison to the previous ANA. A repeat test with a positive result following a negative ANA is seldom associated with a new diagnosis of SARD. Repeat ANA testing is common practice, but has limited clinical utility.

P73

RhoH binding to myosin-9 prevents the intracellular traffic of mitochondria and granules diminishing neutrophil extracellular trap formation

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Aims: RhoH, an atypical Rho GTPase, is not detectable in freshly isolated neutrophils but up-regulated in neutrophils under inflammatory conditions *in vitro* and *in vivo*. Whether and how the aberrant expression of RhoH would affect neutrophil effector functions, such as the formation of neutrophil extracellular traps (NETs), remain to be investigated.

Methods: Neutrophils with different levels of RhoH were activated by diverse stimuli and their capacities to form NETs were evaluated. Subsequently, the involved molecular events in NET formation were analyzed to explain the regulatory role of RhoH.

Results: We demonstrated that blood neutrophils from cystic fibrosis (CF) patients expressed increased levels of RhoH and exhibited a decreased ability to form NETs as compared to control neutrophils. Similarly, induction of RhoH expression in primary mouse neutrophils by GM-CSF treatment or overexpression of HA-tagged RhoH in neutrophils significantly impaired NET formation. Proteomic screening identified myosin-9 as an interacting partner of RhoH. Myosin-9 associated with neutrophil mitochondria and granules via its tailpiece and transported mitochondria and granules towards the cell membrane by sliding along actin filaments upon neutrophil activation. Interestingly, the interaction between RhoH and myosin-9 prevented the intracellular traffic of mitochondria and granules while disruption of their interaction rescued this process.

Conclusions: RhoH negatively regulated NET formation by preventing myosin-9 mediated intracellular traffic of mitochondria and granules.

P74

Establishment of a novel functional mast cell assay for determination of IgE-mediated allergies and treatment responses

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Aim: Clinical management of allergic diseases is hampered by the lack of safe and simple tests to reliably identify culprit allergens and to closely monitor disease activity. Since allergy diagnosis is a complex multistep procedure, there is urgent need for standardized functional *ex vivo* assays allowing objective diagnosis, substantiating treatment choices, and quantifying therapeutic success.

Here, we present a novel functional cell-based assay that relies on passive sensitization of allergic effector cells with patient serum circumventing many current limitations in allergy diagnosis.

Methods: We genetically engineered conditional Hoxb8-immortalized myeloid progenitors from bone marrow of transgenic mice expressing the human high-affinity IgE receptor (FcεRIα). Within 5 days, these cells can be differentiated into mature mast cells ("Hoxb8 MCs") in virtually unlimited numbers. Hoxb8 MCs were passively sensitized with patient sera, activated with various allergens and cellular responses were assessed by flow cytometry.

Results: We demonstrate that the established Hoxb8 MC assay can be used to accurately measure total IgE levels, identify culprit allergens, longitudinally monitor allergen-specific immunotherapy (AIT) and determine the timepoint of tolerance induction upon AIT in patients.

Conclusion: Our results indicate that this novel diagnostic test represents a valuable tool to support clinicians in the identification of IgE-mediated allergies and in the quantification of treatment efficacy as well as duration of therapeutic response.

P75

Neutralization of Middle East Respiratory Syndrome coronavirus through a scalable nanoparticle vaccine

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MERS-CoV continues to cause human outbreaks, so far in 27 countries worldwide following the first registered epidemic in Saudi Arabia in 2012. In this study, we produced a nanovaccine based on virus-like particles (VLPs). VLPs are safe as they lack any replication-competent genetic material, and are used since many years against hepatitis B virus (HBV), hepatitis E virus (HEV) and human papilloma virus (HPV). In order to produce a vaccine that is readily upscalable, we genetically fused the receptor-binding motif (RBM) of MERS-CoV Spike protein into cucumber-mosaic virus-like particles. The employed $CuMV_{TT}$-VLPs represent a new immunologically optimized vaccine platform incorporating a universal T cell epitope derived from tetanus toxin (TT). The resultant vaccine ($mCuMV_{TT}$-MERS) consists of unmodified wild type monomers and genetically modified monomers displaying RBM, both co-assemble in a prokaryotic expression system. $mCuMV_{TT}$-MERS vaccine is self-adjuvanted with ssRNA, a TLR7/8 ligand which is spontaneously packaged during the expression process in E. coli. The ability of the engineered vaccine to bind to MERS-CoV receptor DPP4 was tested in a competitive ELISA. To test the safety and immunogenicity of $mCuMV_{TT}$-MERS, Balb/cOlaHsd mice were primed with 100ug VLPs and boosted on day 28. The developed vaccine induced high anti-RBD and anti-Spike antibodies in a murine model, showing high binding avidity and the ability to completely neutralize MERS-CoV/EMC/2012 isolate, demonstrating the protective potential of the vaccine candidate in dromedaries and humans.

P76

Self-assembling glycoprotein nanoparticle vaccines

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One major reason behind the increase in antimicrobial resistance (AMR) is the overuse of antibiotics, especially in food production animals. Hence, development and deployment of effective anti-bacterial vaccines, targeting a wide range of Gram-negative bacteria, with an added bonus of extremely low cost and simple production can target gaps in the market.

Some of the most effective bacterial vaccines target pathogen-specific sugar structures (glycans) on the bacterial surface and raise an immune response at mucous membranes. The aim is to develop a biotechnological platform that combines novel bacterial glyco-engineering system with virus like particle (VLP) based vaccines, enormously simplifying the production of potent anti-bacterial vaccines. Making it cost efficient is necessary to decrease AMR so farm animals can be immunized.

The novel biotechnological platform consists of lab-safe bacteria that are genetically reprogrammed to synthesize pathogen-specific glycans onto self-assembling viral coat proteins inside the cytosol. The products are ready- to-use vaccine candidates, called glyco-VLPs; spherical nanoparticles that resemble viruses and are decorated with glycans from the pathogen's outer surface. Target antigens are glycoproteins or capsular polysaccharides. The optimization of vaccine application for strong induction of a mucosal antibody response will be performed and candidates will then be tested for protective efficacy in murine models and in domestic pig in pre-clinical and clinical settings, respectively.

P77

Embryonic alveolar macrophage progenitors are critically reliant on epithelial GM-CSF

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Aim: Fetal monocytes that seed embryonic tissues during organogenesis differentiate into specialized tissue-resident macrophages upon exposure to critical niche-specific factors. In the lung, alveolar macrophages (AMs) are known to rely heavily on GM-CSF (encoded by *Csf2*) for their survival. Postnatally, we have unequivocally demonstrated that AM survival is critically reliant not on hematopoietic GM-CSF but on epithelial GM-CSF derived from alveolar epithelial type 2 cells (AT2s). It is not known, however, if differentiating fetal monocytes also require epithelial GM-CSF for AM fate specification.

Methods: To answer this question, we have developed novel transgenic *Csf2* reporter mice that were used to not only profile pulmonary GM-CSF production, but also to selectively delete *Csf2* expression from populations of interest.

Results: Flow cytometry analysis of *Csf2*-reporter kinetics suggests that pulmonary GM-CSF production is first induced between E16.5 and E18.5. At E17.5, constitutive deletion of hematopoietic GM-CSF did not impact the lung fetal monocyte population. Depletion of epithelial GM-CSF, however, revealed a severe perturbation in the developing fetal monocytes, with significant declines in both fetal monocyte numbers and proliferation.

Conclusions: The aforementioned postnatal AM-AT2 relationship thus begins during embryogenesis, where nascent AT2s timely induce GM-CSF expression to support the proliferation and differentiation of fetal monocytes towards an AM fate.

P78

The VEXAS syndrome: Report of 3 new cases

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Introduction: The VEXAS (vacuoles, E1 enzyme, X-linked, autoinflammatory, somatic) syndrome is a recently described X-linked autoinflammatory condition caused by a somatic mutation of the UBA1 gene. It is characterized by an evolving phenotype, including inflammatory processes such as recurrent fever, neutrophilic dermatosis, vasculitis, pulmonary fibrosis, relapsing polychondritis and venous thromboembolism. An important feature, present in almost all cases, is macrocytic anemia with vacuolization of myeloid and erythroid precursors resembling myelodysplastic syndrome (MDS) without fulfilling all diagnostic criteria.

Results: Here, we describe the clinical features and response to therapy in three new cases of the VEXAS syndrome. Two were identified by a review of bone marrow aspirates after a disease course of 4 and 6 years, respectively. The pathognomonic vacuolization of myeloid and erythroid progenitors were present in both cases. The third patient was diagnosed after 4 years of investigations for arthritis, leukocytoclastic vasculitis, pyoderma gangrenosum in the context of MDS. The clinical features and the results of the mutational analysis (p.Met41Val, p.Met41Leu, p.Met41Thr) corresponded well to the initial report, with pulmonary hemorrhage in one patient and cryoglobulinemia in another as new findings. The course of all patients was characterized by a lack of response to steroid-sparing drugs and a high morbidity.

Conclusion: The VEXAS syndrome is an emerging diagnosis in patients with MDS or MDS-like disease with autoinflammatory manifestations of unknown origin.

P79

Blood Monocytes Deliver Iron nanoparticles to Liver in a C-C chemokine receptor type 5-dependent mechanism

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Most of the body iron is utilized for hemoglobin synthesis in erythrocytes, while macrophages and liver cells are involved in iron storage. Iron deficiency may lead to severe clinical manifestations and intravenous iron preparations, such as ferric carboxymaltose (FCM) are routinely administered to treat iron deficiency. FCM nanoparticles (NPs) are composed of a polynuclear Fe(III) oxyhydroxide core surrounded by carbohydrate ligands, stabilizing the nanoparticle.

In this study, we investigated the contribution of monocytes and liver cells in the uptake of FCM NPs injected IV in wild-type mice. Total serum iron showed a peak at 2.5h, while liver iron levels peaked 5h post FCM injection. Analysis of liver Kupffer cells (KCs) and monocytes by flow cytometry showed a decrease of the proportion of liver KCs, reaching the lowest levels 18h post FCM injection. The percentage of liver monocyte increased over time following FCM injection, suggesting monocytes might be recruited from blood to liver following FCM uptake. Furthermore, the percentage of $CCR5^+$ KCs was reduced within 24h post FCM administration, while the percentage of $CCR5^+$ monocytes increased. To investigate the role of CCR5 in monocyte migration, we blocked CCR5 by an inhibitor (Met RANTES). Treatment of mice with Met RANTES prevented the increase of infiltrating monocytes in the liver of FCM-injected mice, suggesting that homing of monocytes into liver is CCR5-dependent. This study revealed that blood monocytes and KCs play a central role for uptake of circulating FCM nanoparticles within 24h of administration.

P80

Novel malaria vaccines based on genetic fusion of Plasmodium falciparum circumsporozoite protein sequences into cucumber-mosaic virus-like particles

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Our aim is to develop a potent malaria vaccine providing a long lasting immune protection. For this, we rely on the proven and highly efficient $CuMV_{tt}$-platform. This Virus-like particle is derived from the cucumber mosaic virus (CuMV) and possesses a universal T helper cell epitope which is based on the tetanus toxin (tt). Mediating hepatocyte invasion, the *Plasmodium falciparum* circumsporozoite protein (*Pf*CSP) is a promising target at the pre-erythrocytic stage of the parasite. Here we screened different vaccine candidates targeting the conserved *Pf*CSP which consist of the central repeats NANP/NVDP, the junctional epitope NPDP and the internal cleavage side.

Mice were immunized with 30 µg of CuMVtt-*Pf*CSP versions using a prime boost regiment with a 2nd immunization at day 21. Serum IgG antibody titers were determined by ELISA on whole *Plasmodium berghei* (*Pb*) parasites expressing *Pf*CSP. To validate the protectivity of our vaccine candidates, immunized mice were challenged by i.v. injection of Pb/*Pf*CSP sporozoites. Parasite-load of erythrocytes was tracked under the microscope by blood smear staining.

High anti-*Pf*CSP IgG antibody titers were obtained in mice immunized with $CuMV_{tt}$ genetically fused either to 19 NANP repeats ($CuMV_{tt}$-19NANP) and to the other variants. High antibody titers correlated with a survival rate of 75% in mice immunized with $CuMV_{tt}$-19NANP, 40% in mice immunized with other variants.

Our preliminary data suggests, that antibodies induced by $CuMV_{tt}$-based vaccines are capable to neutralize *Pf* parasites, thus being interesting candidates for future vaccines.

P81

12 months follow-up of early phase Covid-19 patients with immune disorders and of members of their family. Cellular and humoral evolution

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Aims: Evaluate the lasting immune protection and effects after SARS-CoV-2 infections in healthy individuals and in patients with immune disease.

Methods: 54 convalescent COVID patients from the 1st wave epidemic and 12 COVID negative close relatives accepted to participate. 29% had an auto-immune disease, 31% allergies and 13% had immune deficiencies. Clinical evolution and blood samples were collected monthly for ≥ 12 months. Inflammatory markers and β2microglobulin [β2m] were measured by nephelometry, specific ab anti Spike1 were assayed by immunochromatography and ELISA, neutralizing ab by inhibition ELISA, lymphocyte phenotyping by flow cytometry. Lasting memory T $CD4^+$ and T $CD8^+$ and mB $CD19^+$ were determined after in vitro culture with SARS-CoV-2 recombinant proteins or self-designed peptides.

Results: Along the 12 months of the study, 95% of patients maintained significant antibodies and effective % of neutralising ab (peak: 3rd to 5th month). The persistence of this immunity was corroborated by the presence of circulating memory T $CD4^+$, $CD8^+$ and B $CD19^+$ (month 10 and 12 post COVID). Inflammatory markers returned to pre-infection values within two months after recovery. Elevated β2m, reflecting $CD8^+$ and $CD5616^+$ activation, was observed only during the first month after onset. In 12 patients it was again present upon reactivation during the 2nd wave. A decreased or low CD4/CD8 ratio was observed in all for more than 8 months before slowly returning to initial reference.

Assertion: SARS-CoV-2 immunity and pro-inflammatory profile persist much longer than postulated.

P82

Innate lymphoid cell 2 (ILC2) involvement in mastocytosis

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Aim: Mastocytosis is a heterogeneous group of disorders characterized by expansion and accumulation of mast cells in different organs. It has been recently reported that ILC2s are significantly higher in mastocytosis patients. Nonetheless, ILC2 activation and function in mastocytosis remain poorly studied. The aim of this project is to characterize ILC2-drivers and ILC2 function and phenotype to better define their involvement in mastocytosis.

Methods: Peripheral blood mononuclear cells and serum from healthy donors (HDs) and mastocytosis patients were used to analyze ILC frequencies, phenotype and cytokine production by flow cytometry and multiplexed technologies.

Results: We found that the concentration of soluble ILC2-triggering factors, including mast cell derived PGD2 and IL-33, was elevated in the serum of mastocytosis patients as compared to HDs. As previously reported, circulating ILC2 frequencies, was higher in mastocytosis patients as compared to HDs. Importantly, patients' ILC2s displayed reduced expression of the IL-9R and increased production of IL-13, IL-5, IL-4 and IL-9, a mast cell stimulating cytokine, upon ex-vivo stimulation. Exposure of expanded ILC2s from HDs to the ILC2-triggering factors identified in patients' sera recapitulates our observations obtained in patients.

Conclusions: Our findings highlight a crucial role of ILC2 in supporting the pathophysiology of mastocytosis suggesting that mastocytosis could be considered as an ILC2-triggered disease. Hence, targeting ILC2 effector functions might be exploited for the treatment of patients with mastocytosis.

P84

Effects of anti-COVID restrictive directives on pollen allergy in the Geneva aera in March-April 2020

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Circumstances: During the spring of 2020, March and April benefitted from good weather conditions and tree pollens were very abundant. At the same period, because of the rapid expansion of the SARS-CoV-2 pandemic, strict confinement and sanitary measures were enforced resulting in the cessation of all industrial and commercial activities, in the closure of the airport, car traffic reduction and partial closure of the border.

Methods: Index scores for allergic symptoms and anti-allergic medications were compiled from the Symptoms Report Notebooks of 42 patients with tree pollen allergy. They were compared with scores recorded yearly by patients since 2016. Daily pollen counts (MeteoSuisse) and the "Clinical pollen index" (RNSA network) from 2016 to 2020, sale index of anti-allergic medications and pollution measures from the OCEV, République et Canton de Genève were used for comparison and analysis.

Results: During the months of April and April 2020, there was an impressive reduction of the air pollution never observed before. Despite high pollen counts, analysis of patients' data showed a neat reduction of symptoms scores: both for frequency and severity, as well as in medication use. Global drop in sales of anti-allergic medications also corroborated these observations.

Assertion: It is generally admitted that in pollen allergy, a clear correlation exist between high clinical symptoms scores and high pollen counts. The divergence observed during the spring of 2020 bring stronger evidence that air pollution is an important co-factor in triggering pollen allergic reactions.

P85

Dynamics and functions of monocyte-derived cells at brain barriers during neuroinflammation

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Background: In multiple sclerosis (MS), circulating monocytes invade the central nervous system (CNS) trafficking through different anatomical barriers. Monocytes interact with barrier-associated cells that lead to maturation to monocyte-derived macrophages, driving disease evolution. Surprisingly however, the dynamics of this key event remain understudied.

Aim: We aimed to describe movement and functions of macrophages at the different CNS barriers, and understand how their pro- or anti-inflammatory functions are acquired.

Methods: We employed transgenic mouse models of MS expressing reporter proteins indicative of pro-inflammatory (iNOS) or anti-inflammatory (arginase-1) functions. We used intravital imaging to describe the real-time evolution of inflammation and in vitro paradigms mimicking trafficking of macrophages at brain barriers.

Results: We described the evolution of inflammatory lesions in vivo from pre-clinical stages to the remission phase, showing functional changes in macrophages at a single-cell and population level. We could show barrier-specific differences in macrophage functions and highlight the role of the choroid plexus as an immigration gateway for tissue-invading macrophages. We could also show that, depending on the dominant cytokine environment, interaction with barriers cells can prime macrophages toward a pro- or anti-inflammatory state.

Conclusions: Our work suggests novel mechanisms in the immigration pathways and phenotype acquisition by CNS-invading phagocytes during neuroinflammation.

P86

Retaining memory: Virtual memory CD8+ T cells as an adaptive mechanism of the ageing immune system

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Naïve T cell immunity is impaired during ageing, which impinges immune responses reliant on diversity, including those necessary for efficient vaccinations. As the deterioration of the naïve T cell repertoire is accompanied by an accumulation of virtual memory $CD8^+$ T cells (T_{VM} cells), we aimed to investigate the extent of their contribution to immunity in the elderly. Using $CD8^+$ T cells from P14 young and aged mice, we initially characterized that T_{VM} cells (1) still proliferate upon TCR activation, (2) rely on both glycolysis and OXPHOS and (3) retain their ability to undergo asymmetric cell division, a mechanism known to contribute to T cell memory formation. In adoptive transfer experiments, these features resulted in better survival and re-expansion upon LCMV infection in comparison to their naïve counterparts. Interestingly, the memory pool generated by T_{VM} cells consisted mostly of $KLRG1^+$ $CD127^+$ cells, in contrast to the typical $KLRG1^-$ $CD127^+$ memory derived from naïve T cells. Thus, we compared the transcriptional profile of these two distinct memory populations by RNAseq and observed a unique mixed effector-memory signature in T_{VM}-derived memory cells. Of relevance, these cells were able to survive in absence of antigen and build new memory responses upon antigenic re-challenge. Our results suggest that T_{VM} cells are metabolically adapted to swiftly respond to environmental changes, and might represent an adaptation of the ageing immune system to maintain a memory-like pool of cells in absence of previous antigen encounter, while not compromising effector responses.

P87

mTOR signaling mediates ILC3-driven immunopathology

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Innate lymphoid cells (ILCs) have a protective immune function at mucosal tissues but can also contribute to immunopathology. Previous work has shown that the serine/threonine kinase mammalian target of rapamycin complex 1 (mTORC1) is involved in generating protective ILC3 cytokine responses during bacterial infection. However, whether mTORC1 also regulates IFN-γ-mediated immunopathology has not been investigated. In addition, the role of mTORC2 in ILC3s is unknown. Using mice specifically defective for either mTORC1 or mTORC2 in ILC3s, we show that both mTOR complexes regulate maintenance of ILC3s at steady state and pathological immune response during colitis. mTORC1 and to a lesser extend mTORC2 promote proliferation of ILC3s in the small intestine. Upon activation, intestinal ILC3s produce less IFN-γ in the absence of mTOR signaling. During colitis, loss of both mTOR complexes in colonic ILC3s results in reduced production of inflammatory mediators, recruitment of neutrophils and immunopathology. Similarly, treatment with rapamycin after colitis induction ameliorates the disease. Collectively, our data show a critical role for both mTOR complexes in controlling ILC3 cell numbers and ILC3-driven inflammation in the intestine.

P89

Patterns of Immune Dysregulation in Primary Immunodeficiencies: A Systematic Review

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Aim: Immune dysregulation is as important as susceptibility to infection in defining primary immunodeficiencies (PIDs). Diagnosis of PIDs can be delayed—especially if a patient presents with immune dysregulation. So far there is no large report linking patterns of immune dysregulations to the underlying genetic defects. The objective of this work is to identify immune dysregulatory patterns associated with PIDs and to help clinicians to detect an underlying PID in certain patients with noninfectious inflammatory diseases.

Method: A systematic literature review was performed.

Results: We included 186 articles that reported on n = 745 patients. The most common immune dysregulation category was "autoimmunity" (62%, n = 463), followed by "intestinal disease" (38%, n = 283) and "lymphoproliferation" (36%, n = 268). Most patients (67%) had 1 or more symptoms of immune dysregulation. Autoimmune hemolytic anemia, the most common autoimmune phenotype, was most frequently reported in patients with LPS responsive beige-like anchor protein deficiency (when combined with hypogammaglobulinemia or gastrointestinal symptoms), activation-induced cytidine deaminase deficiency (when combined with autoimmune hepatitis), or RAG1 deficiency (when it was the only symptom of immune dysregulation). Eczema, allergies, and asthma were reported in 34%, 4%, and 4% of the patients, respectively.

Conclusion: Patterns of immune dysregulation may help the physician to recognize specific PIDs. This systematic review provides clinicians with an overview to better assess patients with immune dysregulation.

SYMPOSIUM 1A: BASIC IMMUNOLOGY – AUTOIMMUNITY

OP3

SLAMF7 and CD38 as Possible New Therapeutic Targets on NK Cells for Systemic Lupus Erythematosus

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Introduction: Systemic lupus erythematosus (SLE) is an autoimmune disease of unknown etiology and poorly understood pathophysiology. Natural killer (NK) cells are decreased and dysfunctional in SLE. Here, phenotypic, functional and immunometabolic alterations of SLE NK cells were examined to identify novel therapeutic targets.

Methods: Phenotype and function of cryopreserved NK cell from SLE patients and matched healthy controls (HC), were analyzed by single cell mass cytometry, flow cytometry and by Seahorse XFe96 Analyzer.

Results: SLE NK cells exhibited impaired cytokine production, degranulation, cellular glycolysis and mitochondrial metabolism. Single cell mass cytometry showed that SLE NK cells displayed increased expression of CD38 and altered upregulation of SLAMF7 following activation compared to HC. Engagement of SLAMF7 and CD38 with monoclonal antibodies (mAb) restored NK function and promoted the killing of circulating plasma cells (cPC) in SLE and HC *in vitro*. Mechanically, we showed that anti-SLAMF7 primarily promotes mitochondrial respiration while anti-CD38 enhances glycolysis in NK cells.

Conclusion: SLAMF7 and CD38 are aberrantly expressed or regulated on SLE NK cells and using mAb against these receptors promotes NK cells' mitochondrial metabolism and glycolysis, respectively. In addition, ligation with these mAbs enhances NK cell function (cytokine production, degranulation), thus promoting the killing of cPC and reducing the production of autoantibodies.

Targeting SLAMF7 or CD38 with mAbs could represent future therapeutic targets in patients with SLE.

OP4

Characterization of autoreactive T cells in Guillain-Barré syndrome.

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Guillain-Barré syndrome (GBS) is considered an autoimmune disorder of the peripheral nervous system (PNS) in which the contribution of pathogenic autoreactive T lymphocytes targeting PNS antigens has been strongly supported by *in vivo* studies. However, the underlying immune-mediated mechanisms in humans are far from clear. The overall aim of this study is to gain insights into this issue by investigating the existence and providing an in-depth characterization of the autoreactive T cell response in GBS patients during the acute and recovery phases of the disease. Flow cytometry analysis of *ex-vivo* PBMCs revealed increased frequencies in effector memory and TEMRA subsets among $CD4^+$ and $CD8^+$ T cells in GBS patients, thus pointing to an involvement of T cells in the disease. Notably, by using a recently established sensitive workflow based on *ex vivo* T cell screenings, generation of single T cell clones and TCR sequencing, here we reveal the existence of self-reactive T cells in GBS patients. Memory $CD4^+$ T cells targeting self-antigens of the PNS were detected in all GBS patients analyzed so far, whereas they resulted almost absent in healthy controls. Moreover, by analyzing more than 250 autoreactive T cell clones, we found that these cells show a polyclonal TCR repertoire, target multiple epitopes of the self-antigens with some immunodominant regions and are mostly HLA-DR restricted. Collectively, our data provide the first description of self-reactive T cells directed against PNS myelin proteins in GBS patients, thus opening new perspective for biomedical application.

SYMPOSIUM 1B: CLINICAL IMMUNOLOGY – ALLERGY

OP14

Cytokine dominance in delayed drug hypersensitivity correlates with the clinical picture

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Delayed drug hypersensitivities (DHRs) occur days to weeks after drug intake and manifest mostly as cutaneous exanthem reactions such as maculopapular drug eruption (MPE), although rarely, life-threatening reactions like drug rash with eosinophilia & systemic symptoms (DRESS), Stevens-Johnson syndrome (SJS) or toxic epidermal necrolysis (TEN) may occur. Since drug-induced exanthema can be very heterogenous, it may be difficult to distinguish severe MPE by confluent erythema and slightly elevated liver enzymes from DRESS, therefore additional laboratory parameters may support diagnosis. We aimed to define if the cytokine profiles for these distinct clinical manifestations differed using an *in vitro* lymphocyte activation test (Cyto-LTT) by the culprit drug. Patient cohorts were recruited from two experienced allergologists and grouped by clinical features for MPE (n = 13), DRESS (n = 8) or SJS/TEN (n = 6). We analysed the concentrations of 5 cytokines covering either Th2 dominant (IL-5/IL-13) or Th1 dominant (IFNγ, GranzymeB (GzB) & Granulysin (GL)) reactions after 7 days of culture. Cytokine secretions were highest in DRESS patients due to their systemic symptoms, with stimulation indexes (SI) >10 for all cytokines versus untreated cultures. Although both Th1/2 cytokines are present in all the studied delayed DHRs, there is a dominance of IL-5 for DRESS, IFNγ/GzB for MPE, and GzB for SJS/TEN. In conclusion, the cytokine concentrations and dominance provided by Cyto-LTT could help clinicians identify the type of DHR in ambiguous situations to better guide their medical advice.

OP16

Ligelizumab as add-on therapy for patients with anti-H1-refractory CSU: Primary results of a placebo- and active-controlled phase 2b dose-finding study

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Introduction: This phase 2b study examined the efficacy and safety of ligelizumab in patients with CSU inadequately controlled with H1-antihistamines (H1-AH) alone or in combination with H2-AH and/or leukotriene receptor antagonists.

Method: Patients with moderate to severe CSU (Urticaria Activity Score [UAS7]≥16) were randomized to receive s.c. ligelizumab 24, 72 or 240mg, omalizumab 300mg, or placebo every 4 weeks (q4w) over 20 weeks, or a single dose of ligelizumab 120mg. The primary endpoint was complete hives response (Hives Severity Score [HSS7] = 0) at Wk12. Other endpoints assessed were the percentage of patients with UAS7 = 0 and Dermatology Life Quality Index (DLQI) = 0–1 at Wks 4, 12 and 20.

Results: The primary objective of the study was achieved, with ligelizumab demonstrating a dose-response relationship with respect to HSS7 = 0 at Wk12 ($p<0.001$). HSS7 = 0 response rates at Wk12 were 30%, 51%, and 42% for ligelizumab 24, 72, and 240mg, respectively, vs. 26% for omalizumab, and 0% for PBO. These responses were maintained up to Wk20. High UAS7 = 0 and DLQI = 0–1 response rates were observed as early as Wk4; more patients were symptom-free (UAS7 = 0) and reported marked improvement of their quality of life (DLQI = 0–1) with ligelizumab 72 and 240mg vs omalizumab. Ligelizumab was well-tolerated and the safety profile was comparable with that of omalizumab.

Conclusion: In patients with moderate to severe CSU, ligelizumab exhibited a clear dose response in HSS7 = 0 at Wk12. Compared with omalizumab 300mg, ligelizumab achieved higher efficacy across multiple endpoints and showed comparable safety.

SYMPOSIUM 1C: LABORATORY DIAGNOSTICS

OP9

Quantification of IL-1β with Electrochemical Biosensors by Electrochemical Impedance Spectroscopy (EIS), using Screen Printed Electrodes (SPE).

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IL-1β is a key mediator of the inflammatory response. Overexpression of this cytokine or a defect in its inhibitory system may cause several chronic inflammatory conditions, such as Cryopyrin-Associated Periodic Syndromes (CAPS). These syndromes are commonly caused by gain-of-function mutations in the gene coding for pyrins or cryopyrin, an inflammasomal protein that play key roles in the activation of IL-1 β, leading to its overexpression and to a state of chronic systemic inflammation. Ther main goal of our study is to the quantify IL-1β levels in serum patient samples and to follow therapy progress in patients with these conditions. The problem is, however, that the serum concentrations of this cytokine are in the order of picogram/mL, below the detection range of most routinely used diagnostic techniques, such as Enzyme-Linked Immunosorbent Assay (ELISA).

Electrochemical biosensors have been presented as a reliable possibility to detect cytokines in the order of tens's of fg/ml. In our preliminary results, we incorporate a biorecognition element, composed by mab-anti-IL-1β and Type 2 receptor to detect IL-1β in PBS solutions. The results were obtained by electrochemical circuit fit analysis of EIS, providing a signal/concentration ratio suited to quantify this cytokine in diluted human sera. Sensitivity achieved were IL-1β concentrations of 100 fg/mL, 1000 times higher than conventional ELISA methods. Other advantages, such as low-cost fabrication, small size and portable devices may allow quick and easy to use, making this technology attractive for clinical application.

OP11

Highly specific and reliable in vitro diagnostic analysis of memory T and B lymphocytes in a Swiss cohort of Covid-19 patients

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The 2019 novel SARS-CoV-2 pandemic has claimed many lives and disrupted people's quality of life. Diagnostic tests not only confirm past exposure but offer key information to guide patients' healthcare options. Current diagnostics for SARS-CoV-2 virus presence or antibodies lack evidence of longer-lasting cellular immunity, partly due to more complex cell culture-based techniques compared to serological tests. We aimed to (1) develop an *in vitro* flow cytometric diagnostic immunoassay and (2) determine if lasting memory helper CD4 and cytotoxic CD8 T cell activations are distinct between unexposed and convalescent individuals. Peripheral blood mononuclear cells (PBMCs) isolated from whole blood of unexposed (n = 10) or convalescent (n = 30) individuals were cultured for 5 days with individual SARS-CoV-2 recombinant proteins or self-designed peptides including the spike, membrane and nucleocapsid proteins. Specific activation of memory T cells and B cells against SARS-CoV-2 antigens were determined by the upregulation of activation markers (CD4 T cells: $CD134^{+}$ $CD137^{+}$; CD8 T cells: $CD69^{+}$ $CD137^{+}$; CD19 B cells: $CD38^{+}$). We show both activated T cells and B cells are detectable against all tested antigens, and distinguishable between unexposed and convalescent individuals up to 11 months post-infection. Therefore, with this diagnostic tool, we propose that it would benefit immunocompromised individuals who are unable to mount sustained antibody responses, and to study immune correlates of protection, thus enhancing knowledge of the Covid19 disease in a wider range of patient groups.

SYMPOSIUM 2A: BASIC IMMUNOLOGY – B CELL RESPONSES

OP10

Mild COVID-19 elicits early functional plasmablasts and long-term memory B cells while sustaining neutralizing antibody titers

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Aim: Evaluate the kinetics of antibody, plasmablasts (PB) and memory B cells (MBCs) from day 7 up to 8 months following mild COVID-19.

Methods: This observational study enrolled 31 RT-PCR confirmed acute mild COVID-19 patients longitudinal followed ups at six visits: 0, +7, +14, +28, +56 and +200 days post onset of symptoms (dpos). Antibodies against S1 domain of the spike and N (nucleocapside) proteins of SARS-CoV-2 were evaluated using ELISA, while neutralization was quantified by a commercially available surrogate (sVNT) assay. Specific PB and MBC were assessed by ELISPOT.

Results: During mild COVID-19, anti-S1, anti-N as well as neutralizing antibodies were elicited during the first 3 weeks pos, reached a peak between 20-30 dpos and decayed slowly, however 80% of patients had detectable neutralizing antibodies at +200 dpos. S1-specific IgA and IgM PB reached their peak around +14 days while IgG slow increased. All patients developed anti-S1 IgG MBCs by one month that peaked at 49 dpos and remained stable up to 245dpos; anti-N IgG MBCs kinetics was similar but their magnitude was reduced. We next correlated humoral with cellular immune responses and found that anti-S1 IgG and IgA titers at visits +14 and +28 days correlated with plasmablast, while there was a poor correlation between antibody titers and MBCs at visit +56 days that was lost at visit +200 days.

Conclusion: Mild COVID-19 elicits early and long lasting neutralizing antibodies. Antigen-specific PB correlated with early antibody titers, while specific MBCs frequencies were stable and independent of antibody titers up to 245 dpos.

OP23

Low-affinity but high-avidity interactions may offer an explanation for allergen-cross-reactivity

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Aim: Allergy is global disease with overallfrequencies of >20%. Symptoms vary from irritating local itching to life-threatening systemic anaphylaxis. Even though allergies are allergen-specific, there is a wide range of cross-reactivities (e.g. apple and latex) that remain largely unexplained. Given the abilities of low affinity IgG antibodies to inhibit mast cells activation, here we elucidate the minimal affinity of IgE antibodies to induce type I hypersensitivity.

Methods: Three mature (high affinity) IgE antibodies recognizing three distinct epitopes on Fel d 1, the major cat allergen, were back-mutated to germline conformation, which bind to Fel d 1 with low affinity. The ability of these IgE antibodies to activate mast cells *in vitro*and *in vivo*were tested.

Results: We demonstrate that affinities as low as 10^{-7}M are sufficient to activate mast cells *in vitro*and drive allergic reactions *in vivo*. Low affinity IgE antibodies are able to do so, since they bind allergens bivalently on the surface of mast cells, leading to high-avidity binding.

Conclusions: These results suggest that the underlying mechanism of allergen-cross-reactivity may be low-affinity but high-avidity binding between IgE antibodies and cross-reactive allergen.

SYMPOSIUM 2B: CLINICAL IMMUNOLOGY – INFLAMMATION

OP17

Investigation of the PI3K-dependent inflammation in chronic inflammatory pulmonary disease

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Aim: Airway inflammatory diseases like asthma and chronic obstructive pulmonary disease (COPD) are common disorders of increasing prevalence, for which no curative therapies currently exist. The phosphoinositide-3 kinase (PI3K) is often hyperactivated in these chronic lung inflammatory disorders (CLID), and it is therefore a candidate therapeutic target.

Methods: As a model of CLID, we used mice with a genetic deficiency in a phosphatase regulating the PI3K pathway, which develop a spontaneous lung inflammation. To validate our results from the mouse model, we used frozen or fresh lung samples from COPD patients. We performed flow cytometry, transcriptomic analysis and cell transfer studies to study lung-infiltrating immune populations.

Results: In the mouse model, we found that genetic ablation of IL-33 signaling or of lymphoid immune cells prevented lung disease development in animals with hyperactivated PI3K pathway. However, lung infiltrations were re-established upon intranasal transfer of a specific lymphocyte population into CLID-rescued mice. Lack of PI3K pathway-regulating phosphatase correlated with a hyperactivated phenotype of specific lymphocytes populations. In humans, expression of the PI3K pathway-regulating phosphatase was inversely correlated to COPD progression.

Conclusion: By identifying the central role of a specific inflammatory pathway and a particular immune cell type for PI3K-dependent chronic lung inflammatory, our study underpins new potential targets for asthma or COPD.

OP22

Alveolar macrophages strictly rely on GM-CSF from alveolar epithelial type 2 cells after birth and throughout adulthood

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Aims. Programs defining tissue-resident alveolar macrophage (AM) identity depend on local environmental cues. GM-CSF (CSF2) is a crucial niche signal for the development of AMs, which are absent in mice deficient in that signaling axis. However, evidence to functionally link components of the intercellular crosstalk between AM precursors and their niche remains scarce.

Methods & Results. Using an in-house created GM-CSF reporter mouse (*Csf2*$^{fl\text{-}tdTom}$), we profiled pulmonary GM-CSF expression, which we detected in both immune cells, including group 2 innate lymphoid cells and γδ T cells, as well as alveolar epithelial cells type 2 (AT2s). AT2 lineage-specific constitutive (*SPC*^{Cre};*Csf2*$^{fl\text{-}tdTom}$) and tamoxifen-inducible (*SPC*$^{Cre\text{-}ERt2}$;*Csf2*$^{fl\text{-}tdTom}$) *Csf2* deletion revealed the non-redundant function of AT2-derived GM-CSF in establishing the postnatal AM compartment and maintaining AMs in adult lungs.

Conclusion. Our results unequivocally demonstrate that AMs are critically reliant not on hematopoietic GM-CSF but rather on epithelial GM-CSF derived from AT2s, both for development and for maintenance of the mature AM population.

SYMPOSIUM 2C: CLINICAL IMMUNOLOGY – IMMUNE REGULATION

OP5

c-Maf expression induces memory-like features in mouse and human ILC2 enforcing their type 2 functional identity

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Group 2 innate lymphoid cells (ILC2s) are involved in type 2 inflammatory diseases such as allergy. In response to repeated allergen inhalations, lung epithelial cells secrete innate cytokines that activate ILC2s to release interleukin-5 (IL-5) and interleukin-13 (IL-13) resulting in eosinophil infiltration, mucus hyper-production, and airway hyperreactivity. Beside GATA3, very little is known about the role of specific transcription factors in the regulation of ILC2 functions. Here we identify c-Maf as an activation-induced, crucial regulator of ILC2 function and identity. Both mouse and human c-Maf deficient ILC2s are impaired in IL-5 and IL-13 production. Transcriptomic analysis revealed not only a reduced expression of type 2 associated genes in the absence of c-Maf, but also of genes involved in innate memory formation. Indeed, mouse and human c-Maf deficient ILC2s failed to respond more vigorously upon re-challenge. Our results indicate that c-Maf is a core node that connects immature to primed/trained ILC2s.

OP7

CD85k Contributes to Regulatory T Cell Function in Chronic Viral Infections

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Regulatory T cells (Tregs) prevent excessive immune responses and limit immune pathology upon infections. To fulfill this role in different immune environments elicited by different types of pathogens, Tregs undergo functional specialization into distinct subsets. During acute type 1 immune responses, type 1 Tregs are induced and recruited to the site of ongoing Th1 responses to efficiently control Th1 responses. However, whether a similar specialization process also takes place following chronic infections is still unknown. In this study, we investigated Treg specialization in persistent viral infections using lymphocytic choriomeningitis virus (LCMV) and murine cytomegalovirus (MCMV) infection as models for chronic and latent infections, respectively. We identify CD85k as a Th1-specific co-inhibitory receptor with sustained expression in persistent viral infections and show that recombinant CD85k inhibits LCMV-specific effector T cells. Furthermore, expression of the CD85k ligand ALCAM is induced on LCMV-specific and exhausted T cells during chronic LCMV infection. Finally, we demonstrate that type 1 Tregs arising during chronic LCMV infection suppress Th1 effector cells in an ALCAM-dependent manner. These results extend the current knowledge of Treg specialization from acute to persistent viral infections and reveal an important functional role of CD85k in Treg-mediated suppression of type 1 immunity.

SYMPOSIUM 3A: BASIC IMMUNOLOGY – TUMOR IMMUNOLOGY

OP2

HLA-DRB1*04 associated chronic inflammation and extracellular matrix-specific autoimmunity following inadvertent periarticular influenza vaccination

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Aim: Inadvertent vaccine injection into periarticular shoulder tissue can cause inflammatory tissue damage ('shoulder injury related to vaccine administration, SIRVA). Thus, this accident provides a human model to study if vaccine induced pathogen-specific immunity accompanied by an inflammatory insult may trigger autoimmunity.

Methods: We studied 16 otherwise healthy adults with suspected SIRVA following a single influenza immunization campaign. We performed ultrasound, immunophenotypic analyses, HLA-typing, and antigen-specific immunoassays. Vaccine-related bone-toxicity and T cell/osteoclast interactions were assessed *in vitro*.

Results: In 12/16 we found inflammatory tissue damage on imaging, including bone erosions in 6. Tissue damage was associated with a strong peripheral blood T and B cell activation and extracellular matrix-reactive autoantibodies (autoantigen microarray). Subjects with erosions were HLA-DRB1*04 positive and showed extracellular matrix-reactive HLA-DRB1*04 restricted T cell responses targeting heparan sulfate proteoglycan. Antigen-specific T cells potently activated osteoclasts via RANK/RANK-L, and the osteoclast activation marker Trap5b was high in sera of patients with an erosive shoulder injury. *In vitro*, the vaccine component alpha-tocopheryl succinate recapitulated bone toxicity.

Conclusion: Vaccine misapplication, genetic predisposition, and vaccine components contribute to the inflammatory mechanism of SIRVA. Despite autoreactive B and T cell responses, SIRVA was not associated with progression to autoimmune disease during follow-up.

OP24

PD-L1 Incorporation within HIV Virions Contribute to Functionally Impair T-Follicular Helper Cells

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Background: Lymph node germinal center (GC) T follicular helper (Tfh) cells are functionally impaired during HIV infection despite the relatively low level of immune checkpoint ligand (IC-L) expression in GC area. We therefore hypothesized that the source of IC-Ls interfering with Tfh cell functionality might be dependent on membrane-bound extracellular vesicles such as exosomes and/or HIV virions.

Results: We showed that HIV virions represent the major source (>70%) of PD-L1$^+$ extracellular vesicles as compared to exosomes in plasma of viremic HIV-infected individuals (P<0.01), which translated into a significant increase of soluble plasmatic PD-L1 levels (P<0.01). Furthermore, PD-L1 incorporation within plasmatic HIV virions was more frequently detected than HLA-DR (PD-L1 = 37.5%; HLA-DR = 23.3%; P<0.01), demonstrating the preponderance of this phenomenon *in vivo*. Since HIV virions can circulate throughout the body *via* the blood and lymphatic circulation and accumulate at specific sites such as the follicular dendritic cell network, we therefore assessed the potential impact of PD-L1$^+$ virions on CD8 T cell and Tfh cell functions *in vitro*. We showed that *in vitro* produced PD-L1$^+$ HIV virions, but not PD-L1$^-$ HIV virions, significantly reduced CD8 T cell and Tfh cell proliferation (P<0.05), which was fully restored in presence of anti-PD-L1/2 blocking MAb treatment.

Conclusion: Taken together, the present study unveils a mechanism by which HIV specifically exploits the regulatory potential of IC-Ls to systemically suppress the immune system during the course of HIV infection.

SYMPOSIUM 3B: CLINICAL IMMUNOLOGY – LINDENMANN SYMPOSIUM FOR INNATE IMMUNITY

OP8

Autophagy proteins in the restriction of Kaposi's Sarcoma-Associated Herpesvirus Entry

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Autophagy is an evolutionarily conserved cellular degradation process. When microorganisms enter the cytosol, defense mechanisms are triggered that use autophagy cargo receptors to direct the pathogens via xenophagy to lysosomal degradation. One such pathogen and the model organism in this study is Kaposi's sarcoma-associated herpesvirus (KSHV), which is a human oncogenic γ-herpesvirus associated with several malignancies. Numerous studies demonstrate the antiviral role autophagy plays upon lytic reactivation of KSHV, however little is known about the role of autophagy upon KSHV's entry into host cells. Using U-2 OS human epithelial cells, we show that the lipidation of microtubule-associated protein light chain 3 (LC3) is induced upon KSHV entry. We show that several autophagy-related proteins (ATG) are recruited to endocytosed KSHV particles, as well as autophagy receptors p62 and nuclear dot protein 52 kDa (NDP52). We also show that when autophagy is chemically inhibited at the time of KSHV entry, KSHV infection seems to increase, indicating that autophagy has an antiviral role on KSHV. We also observe that galectin-8 is recruited to KSHV upon entry. Thus, we hypothesize that galectin-8 may be recruited to glycans exposed on virus-damaged endosomes, and that NDP52 then binds to galectin-8, thereby recruiting the autophagy machinery to clear the damaged endosome and the pathogenic content therein.

OP18

Multisystem Inflammation and Susceptibility to Viral infections in Human ZNFX1 Deficiency

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Recognition of viral nucleic acids is one of the primary trigger for a type I interferon-mediated antiviral response. Inborn errors of type I interferon immunity can be associated with increased susceptibility to viral infections. NFX1-type zinc-finger-containing 1 (ZNFX1) is an interferon-stimulated dsRNA sensor that was recently found to restrict the replication of RNA viruses in mice. ZNFX1's role in the human immune response is not known. We studied 15 patients from 8 families with an autosomal recessive immunodeficiency characterized by severe infections by both RNA and DNA viruses and virally triggered inflammatory episodes with hemophagocytic-lymphohistiocytosis-like disease, early-onset seizures, renal and lung disease. Deleterious homozygous and compound heterozygous ZNFX1 mutations were identified in all 13 analyzed patients. Stimulation of patient-derived cells with synthetic ds nucleic acids was associated with a deregulated pattern of expression of interferon-stimulated genes (ISGs), linked to changes in the half-life of ISGs mRNA, resulting in poorer clearance of virus infections. Therefore, ZNFX1 has an important regulatory role in responses to ds nucleic acids stimuli following viral infections. ZNFX1 deficiency predisposes to severe viral infections and multisystem inflammatory diseases. Therefore, clinicians should screen for biallelic ZNFX1-mutations in patients with severe viral infections and signs of virally triggered hemophagocytic-lymphohistiocytosis-like disease with hepatitis, encephalopathy, interstitial lung disease, and/or microangiopathy.

SYMPOSIUM 3C: CLINICAL IMMUNOLOGY – NOVEL BIOLOGICS IN IMMUNOTHERAPY

OP1

Development of a compartment locked IL-12 version with increased tissue retention and minimal peripheral exposure for local glioblastoma therapy

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Recent clinical studies in glioblastoma (GBM) confirmed the potency of local IL-12 therapy, but also brought back safety concerns of systemic toxicity. Fusion with an IgG4 Fc region increases the tissue retention of IL-12; but could also confer export into the blood and subsequent systemic recycling through the neonatal Fc receptor (FcRn), leading to potentially toxic IL-12 serum levels.

We assessed the expression of FcRn in human and murine GBM and its role in IL-12Fc tissue retention and systemic exposure upon local delivery. Human or murine IL-12Fc was injected in GBM-bearing or naïve wt or FcRn-humanized mice continuously or as bolus via convection-enhanced delivery (CED). We screened combinations of amino-acid substitutions at the (IL-12)Fc: FcRn binding interface to abolish interaction. Brain and blood concentrations were assessed via ELISA or cytokine bead arrays. FcRn affinity was measured by SPR/ELISA and bioactivity tested on PBMCs and human GBM explant cultures.

FcRn is upregulated in human and mouse GBM and contributes to brain export and peripheral recycling of IL-12Fc in the blood. IL-12Fc with abrogated FcRn binding is fully functional and appears brain compartment locked (CL) as it exhibits enhanced tissue retention and reduced serum levels upon local injection. Compared to non-modified IL-12(Fc), CL IL-12Fc shows significantly higher efficacy at negligible systemic footprint in late stage murine GBM.

The novel CL Fc-modifications may present a promising avenue to increase efficacy and tolerability of biologics for local therapy of CNS diseases also beyond GBM.

OP6

Anti-CD20 rituximab IgG1, IgG3 and IgG4 but not IgG2 subclass differentially trigger Ca2+ mobilization and cytotoxicity in human NK cells

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Antibody-dependent cellular cytotoxicity (ADCC) plays a key role in transplant rejection, cancer immunotherapy, and autoimmunity. CD16 (*FCGR3A*) expressed on Natural killer (NK) cells mediates this effector function. However, molecular interactions of IgG subclasses with CD16 remain elusive.

Aim: To examine the role of IgG subclasses and the *FCGR3A* V158F single nucleotide polymorphism (SNP) on Ca^{2+} signaling and NK cell-mediated ADCC against Daudi target cells induced by anti-CD20 rituximab *in vitro*.

Methods: Human NK cells were purified from donors with different *FCGR3A* V158F SNP. Calcium mobilization was analyzed with calcium 6-QF-labelled NK cells, while binding, cytokine production, and degranulation by flow cytometry, ADCC by non-radioactive TDA release assays.

Results: Induction of Ca^{2+} signaling, degranulation, intracellular cytokine production, and ADCC was observed for IgG1 and IgG3, to a lesser degree for IgG4, but not for IgG2. Compared to carriers of the low-affinity FF variant of the V158F SNP, binding of IgG1 and IgG3 to NK cells from VV and VF donors was 2 to 3-folds higher. Variations of *FCGR3A* SNP among the eight tested donors revealed no significant differences of Ca^{2+} signaling and degranulation; however, ADCC was weaker in donors with the low-affinity FF variation.

Conclusions: This is the first study correlating Ca^{2+} signaling and NK cell-mediated ADCC triggered by the four IgG subclasses with the *FCGR3A* V158F SNP. Our findings indicate important differences in the interactions of IgG subclasses with CD16 but no major impact of the *FCGR3A* V158F SNP.

SYMPOSIUM 4A: BASIC IMMUNOLOGY – ZINKERNAGEL SYMPOSIUM ON ANTI-VIRAL IMMUNOLOGY

OP13

Human tissue resident memory T cells arise during Epstein Barr virus infection in a humanized mouse model

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Epstein Barr virus (EBV) contributes to up to 2% of all tumors worldwide. At the same time, more than 90% of healthy individuals carry EBV persistently without showing clinical symptoms. A major contribution to the control of viruses are immune memory cells. Specifically, memory $CD8^+$ T cells can recognize EBV-infected cells during both latent and lytic infection. However, a detailed understanding of how different memory $CD8^+$ T cells develop during EBV infection and which phenotype they acquire is still lacking.

Using humanized mice, we established an intranasal infection model with Luciferase-carrying EBV. This enables us to visualize the virus location in different tissues and relate it to the memory $CD8^+$ T cell phenotype revealed by flow cytometry and single-cell transcriptomics.

We found that EBV initially locates to the nasal-associated lymphoid tissues (NALT). The murine NALT represents the predominant lymphoid organ in the nasal cavity and is equivalent to human tonsils, where EBV can induce periodic waves of lytic replication. We discovered that only in presence of EBV in the NALT, tissue resident memory $CD8^+$ T cells (TRM cells) establish.

Since resident memory T cells are thought to react rapidly upon reencounter of the antigen and orchestrate the local immune response accordingly, we suggest that these TRM cells can control EBV upon secondary infection and local reactivation. A deeper understanding of these cells will provide valuable insights into how individuals can control persistent viral infections throughout life.

OP15

Viral Infections License CD4+ T Cells for Antigen-Independent Recruitment and Activation

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Epidemiological data suggest that previous infections can alter an individual's susceptibility to unrelated diseases. Nevertheless, the underlying mechanisms are not completely understood. One key feature of the immune system that is likely contributing to changes in susceptibility is the formation of memory. To study the potential contribution of memory CD4+ T cells to heterologous immunity, we challenged mice with a local bacterial pathogen after they had cleared an acute viral infection. We identified a subset of memory CD4+ T cells that were preferentially recruited to the site of infection and reduced the bacterial burden through IFN-γ secretion as early as three days post infection. We could confirm the antigen-independent nature of this innate-like cytokine response by utilizing TCR-transgenic memory CD4+ T cells. Besides helping the host to clear a pathogenic challenge, these virus-specific memory CD4+ T cells could also accelerate disease onset in an autoimmune model of multiple sclerosis. Taken together, we uncovered that infection-induced changes in T helper cells can license them to mount an innate-like response that alters susceptibility to heterologous diseases.

SYMPOSIUM 4B: CLINICAL IMMUNOLOGY – VACCINATION

OP20

Isocitrate dehydrogenase 2 inhibition induces memory CD8+ T cells with enhanced antitumor function

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The differentiation into effector and memory $CD8^+$ T cell subsets was shown to be associated with specific metabolic pathways to sustain their bioenergetics needs. Although T cell glucose utilization is well characterized, the reasons of enhanced glutamine usage upon T cell activation remain unclear.

We found that effector $CD8^+$ T cells, in addition to oxidizing, also reductively carboxylate glutamine, a pathway described in cancer cells to maintain rapid lipid synthesis for cell proliferation. Interfering with reductive carboxylation by genetic deletion of isocitrate dehydrogenase 2 (IDH2), the enzyme mediating this reaction in the mitochondria, led to increased T cell memory differentiation upon infection in mice. Pharmacological IDH2 inhibition during *in vitro* $CD8^+$ T cell expansion also induced memory features and strongly enhanced antitumor activity upon adoptive cell transfer (ACT) into tumor-bearing mice. Mechanistically, IDH2 inhibition caused a disequilibrium in metabolites associated with histone modifications, which increased chromatin accessibility at genes required for memory formation and function. Restoring this metabolite balance or preventing the epigenetic modifications abrogated the enhanced T cell memory differentiation and antitumor activity induced by IDH2 inhibition.

These results propose a novel strategy to promote stable memory T cell differentiation by epigenetic processes induced by metabolic reprogramming during T cell expansion and might be exploited for ACT immunotherapy against cancer.

OP21

Bedside formulation of a personalized multi-neoantigen vaccine against mammary carcinoma

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Background: Neoantigens are attractive targets for cancer immunotherapy. The current study aimed at developing an optimized personalized multi-target vaccine using short or long neoantigenic peptides utilizing virus-like particles (VLPs) as an efficient vaccine platform.

Methods: Here we identified mutations of murine mammary carcinoma cells by integrating mass spectrometry-based immunopeptidomics and whole exome sequencing. Neoantigenic peptides were synthesized and

covalently linked to virus-like nanoparticles using a Cu-free click-chemistry method for easy preparation of vaccines against mouse mammary carcinoma.

Results: As compared to short peptides, vaccination with long peptides was superior in the generation of neoantigen-specific $CD4^+$and $CD8^+$T cells which readily produced IFN-gand TNF-a. The resulting anti-tumour effect was associated with favourable immune re-polarization in the tumour microenvironment through reduction of myeloid-derived suppressor cells. Vaccination with long neoantigenic peptides also decreased post-surgical tumour recurrence and metastases, and prolonged mouse survival, despite the tumour's low mutational burden.

Conclusion: Integrating mass spectrometry-based immunopeptidomics and whole exome-sequencing is an efficient technique for identifying neoantigenic peptides. A multi-target VLP-based vaccine shows a promising anti-tumour results in an aggressive murine mammary carcinoma cell line. Future clinical application using this strategy is readily feasible and practical, as click-chemistry coupling of personalized synthetic peptides to the nanoparticles can be done at the bedside directly before injection.

SYMPOSIUM 4C: CLINICAL IMMUNOLOGY – MICROBIOTA

OP12

Epithelium-autonomous NAIP/NLRC4 prevents TNF-driven inflammatory destruction of the gut epithelial barrier in Salmonella-infected mice

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Aim: The gut epithelium is a critical protective barrier. Its NAIP/NLRC4 inflammasome senses infection by Gram-negative bacteria, including *Salmonella* Typhimurium (*S.*Tm) and promotes expulsion of infected enterocytes. During the first ~12-24h, this reduces mucosal *S.*Tm loads at the price of moderate enteropathy. It remained unknown how this NAIP/NLRC4-dependent tradeoff would develop during subsequent infection stages. We hypothesized that the prompt NAIP/NLRC4-dependent removal of infected enterocytes is crucial to avoid an overshooting pro-inflammatory response of the gut mucosa causing sever enteropathy at late stage infection.

Methods: To test this hypothesis, we orally infected full-body and tissue-specific knockout mice with *S.*Tm and assessed the impact and compartmentalization of the mucosal NAIP/NLRC4 defense over time using fluorescent microscopy.

Results: In NAIP/NLRC4-deficient mice, *S.*Tm elicited severe enteropathy within 72h, characterized by elevated mucosal TNF (>20pg/mg) production from bone-marrow-derived cells, reduced regeneration, excessive enterocyte loss, and a collapse of the epithelial barrier. TNF-depleting antibodies prevented this destructive pathology. In hosts proficient for epithelial NAIP/NLRC4, a heterogeneous enterocyte death response with both apoptotic and pyroptotic features kept *S.*Tm loads persistently in check, thereby preventing this dire outcome altogether.

Conclusions: Our results demonstrate that immediate and selective removal of infected enterocytes, by locally acting epithelium-autonomous NAIP/NLRC4, is required to avoid a TNF-driven inflammatory hyper-reaction that otherwise destroys the epithelial barrier.

OP19

Aryl hydrocarbon receptor (AhR) signaling in the host response directed against the skin commensal yeast Malassezia during health and disease

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The mammalian skin is densely populated with microbes. The lipophilic yeast *Malassezia* is by far the most abundant member of the skin mycobiome, representing over 90% of all commensal skin fungi. Our understanding of the interaction between *Malassezia* and the host remains incomplete, both in the healthy and in the diseased skin. We found that *Malassezia furfur*, a species metabolizing tryptophan into indoles, activates the aryl hydrocarbon receptor (AhR) in human keratinocytes. Importantly, AhR induction and cytokine production correlated with indole production in *M. furfur* strains isolated from seborrheic dermatitis patients in comparison to strains from healthy donors, which do or do not produce indoles, respectively, suggesting a role of indole production and AhR signaling in disease. The relevance of AhR signaling in the fungus-host interplay was further confirmed in an *in vivo* experimental setting. Association of mouse ear skin with indole-producing *M. furfur* revealed that AhR signaling affects fungal control, neutrophile recruitment and induction of the antifungal T cell response in the host. These results lay the foundation for establishing the mechanism by which specific *Malassezia* determinants affect antifungal immunity, with implications for therapeutical approaches targeting AhR signaling or the tryptophan metabolic pathway in *Malassezia*-associated disorders.

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