swiss medical weekly

A diamond open access online journal | established in 1871 |
published by the SMW supporting association | www.smw.ch

Supplementum 270
ad Swiss Med Wkly 2023;153
August 21, 2023

Abstracts of the Annual congress of the Swiss Society for Allergology and Immunology

Bern (Switzerland), August 24–25, 2023

ANNUAL CONGRESS OF THE
SWISS SOCIETY OF ALLERGOLOGY AND IMMUNOLOGY

TABLE OF CONTENTS

SHORT COMMUNICATION SESSION – BASIC IMMUNOLOGY I

FC 1

Heme-mediated reprogramming on guiding CD8+ T cell exhaustion in response to mitochondrial dysfunction

Y. Xu[1], J. J. Peng[1], Y. M. Chuang[1], M. Akrami[2], A. Zippelius[2], Z. Xiao[3], R. Roychoudhuri[4], P. C. Ho[1]

1 Epalinges; 2 Basel; 3 Xi'an CN; 4 Cambridge GB

Aims: Impaired mitophagy leads to dysfunctional mitochondria accumulation and tumor-infiltrating T lymphocytes (TILs) exhaustion. However, the mechanisms connecting the accumulation of dysfunctional mitochondria and T cell exhaustion remain poorly understood. We speculate that cells employ alternative strategies to remove depolarized mitochondria, potentially inducing $CD8^+$ T cell exhaustion.

Methods: 1) a degron-mCherry reporter and a regulatory heme (RH) detection assay were developed to examine protein degradation. 2) the correlation between RH and $CD8^+$ T cell differentiation was investigated by detecting RH concentration and BACH2 and BLIMP1 expression in exhausted $CD8^+$ T cells. 3) hemin, a heme mimic, was used to assess the effect on $CD8^+$ T cell exhaustion. 4) the heme-binding site mutated BACH2 was utilized to explore the mechanism of heme-induced exhaustion. 5) PGRMC2, responsible for heme import into the nucleus, was knocked out to verify the impact on TILs' exhaustion.

Results: $CD8^+$ T cells with dysfunctional mitochondria engage mitochondrial protein degradation, leading to increased RH concentration. RH correlates with TILs differentiation, and exogenous heme directly induces the BACH2-BLIMP1 transcriptional networks, which is essential for commitment of T cell exhaustion. Targeting PGRMC2 to reduce RH nuclear distribution ameliorates $CD8^+$ T cell exhaustion and enhancing mitochondrial fitness.

Conclusions: Heme-mediated reprogramming guides $CD8^+$ T cell exhaustion in response to mitochondrial dysfunction. Manipulating heme signaling can be exploited to restore mitochondrial fitness and tailor T cell-based cellular therapies.

FC 2

Immunoproteasome inhibition attenuates experimental psoriasis

M. Basler[1], M. Del Rio Oliva[2], M. Mellett[3]

1 Kreuzlingen; 2 Konstanz DE; 3 Zürich

Aim: Psoriasis is an autoimmune skin disease, which is driven by the IL-23/IL-17A axis. The immunoproteasome is a special form of the 26S proteasome in which the standard catalytically active β-subunits (β1c, β2c, and β5c) are replaced by low molecular mass polypeptide (LMP)2 (β1i), multicatalytic endopeptidase complex-like (MECL)-1 (β2i) and LMP7 (β5i). It has been shown that Immunoproteasome inhibition is a promising strategy in reducing IL-23 secretion and suppressing Th17 cell development. Therefore, the therapeutic potential of immunoproteasome inhibition in psoriasis pathogenesis was assessed.

Methods: The therapeutic use of ONX 0914, a selective inhibitor of the immunoproteasome, was investigated in *Card14ΔE138*$^{+/-}$ mice, which spontaneously develop psoriasis-like symptoms, and in the imiquimod murine model.

Results: In both models, treatment with ONX 0914 significantly reduced skin thickness, inflammation scores, and pathological lesions in the analyzed skin tissue. Furthermore, immunoproteasome inhibition normalized the expression of several pro-inflammatory genes in the ear and significantly reduced the inflammatory infiltrate, accompanied by a significant alteration in the $\alpha\beta^+$ and $\gamma\delta^+$ T cell subsets.

Conclusions: ONX 0914 ameliorated psoriasis-like symptoms in two different murine psoriasis models, which supports the use of immunoproteasome inhibitors as a therapeutic treatment in psoriasis.

FC 3

NFAT5 induction by the tumor microenvironment enforces CD8 T cell exhaustion

G. Verdeil[1], L. Tillé[1], D. Cropp[1], M. Charmoy[1], P. Reichenbach[1], M. Andreatta[2], T. Wyss[2], I. Crespo[2], J. Lourenco[2], M. M. Leblond[1], C. Lopez-Rodriguez[3], D. E. Speiser[1], G. Coukos[2], M. Irving[1], S. Carmona[2], W. Held[1]

1 Epalinges; 2 Lausanne; 3 Barcelona ES

Persistent exposure to antigen during chronic infection or cancer renders T cells dysfunctional. The molecular mechanisms regulating this state of exhaustion are thought to be common in infection and cancer, despite obvious differences in their microenvironments. We discovered that NFAT5, an NFAT family member lacking an AP-1 docking site, is highly expressed in exhausted T cells responding to chronic infection and tumors but is a central player selectively in tumor-induced exhaustion. While NFAT5 overexpression in CD8+ T cells reduced tumor control, NFAT5 deletion improved tumor control by promoting the accumulation of tumor-specific CD8 T cells that expressed less TOX and PD-1 and produced more cytokines specifically among precursor exhausted cells. Conversely, NFAT5 did not promote T cell exhaustion during chronic infection. While NFAT5 expression was induced by TCR triggering, its transcriptional activity was specific to the tumor microenvironment and required hyperosmolarity. NFAT5 thus promotes CD8 T cell exhaustion in a tumor-selective fashion.

FC 4

T helper 1 cell differentiation and activation, a multi-omics approach

J. Koch[1], P. Westermann[1], A. Ruggia[1], C. Beha[1], M. Imeri[2], S. Schmelzer[1], K. Froehlich[3], A. Heider[1], C. Scheckel[2], K. Baerenfaller[1]

1 Davos; 2 Zurich; 3 Basel

The activation and differentiation of naïve T cells into T helper (Th) cells are precisely controlled processes. The translation of peptides from short open reading frames (sORFs) on RNAs that are annotated as non-protein coding (ncRNAs) can impact cells in multiple ways. Our aim is to elucidate the role of short ORF-encoded polypeptides (SEPs) during Th1 cell differentiation and activation and to determine the effect of translational regulation.

We are using ribosome profiling and RNA sequencing of human *ex vivo* differentiated Th1 cells compared to unstimulated naïve T cells to detect so far unknown translation events, focusing on SEPs, and estimate translation efficiencies of RNAs during differentiation and after activation. Mass spectrometry (MS) based methods are then used to confirm our results on the protein level.

Ribosome footprints, an indicator of translation, could be detected in known coding sequences as well as on ncRNAs. Resulting promising SEPs were validated by MS. Additionally, the analysis of differential translation efficiencies between Th1 and

unstimulated naïve T cells resulted in the identification of transcripts that were regulated in translation, and led us to further investigate the prenylation pathway.

The multi-omics approach presented here promises to provide valuable insights into regulatory processes in Th cell differentiation and activation. Identifying differentially expressed proteins or novel peptides, and additional mechanisms of post-transcriptional gene regulation could provide suitable targets for the treatment of immune diseases.

FC 5

Identification of carbonic anhydrase as a critical regulator of skin inflammation and barrier integrity in atopic dermatitis

Y. Mitamura[1], N. Serhan[2], P. D'avino[1], M. Li[1], N. Gaudenzio[2], C. A. Akdis[1]

1 Davos; 2 Toulouse FR

Atopic dermatitis (AD) is a chronic skin disease characterized by type 2 inflammation. Carbonic anhydrase (CA) an enzyme that regulates tissue acid balance is increased in keratinocytes in AD. We aimed to investigate how increasing CA enzyme affects type 2 inflammation and barrier function in AD skin. Bulk, single-cell, and spatial RNA-seq of lesional and non-lesional skin of AD patients were analysed. In addition, the expression of CA enzymes and barrier-related molecules were investigated in reconstructed epidermis (Episkin®) and *ex-vivo* human skin (NativeSkin®) treated with IL-4, IL-13, and IL-22. Their influence on epithelial barrier integrity was determined by electrical impedance spectroscopy. The effect of CA2 inhibitor treatment was investigated in MC903-induced AD-like model in mice and in *ex-vivo* samples. CA2 is increased in keratinocytes and correlate with pro-inflammatory genes in AD patients, type 2 cytokine-stimulated- Episkin and -NativeSkin. IL-4, IL-13, and IL-22 induced barrier impairment in NativeSkin and the inhibition of CA2 partially recovered the downregulation of electric impedance induced by cytokines. In line with these findings, in vivo inhibition of CA2 significantly improved AD-associated pathological features in mice. As a conclusion, we demonstrate that type 2 cytokines are potent enhancers of CA2 expression across species and specific inhibition of CA2 can suppress allergic skin inflammation. Our results thus identify the enzyme CA2 as a promising new druggable target in AD, and perhaps other skin inflammatory conditions.

SHORT COMMUNICATION SESSION – BASIC IMMUNOLOGY II

FC 11

IL-17RB in tuft cells controls IL-25-mediated tonic ILC2 activation to maintain their proliferative potential

C. Schneider[1], X. Feng[1], T. Andersson[1], J. Gschwend[1], P. Flüchter[1], N. Brander[1], R. Kroeschell[1]

1 Zürich

The intestinal tuft cell-ILC2 circuit initiates type 2 immunity and gut remodeling following worm infection. Specifically, tuft cell-derived IL-25 and leukotrienes trigger ILC2 activation following helminth colonization. In turn, ILC2s secrete IL-13 to promote tuft and goblet cell hyperplasia. While rapidly engaged upon chemosensory tuft cell stimulation, the circuit shows little homeostatic activity, suggesting the presence of regulatory mechanism that prevent inappropriate circuit activation.

Here, we detected IL-17RB, the specific component of the heterodimeric IL-25 receptor complex, on both ILC2s and tuft cells. Absence of IL-17RB specifically in ILC2s resulted in reduced circuit activation. Conversely, deletion of *Il17rb* in tuft cells spontaneously induced the activation of ILC2s, as reflected by an increase in basal IL-13 expression. Notably, the tuft cell–ILC2 circuit in $Vil1^{Cre}$;$Il17rb^{flox}$ mice remained unresponsive to succinate treatment as revealed by an impaired proliferative response in ILC2s. A similar hypoproliferative state was found in wild-type mice following prolonged circuit activation with luminal succinate.

Our studies identified a dichotomous role of IL-17RB. While IL-17RB in ILC2 is required for their IL-25-mediated activation, tuft cell IL-17RB functions to limit steady state availability of IL-25 to ILC2s. Such regulation maintains the circuit in a highly dynamic state, preventing unwanted activation of ILC2s and enabling appropriate proliferative capacity in ILC2s when luminal agonists are present. The hypoproliferative state of ILC2s may represent physiological adaptation to conditions of persistent circuit activation.

FC 12

Novel mechanisms in the regulation of serum IgE levels and anaphylaxis

P. Engeroff[1], M. Vogel[1], K. Plattner[1], M. F. Bachmann[1]

1 Bern

Aim: Immunoglobulin E (IgE) can trigger powerful inflammatory cascades from mast cells and basophils that can be useful in the fight against certain pathogens but if not properly controlled, can cause type I hypersensitivity and anaphylaxis. The potency of IgE requires for stringent mechanisms that keep the serum levels of IgE in check and thereby limit the extent of mast cell and basophil sensitization. It has previously been observed that surprising levels of IgG anti-IgE autoantibodies are present in mice and humans, however their function has never been clear.

Methods: To investigate the role of those antibodies in greater detail, we have combined *in vitro* IgE receptor binding and activation assays with *in vivo* IgE serum kinetic studies and different mouse models of passive and active systemic anaphylaxis.

Results: IgG anti-IgE autoantibodies recognize glycan structures on IgE and functionally facilitate serum IgE clearance in mice. Interestingly, the IgG-IgE complexes are cleared via the low-affinity IgE receptor CD23. The important role for CD23 in clearing IgE is further supported by the fact that in contrast to FcεRI that binds free IgE with high affinity, CD23 preferentially binds complexed IgE over free IgE. In turn, once the IgE is complexed it fails to sensitize and activate FcεRI.

Conclusions: In summary, we show that glycan-specific IgG anti-IgE autoantibodies and CD23 work together to remove IgE from the serum thus preventing IgE sensitization as well as anaphylaxis upon allergen challenge. We have thus unravelled a novel physiological pathway of IgE serum control.

FC 13

The b-Carboline harmine is an immunomodulator in Leishmania infection favoring the balance towards protective immune response

S. Peyvandi[1], A. Ives[1], Y. Hayoun[1], F. Tacchini Cottier[1], F. Prevel[1], Q. Lan[2], A. Chabloz[1]

1 Epalinges; 2 Helsinki FI

Leishmania are obligate intracellular parasites that cause *Leishmaniasis*. Current anti-leishmanial therapies are often toxic with side effects and mostly target the parasites and not the host response. bcarbolines are chemical components that were shown to improve MHC-I dependent antigen presentation in poorly immunogenic cancer cells. Here, we investigated the impact of synthetic bcarbolines (ACB) on the immune response and control of the parasite load using a drug-resistant *Leishmania* spp causing non healing lesion despite a strong Th1 response.

Our results showed that ACB reduce parasite burden in antigen presenting cells including macrophages *in vitro*. Furthermore, ACB1801 increased the expression of MHC-II and co stimulatory molecule on *Leishmania* infected DCs suggesting better immune response. In line with these results, ACB1801 treatment *in vivo* showed a major impact on lesion development and parasite burden in *L. major* infected C57BL/6 mice. RNAseq analysis of draining LNs highlighted an enrichment of TNF-a, IFN-g and MHC II Ag presentation signatures in ACB treated mice. These results were confirmed by flow cytometry showing an increase of CD4+ IFN-g^{+}TNF-a producing T cells and a decrease of IL-10^{+}FoxP3^{-} T cells. This resulted in higher iNOS activity in macrophages in ACB treated mice. ACB bcarbolines were further shown to contributes to the downregulating Aryl hydroxyl receptor (AhR) signaling which results decrease of immunosuppressive cytokines.

Collectively, we show that treatment with ACB-drug improved significantly host protective immune responses and reduced the diseases pathology.

FC 14

IL-9 sensitizes human pathogenic Th2 cells to pro-inflammatory IL-18 signals in atopic dermatitis

S. Schärli[1], F. Luther[1], O. Steck[1], J. P. Thyssen[2], N. L. Bertschi[1], C. Schlapbach[1]

1 Bern; 2 Hellerup DK

Background: Pathogenic CRTh2^{+} T helper 2 (pTh2) cells are crucial contributors to the pathogenesis of atopic dermatitis (AD) by secreting IL-13 and IL-22. Yet, IL-18 as one putative upstream regulator of pTh2 cells, is linked to AD pathogenesis by multiple lines of evidence, however, its role in activating pTh2 cells in AD skin is unknown.

Objective: We investigated the role of IL-18 in human Th2 responses in AD by analyzing pTh2 cells from AD patients, and *ex vivo* skin explants of human lesional AD skin.

Results: We first investigated the signals that induce interleukin 18 receptor (IL-18R) expression on Th2 cells. Of all the cytokines which pTh2 cells express the receptor for, only IL-9 was able to induce high levels of IL-18R. Consistently, IL-9R^{+}/IL-18R^{+} pTh2 cells were strongly increased in the blood of AD patients. Functionally, stimulation of circulating pTh2 cells with IL-18 induced secretion of IL-13, an effect that was significantly enhanced by co-stimulation with IL-9. Mechanistically, IL-18 induces rapid and strong activation of both NF-κB and AP-1 signaling in pTh2 cells. In human skin explants from lesional AD skin, neutralization of IL-18 downregulated IL-13 and IL-22 secretion from pTh2 cells. Finally, IL-18 protein levels correlated positively with *IL13* expression in lesional AD skin.

Conclusion: Collectively, our data reveal a previously underappreciated role of IL-9 and IL-18 as positive regulators of Th2 cell responses in human AD. These findings may guide future therapeutic approaches aiming at inhibiting aberrant activation of pTh2 cells in human skin.

FC 15

Targeted removal of macrophage-secreted interleukin-1 receptor antagonist protects against lethal Candida albicans sepsis

H. Gander-Bui[1], J. Schäfli[1], J. Baumgartner[1], S. Walthert[1], V. Genitsch[1], G. van Geest[1], J. A. Galván[1], C. Cardozo[1], C. G. Martinez[1], M. Grans[2], R. Bruggmann[1], H. C. Probst[2], C. Gabay[3], S. Freigang[1], S. Muth[2]

1 Bern; 2 Mainz DE; 3 Geneva

Invasive fungal infections are associated with high mortality rates; and the lack of efficient treatment options emphasizes an urgency to identify underlying disease mechanisms. We report that disseminated *Candida albicans* infection is facilitated by interleukin-1 receptor antagonist (IL-1Ra) secreted from macrophages in two, temporally and spatially distinct waves. Splenic CD169+ macrophages release IL-1Ra into the bloodstream, impeding early neutrophil recruitment. IL-1Ra secreted by monocyte-derived tissue macrophages further impairs pathogen containment. Therapeutic IL-1Ra neutralization restored the functional competence of neutrophils, corrected maladapted hyper-inflammation, and eradicated the otherwise lethal infection. Conversely, augmentation of macrophage-secreted IL-1Ra by type I interferon severely aggravated disease mortality. Our study uncovers how a fundamental immunoregulatory mechanism mediates the high disease susceptibility to invasive candidiasis; and suggests that interferon-stimulated IL-1Ra secretion may exacerbate fungal dissemination in human patients with secondary candidemia. Macrophage-secreted IL-1Ra should be considered as an additional biomarker and potential therapeutic target in severe systemic candidiasis.

SHORT COMMUNICATION SESSION – CLINICAL IMMUNOLOGY

FC 6

Allergen-targeting antibodies for the treatment of peanut and pollen allergies

N. Wuillemin[1], D. Bieli[1], C. Arena[1], P. Gasser[1], S. Götschi[1], R. Scheibling[1], T. Ilca[1], M. Paolucci[2], P. Johansen[2], D. Bouis[3], O. Martin-Rodriguez[3], K. Ho Wang Yin[4], B. Ballmer-Weber[2], A. Köhli[2], T. Sonati[1], A. Barberis[1], N. Pengo[1], T. Hauser[2]

1 Schlieren; 2 Zurich; 3 Archamps FR; 4 Archamps

Allergies rise worldwide, with peanut allergy being the major cause of fatal food-induced anaphylaxis, while pollen allergies impact up to 30% of the population. Traditional allergy treatment methods rely on desensitization to the specific allergen.

To make allergy treatment safer and faster, Mabylon developed an anti-allergen drug discovery pipeline, which allows rapid generation of effective patient-derived antibodies.

Allergen-targeting antibodies block IgE binding to the allergens and prevent effector cell activation. We have shown efficacy of such an approach for both peanut and pollen allergy in vitro and in humanized mouse models. One limitation of anti-allergen antibody therapies, as demonstrated by our research and others, is the need of antibody cocktails to be effective, which can be costly to produce and may involve additional regulatory considerations. Thus, we are developing engineered multi-specific antibodies to create highly effective, single antibody-based therapeutics. We also investigated a combinatorial approach in which allergen-targeting antibodies are combined with allergen immunotherapy. In vitro, the allergen-targeting antibodies in combination with immunotherapy are able to induce a tolerogenic cytokine milieu.

In conclusion, with the premise of good safety profile and rapid onset of protection, Mabylon's allergen-targeting antibodies have the potential to become a novel therapy for the treatment of peanut and pollen allergies. The engineering of antibody cocktails to one multi-specific molecule allows targeting multiple allergies at once.

FC 7

Evaluation of extracellular vesicles as noninvasive early predictive markers for severe COVID-19

P. A. Martinez Murillo[1], M. Ravi[2], K. Eriksson[2], S. Adapa[3], O. Schildgen[4], M. L. Merten[4], I. Ghiran[5], C. Karagiannidis[4], P. Krebs[6], D. Kressler[2], M. Stumpe[2], R. Jiang[3], P. Y. Mantel[1]

1 Davos; 2 Fribourg; 3 Florida US; 4 Cologne DE; 5 Boston US; 6 Bern

There is an urgent need to identify novel biomarkers to detect as early as possible severe cases of COVID-19. Extracellular vesicles (EVs) contain cargoes derived from the cell of origins that include proteins, lipids, and nucleic acids. As a consequence of cellular activation, EVs concentration is often increased during disease and specific EV biomarkers can be found in biofluids. We investigated the EV composition in the lungs and matching plasma of severe COVID-19 patients by using mass spectrometry-based proteomics. We optimized the isolation of EVs from small amount of plasma and broncho-alveolar lavage (BAL) and characterized them. Next, we performed proteomics in two independent cohorts (discovery and validation) to identify a "COVID-19 severe signature" common in BAL and plasma. Proteomics of EVs isolated from BAL showed that COVID-19 positive samples had a higher median of unique proteins detected (1518, CI 118-2027; n=49). The 12 conventional EVs markers examined were found in all our BAL samples and were present in $\geq$70% or $\geq$85% of negative and positive COVID-19 samples respectively. Overall characterization of COVID lung proteome shows infection signatures. Gene set enrichment analysis showed cellular pathways specifically involved in COVID infections including inflammation and tissue remodeling. Different machine learning models can all predict COVID infection based on proteome signatures. Next, plasma EV proteome will be analyzed to find a common signature together with the BAL, that will be validated in the second cohort.

FC 8

Safety and efficacy of a respiratory syncytial virus vaccine (mRNA-1345), against a spectrum of symptomatic disease in adults aged ≥60 years

G. L. Chen[1], J. Goswami[1], E. Wilson[1], S. K. Stoszek[1], R. Mithani[1], S. Mehta[1], A. Kapoor[1], W. Huang[1], L. Lan[1], J. Du[1], L. El Asmar[1], C. A. Panozzo[1], P. Ghaswalla[1], B. M. Francis[1], A. K. Simorellis[1], C. A. Shaw[1], J. M. Miller[1]

1 Cambridge, MA US

We present an interim analysis from a pivotal phase 2/3 randomized, double-blind, placebo-controlled, case-driven clinical trial (NCT05127434) in adults aged ≥60 years assessing mRNA-1345, an investigational mRNA vaccine encoding the RSV prefusion stabilized F glycoprotein.

Participants were randomized 1:1 to receive mRNA-1345 50 µg or placebo. Primary efficacy endpoints were prevention of a first episode of RSV-associated lower respiratory tract disease [RSV-LRTD] with ≥2 or ≥3 lower respiratory symptoms between 14 days and 12 months post-injection; secondary efficacy endpoints include RSV-associated acute respiratory disease [RSV-ARD] with ≥1 respiratory symptom between 14 days and 12 months post-injection.

mRNA-1345 was well-tolerated; no safety concerns were identified (solicited local adverse reactions [Ars]: mRNA-1345=58.7%; placebo=16.2%; solicited systemic ARs: mRNA-1345=47.7%; placebo=32.9%). Primary efficacy endpoints were met in the per-protocol efficacy set (n=35,088), including a vaccine effectiveness [VE] of 83.7% (95.88% CI, 66.0-92.2; $P<0.0001$) against RSV-LRTD with ≥2 symptoms and VE of 82.4% (96.36% CI, 34.8-95.3; $P=0.0078$) against RSV-LRTD with ≥3 symptoms. For the secondary efficacy endpoint, VE was 68.4% (95% CI, 50.9-79.7) against RSV-ARD.

Symptom distribution between mRNA-1345 and placebo recipients, and additional efficacy analyses by RSV subtype will be discussed. mRNA-1345 had a favourable safety and tolerability profile in adults aged ≥60 years and is efficacious in preventing a spectrum of symptomatic RSV disease including ARD and LRTD.

FC 9

CD70 targeting bi-specific Natural Killer cell Engager (BIKE) eliminate acute myeloid leukemia cells

M. Boy[1], H. Bratschi[1], C. Riether[1], A. Ochsenbein[1]

1 Bern

Introduction: Monoclonal antibodies (mAbs) in cancer immunotherapy often act by Antibody-Dependent Cellular Cytotoxicity (ADCC) and are dependent of the interaction between their Fc region and the FcYRIII (CD16) on NK cells. However, increased levels of plasma IgG in cancer patients reduces the efficiency of mAbs through competitive binding of CD16. Thus, te development of CD16 BiKEs may show superior efficiency compared

to mABs. Here we developed a new CD70 targeting BiKE and compared its efficacy in vitro to a CD70 targeting mAb.

Materials and Methods: Whole PBMCs and purified NK cells were co-cultured with different $CD70^{WT\ or\ KO}$ cells lines in presence of the different antibody constructs. Expression of CD107a, CD16, INFy and TNF were determined as well as NK cell activation and cytotoxicity by flow cytometry and calcein release assay

Results: BiKE CD70 efficiently activated purified NK cells in presence of $CD70^{WT}$ AML cells in vitro. In the presence of whole PBMCs, BIKE showed superior activation of NK cells compared to a CD70 mAb. Moreover, we observed low CD16 shedding upon activation of NK cells by BiKE..

Conclusions: Our results indicate that a CD70 targeting BiKE efficiently eliminates AML cells in vitro. Persistence of CD16 expression could allow rechallenging of NK cells with BiKE.

FC 10

Interleukin-2 immunotherapy reveals human regulatory T cell subsets with distinct functional and gatekeeper features

M. E. Raeber[1], D. Caspar[1], Y. Zurbuchen[1], N. Guo[2], J. Schmid[1], J. Michler[3], U. C. Steiner[1], A. E. Moor[3], F. Koning[2], O. Boyman[1]

1 Zurich; 2 Leiden NL; 3 Basel

Due to its stimulatory potential for immunomodulatory $CD4^+$ regulatory T (Treg) cells, low-dose interleukin-2 (IL-2) immunotherapy has recently gained considerable attention for treatment of various autoimmune diseases. Although early-stage clinical trials have correlated expansion of circulating Treg cells with clinical response to IL-2 treatment, detailed mechanistic data on responding Treg cell subsets and effects on other immune cells are lacking. In this investigator-initiated phase-2 clinical trial of low-dose IL-2 immunotherapy in systemic lupus erythematosus (SLE) patients, we performed an in-depth study of circulating and cutaneous immune cells by imaging mass cytometry, high-parameter spectral flow cytometry, bulk and single-cell RNA sequencing with cellular indexing, and targeted serum proteomics, building a comprehensive atlas of *in vivo* human immune responses to IL-2. Low-dose IL-2 stimulated various circulating immune cells, including Treg cells with skin-homing properties that appeared in the skin of SLE patients in close interaction with endothelial cells, whereas other cutaneous immune cells remained unchanged. Analysis of surface proteins and transcriptomes detected different IL-2-driven Treg cell programs, including highly proliferative $CD38^+$ $HLA\text{-}DR^+$, activated gut-homing $CD38^+$, and skin-homing $HLA\text{-}DR^+$ Treg cells. These data identify functionally distinct Treg cell subsets in human blood and skin, including those most responsive to IL-2 immunotherapy, thus providing unprecedented insight into human IL-2-responsive immune cells.

SHORT COMMUNICATION SESSION – SWISS YOUNG IMMUNOLOGISTS SOCIETY

FC 16

IL-9 induces a type-1 interferon signature in pathogenic skin-tropic Th2 cells

N. L. Bertschi[1], A. Vallone[1], F. Luther[1], O. Steck[1], S. Schaerli[1], C. Bazzini[1], I. Keller[1], J. M. Nuoffer[1], L. Borradori[1], D. Simon[1], C. Schlapbach[1]

1 Bern

Aim: IL-9 is a pleiotropic cytokine, for which an overarching role in humans remains elusive. Both interleukin 9 (IL-9) and its receptor, IL-9R, are specifically expressed by skin-tropic T helper 2 (T_H2) cells. This suggests that IL-9 signals play an important role in cutaneous immunity and allergy. Yet, the mechanism of action of IL-9 remains incompletely understood. Here, we aimed at deciphering the effect of IL-9 signals on T_H cells in allergic skin inflammation.

Methods: We isolated human $IL\text{-}9R^+$ T_H2 cells from blood and skin of acute allergic contact dermatitis and performed transcriptional profiling after stimulation with IL-9.

Results: IL-9 induced differential expression of approx. 800 genes in T_H2 cells. Upregulated genes were associated with conventional T_H2 immune response. Surprisingly, it also showed a strong induction of i) interferon-stimulated genes (ISGs) such as *IFIT3*, *RSAD2*, and *OAS1*, and ii) subunits of ISGF3 and DTX3L-PARP9. The latter are powerful transcription factor complexes that amplify type-1 interferon responses. Since ISG expression is mediated by STAT1 phosphorylation, we next investigated IL-9R signaling in detail. Interestingly, we found that signal transduction by IL-9R indeed leads to a unique STAT1 phosphorylation profile that is not comparable to other members of the common gamma-chain-cytokine receptor family.

Conclusion: We show that IL-9 signals are able to induce a type-1 interferon signature in T_H2 cells through a unique STAT1 signaling profile. This suggests that IL-9 enhances interferon-dependent immunity in type-2 inflammation and plays an important role in protecting against viral infections.

FC 17

IL-23 is dispensable for differentiation of Th1* and maintenance of Th1* and Th17 memory T cells

J. Puchan[1], Q. Philippot[2], J. Bohlen[2], F. Mele[3], D. Latorre[1], J. Bustamante[2], A. Puel[2], S. Boisson-Dupuis[2], J. L. Casanova[4], F. Sallusto[1]

1 Zürich; 2 Paris FR; 3 Bellinzona; 4 New York US

Cytokines are important cues that guide differentiation of naïve T cells in response to different pathogens. IL-23 has been implicated in the differentiation and maintenance of the human helper T cells subsets Th1* and Th17, which develop in response to mycobacteria and fungi, respectively. Its exact function in this process is however still unclear.

To understand the role of IL-23 in human helper T cells, we therefore studied patients with different mutations that impair or fully abrogate IL-23 signaling. These patients develop infections with weakly virulent mycobacteria, and in some cases fungal infections. Through naïve T cell priming with mycobacteria, we found that IL-23 signaling is neither required for development of Th1* cells expressing appropriate chemokine receptors nor for optimal cytokine production in primed cells in response to restimulation.

We also found that abrogation of IL-23 signaling did not impair the functionality of the memory helper T cell compartment of

these patients, measured by frequency and cytokine production of antigen-specific Th1* and Th17 cells. This may explain why the patients, which have deficits in their innate immune response to mycobacteria, do not develop recurrent infections.

Our results imply that IL-23 signalling is dispensable or at least redundant for the development and function of Th17 and Th1* cells and raise the question which signals are essential to differentiate naïve T cells into these subsets.

FC 18

Recapitulation of human coloncytes in situ by human colon organoids in vitro and after orthologous transplantation

A. Hausmann[1], F. Post[1], C. Steenholdt[1], A. Mund[1], O. H. Nielsen[1], M. Mann[1]

1 Copenhagen DK

The intestinal epithelium plays a central role in human health and disease, and several chronic inflammatory disorders associate with a weakened epithelial barrier. The organoid model allows the cultivation, propagation and analysis of non-immortalized intestinal epithelial cells and has been instrumental in studying epithelial behavior in homeostasis and disease. Recent advances allow the transplantation of human organoids into mice to study epithelial cell behavior within the intestinal tissue context. It remained unclear how realistically human colonic organoids cultured in vitro or transplanted into the murine colon recapitulate protein expression of human colonocytes in vivo, and how the transplantation influences the mucosal microenvironment. To address this, we employ state-of-the-art deep visual proteomics to compare human colonic epithelial stem and differentiated cells in human and mouse and compare them to organoids cultured in vitro. In addition, we sample immune and mesenchymal cells from the lamina propria in the three in vivo conditions to obtain information about changes in the mucosal microenvironment. Taken together, this will provide important insights into cellular functions which are realistically recapitulating human colonocytes in these models.

FC 19

Clonal structure, stability and dynamics of human memory B cells and circulating plasmablasts

G. E. Phad[1], D. Pinto[1], M. Foglierini[1], M. Akhmedov[1], R. L. Rossi[2], E. Malvicini[2], A. Cassotta[1], C. Silacci Fregni[1], L. Bruno[2], F. Sallusto[1], A. Lanzavecchia[2]

1 Bellinzona; 2 Milan IT

Memory B cells persist for a lifetime and rapidly differentiate into antibody-producing plasmablasts and plasma cells upon antigen re-encounter. The clonal relationship and evolution of memory B cells and circulating plasmablasts is not well understood. Using single-cell sequencing combined with isolation of specific antibodies, we found that in healthy donors, the memory B cell repertoire was dominated by large IgM, IgA and IgG2 clonal families, whereas IgG1 families, including those specific for recall antigens, were of small size. Analysis of multiyear samples demonstrated stability of memory B cell clonal families and revealed that a large fraction of recently generated plasmablasts was derived from long-term memory B cell families and was found recurrently. We also demonstrate the continuous generation of plasmablasts specific for recall antigens such as measles virus or tetanus toxoid in the absence of recent exposure. These findings support a model where polyclonal bystander activation of memory B cells continuously generates plasmablast at low rate, thus contributing to the maintenance of bone marrow plasma cells and serum antibody levels. Collectively, this study provides a systematic description of the structure, stability and dynamics of the human memory B cell pool and suggests that memory B cells may be active at any time point in the generation of plasmablasts.

FC 20

Iteratively stimulated T cells as tools to understand mechanisms of nonlymphoid recirculation

M. Künzli[1], A. Soerens[1], S. D. O'flanagan[1], V. Joag[1], C. Quarnstrom[1], M. Scott[1], D. Masopust[1]

1 Minneapolis US

Memory T cell migration facilitates whole-body immunosurveillance and protection against recurrent infections and cancer. In the absence of inflammation, tissue resident memory T cells (TRM) patrol non-lymphoid tissues (NLT) while effector and central memory T cells are mostly restricted to the blood and the blood/lymph system respectively. Memory T cells that retain the ability to recirculate through all compartments are rare and poorly characterized. Thus, the function of these cells as well as their non-lymphoid tissue entry and egress requirements under homeostatic conditions are not well defined.

Using a heterologous prime-boost immunization scheme and adoptive transfers, we have generated an abundant murine memory T cell population that has been exposed to >50 viral infections over the last 10 years. We termed these cells iteratively stimulated T cells (ISTCs).

Parabiosis experiments revealed that ISTCs acquired pan-migration properties: they can be found in blood, non-lymphoid tissues, lymph nodes, and thoracic duct lymph (TDL) of the naïve parabiont. ISTCs stably persist in NLTs like the lung which are usually associated with short-lived TRM populations, and mice harboring recirculating ISTCs are protected from lethal Influenza challenge.

These data indicate that establishing recirculating memory T cells might provide a new strategy to generate long-lasting immunity in tissues like the lung. Single cell RNA-sequencing revealed tissue entry and egress target molecules that will be tested in *in vivo* CRISPR-Cas9 screens to elucidate mechanisms of T cell recirculation.

POSTERS

P1

Anaphylaxis with cardiac arrest due to tranexamic acid allergy

A. Neff[1], K. Scherer Hofmeier[2], A. Grünewald[3], T. Heidegger[4], T. Girard[5], B. Ballmer-Weber[1]

1 St.Gallen; 2 Aarau; 3 Luzern; 4 Grabs; 5 Basel

Aim: to raise awareness for a rare but important elicitor of perioperative anaphylaxis

Case history: A 65-year old woman developed an erythema, hypotension and cardiac arrest with following resuscitation during induction of anaesthesia for a planned orthopaedic procedure. The reaction happened briefly after the intravenous application of 1000mg tranexamic acid. Tryptase level after reaction was 38ug/l (baseline tryptase: 5.25ug/l).

Results: Skin testing with all drugs administered before the reaction (propofol, fentanyl, remifentanil, rocuronium, cefuroxime, lidocaine, dexamethasone) were negative, as well as in vitro results for chlorhexidine, morphine, suxamethonium, ethylene oxide and latex. However, basophil activation test (BAT) with tranexamic acid was highly positive (stimulation index (SI) 32.7) confirming the diagnosis of anaphylaxis to tranexamic acid. Skin testing with tranexamic acid was omitted due to the severity of the reaction.

Conclusions: Tranexamic acid is a synthetic lysin-derivative and acts as an antifibrinolytic agent. It blocks the binding site of plasminogen to fibrin. It is used therapeutically in postpartum haemorrhage and for prophylaxis or therapy in many surgeries with increased blood loss. Anaphylaxis to tranexamic acid is rarely reported in the literature. To date, only 8 cases of anaphylaxis to tranexamic acid have been published. It is important to be aware of the allergenic potential of tranexamic acid. Avoidance of other lysine derivatives such as aminocaproic acid is recommended.

P2

Generating a Virus Like Particles-based vaccine against IgE

Z. Gharailoo[1], K. Plattner[1], M. Vogel[1], M. Bachmann[1]

1 Bern

Immunoglobulin E (IgE) plays a significant role in type I allergy which is characterized by the induction of IgE responses towards harmless antigens. Cross-linking by allergens of IgE bound to its high-affinity receptor (FcεRI)- expressed on mast cells and basophils- leads to cellular activation, degranulation and releasing inflammatory mediators. As this receptor cross-linking plays a key role in initiating allergic reactions, we aimed at developing a vaccine targeting IgE antibodies by inducing a protective anti-IgE immune response which blocks IgE-FcεRI interaction. Because IgE-Fc is the region responsible for its effector functions as the binding site for FcεRI receptor is located in Cε domains, we generated three vaccines by coupling three IgE-Fc fragment (Cε1-4, Cε2-4 and Cε3-4) to virus-like particles (VLPs) derived from cucumber-mosaic virus which contains a universal Tetanus toxoid epitope ($CuMV_{TT}$). The immunogenicity of the vaccines was tested by immunizing mice with either the three vaccines or the VLP control. In comparison with the control vaccine, mice immunized with the IgE-vaccines had high titer of IgG anti-IgE response in the sera and more anti-IgE secreting plasma cells in lymphatic organs. Moreover, immunization with two of the three vaccines (Cε2-4 and Cε3-4) leads to reduction of free IgE in serum and suppressed systemic anaphylaxis upon allergen challenge in naïve and allergen sensitized mice. These data showed that active immunization against IgE using VLPs has the potential to become a promising therapeutic method against IgE-mediated allergy.

P3

Investigation of anaphylaxis in mouse models for mastocytosis

L. Clauss[1], M. Konantz[1]

1 Basel

Mastocytosis is characterized by expansion and activation of mast cells (MC) in various organs, particularly in skin and bone marrow (BM), associated with a mutation in the gene KIT (KITD816V in >90% of patients). Patients with mastocytosis suffer from symptoms of MC degranulation, including anaphylaxis, that can also be life-threatening.

Our lab utilizes novel mouse models expressing the mKitD814V mutation, the homologue of hKITD816V, in MC (Mcpt5Cre x $KitD814V^{fl}$) or all BM-derived hematopoietic cells (SclCreER x $KitD814V^{fl}$) to study non-advanced vs. advanced systemic mastocytosis, respectively. We assess the number of skin and organ MC as well as serum Mcp1 level. To study anaphylaxis, we sensitize mice with IgE@DNP and induce anaphylaxis 24h later with DNP-HSA. Anaphylaxis is then measured by the drop of body temperature.

In both mouse lines, MC in the skin accumulate compared to control littermates. In the Scl-Cre-ER model, there is a continuous increase of skin MC 30 weeks before mice had to be sacrificed, while in Mcp5-Cre mice, MC only increase initially, but do not rise further. This is accompanied by an increase of Mcp1 serum level. When inducing systemic anaphylaxis, we observe temperature drops of Δ6°C in mutant vs. Δ4°C in control mice after 40 minutes. Mutant mice recover slower (>2h20) than control mice (2h) until being back to the initial temperature, respectively.

Our data indicate that our mouse models allow studying mastocytosis, including anaphylaxis. We will next explore the effect of KIT tyrosine kinase inhibitors on various MC functions in these models.

P4

Mass spectrometry-based identification of allergen proteins involved in seafood-related allergic reactions

E. Barletta[1], P. Westermann[1], K. Fröhlich[2], M. C. Brüggen[3], P. Schmid-Grendelmeier[3], K. Baerenfaller[1]

1 Davos Wolfgang; 2 Freiburg DE; 3 Zurich

Shellfish are one of the most common causes of food allergies and a major cause of food-induced anaphylaxis. Sensitization and subsequent reactions occur most frequently upon ingestion. However, they can also occur because of skin contact. Shrimps are, among all, the most consumed type of seafood worldwide, and for that, it is important to identify and characterize all possible allergens. A bottom-up proteomics approach, LC-MS/MS coupled with Parallel Reaction Monitoring (PRM) technique, is used to acquire high-resolution full MS/MS spectra for each target allergen peptide. Total protein extracts from shrimp (*Penaeus monodon* and *Penaeus vannamei*) were isolated and processed through in-gel tryptic digestion of SDS-PAGE gel fractions or using PreOmics columns with or without

fractionation. Resulting peptides were then collected and purified prior to LC-MS/MS analysis and the MS raw files were processed by the SEQUEST algorithm within the protein database for decapods (TaxID = 6683). In all shrimp samples, it was possible to accurately identify our proteins of interest. Tropomyosin proteins specific for shrimp, prawns, lobster, and crab were identified in our discovery workflow sharing a sequence identity between 89% and 100%. To support our findings, a PRM analysis was then performed looking for all shrimp unique tropomyosin peptides analyzed within the Skyline open-source software. The results obtained suggest the reproducibility of this proteomics workflow, so as to be used not only in the identification of other important allergens in seafood-related allergic reactions but also of allergens involved in other types of allergic diseases.

P5

Inflammatory dendritic cells (inf DCs): a role in the pathogenesis of nephrotic syndrome (NS)?

D. de Haro[1], C. Obregón[1], U. Simeoni[1], H. Chehade[1]

1 Lausanne

Nephrotic syndrome (NS) is a glomerular disease characterized by increased permeability of the filtration barrier. Although structural damages have been identified, the underlying immune mechanism is largely unknown. *The aim of this study* is to characterize different subsets of dendritic cells (DCs) in urine and blood samples from patients with NS and to associate them with the development of the disease.

Urine and blood samples were collected from children with NS (n=7) and compared with controls (n=10). Cells were analyzed by FACS and different DC subsets were identified as DC1(CD141), DC2 (CD1c), inf DC ($CD1c^+CD14^+$) and monocyte-derived DC (ModDC), as well as CD4 and CD8 T cells, NK, neutrophils and macrophages.

Using PCA we were able to distinguish NS from controls in both urine and blood samples, suggesting that the FACS panel is relevant to identify a cellular signature in NS patients. CD4 and CD8 T-cell populations, although significantly increased in the blood of patients with NS, urine T-cell frequencies are very low. This result contrasts with the observation that the frequency of HLA-DR population increased in the urine of patients with NS. Importantly, increased frequencies of DC1, DC2, inf DCs and ModDC can be found in urine compared to blood samples; only inf DCs were significantly increased in NS, suggesting that this inflammatory phenotype that develops locally in the kidney may be involved in the pathogenesis of NS.

This result constitutes an important finding not only for the diagnostic value of urine samples, but mainly as the first reference of inf DCs in NS.

P6

Versatile and scalable nanoparticle vaccine as a scaffold against newly emerging influenza viruses

M. Kopf[1], A. Pardini[2], D. Rothen[2], P. S. Krenger[2], A. C. Vogt[2], R. Josi[2], M. Vogel[2], M. F. Bachmann[2]

1 Zürich; 2 Bern

Aim: The public's health is still being endangered by influenza, a respiratory infection that is both acute and highly contagious. Here, we assessed the immunogenicity and efficacy of a vaccine candidate for influenza virus based on the virus-like particles AP205 (AP205-VLPs) displaying the HA head domain (HA1) of A/PR8/H1N1 which is a pandemic murine-adapted strain.

Methods: The HA1 was genetically linked to the N-terminus of AP205 dimerized capsid proteins. Following that, we inoculated mice intranasal, subcutaneous and assessed IgG, IgG Subclasses, IgA and avidity of IgG and IgA. For the challenge, vaccinated mice were infected with a 2xLD50 dose of the A/PR8/H1N1 strain.

Results: We found that administration i.n and s.c of HA1-AP205dim elicited strong and comparable specific IgG responses in serum of immunized mice. Preliminary experiments showed that intranasal immunization also resulted in local IgA and IgG production in the lung. Furthermore, we demonstrated that i.n and s.c immunizations of mice with HA1-AP205 vaccines prevent body weight loss and provide complete protection against a lethal A/PR8/H1N1 virus challenge.

Conclusions: These findings suggest that i.n. and s.c. delivery of AP205-VLP displaying HA1 antigen can result in an effective antibody-based vaccination against influenza infection. This opens the door for subsequent HA1 vaccine development not only in humans but also animal health, because AP205-VLP based vaccines have the potential to be generated through bacterial expression in a large-scale production for immunization programs.

P7

Influence of antigen density and TLR ligands on preclinical efficacy of a VLP based vaccine against peanut allergy

P. Krenger[1], R. Josi[1], J. Sobczak[1], T. Velazquez[2], I. Balke[3], M. Skinner[2], M. Kramer[2], C. Scott[2], S. Hewings[2], M. Heath[2], A. Zeltins[3], M. Bachmann[1]

1 Bern; 2 Worthing GB; 3 Riga LV

Aim: VLP-Peanut is a new vaccine candidate for peanut allergy recently enrolled in a phase I clinical study. The vaccine is based on cucumber mosaic virus-like particles ($CuMV_{TT}$-VLPs) that are genetically fused with one of the major peanut allergens, Ara h 2 ($CuMV_{TT}$-Arah2). Here, product characterization studies were undertaken to better understand the basis of the vaccine's immunogenicity and protective efficacy.

Design & methods: Focusing on the role of the prokaryotic RNA encapsulated within VLP-Peanut, a VLP-Peanut batch with reduced RNA content (VLP-Peanut low RNA) was produced. Immunogenicity and peanut allergen tolerability studies were conducted with VLP-Peanut low RNA and VLP-Peanut in WT as well as TLR 7 KO mice. Additionally, the link between Ara h 2 antigen density and immunogenicity of VLP-Peanut was analyzed on the basis of a newly established mass spectrometry based method.

Results: We observed a TLR 7 dependent formation of IgG2c subclass and high avidity IgG antibodies specific for Ara h 2 after VLP-Peanut vaccination. Reducing the RNA content resulted in diminished Ara h 2 specific IgG responses, followed by a significantly impaired peanut allergen tolerability. Furthermore, a strong correlation could be established between the number of Ara h 2 antigens displayed on VLP-Peanut particle's surface and the vaccine's efficacy.

Conclusions: These findings suggest that the prokaryotic RNA in VLP-Peanut and antigen density of Ara h 2 on the particle's surface are key contributors to the immunogenicity and the protective capacity of the VLP-Peanut vaccine.

P8

Heterologous prime-boost vaccination with a peptide-based vaccine and viral vector improves antitumor therapy

F. Alfei[1], E. Belnoue[1], M. Rossi[1], M. Derouazi[1], L. Devy-Dimanche[1], M. L. Santiago-Raber[1]

1 Geneva

Immunotherapy has revolutionized the treatment of cancer; however, a substantial number of patients don't respond to the treatments and have a poor clinical outcome. The low response rate to the therapies is due to the low number of tumor infiltrating T-cells and the presence of exhausted T-cells and other immune suppressive immune cells. At AMAL, we have developed a prime-boost regimen which can increase number and functionality of tumor infiltrating T-cells. The regime is composed of prime with the therapeutic vaccine KISIMA™ and boost with an oncolytic vesicular stomatitis virus variant (VSV-GP). The therapeutic vaccine KISIMA is made as a single chimeric fusion protein, containing a proprietary cell-penetrating peptide (CPP) for antigen delivery, a proprietary Toll-like receptor (TLR)- peptide agonist with self-adjuvant properties and a modulable multi-antigenic domain (Mad). VSV-GP is chimeric viral vector carrying the same antigen included in the Mad domain of the vaccine, able to trigger a robust innate response and boost antigen specific T-cells primed by the KISIMA vaccine. This innovative prime-boost strategy has been tested in several mouse models, showing enhance tumor control and survival of tumor bearing mice. The great efficacy reached by the KISIMA-VSV strategy is due to the extensive reshaping of the tumor microenvironment and the significant increase of T-cell functionality. The promising pre-clinical results have granted the enter in a phase I clinical trial for the KISIMA-VSV prime-boost strategy for the treatment of colorectal cancer (NCT04046445).

P9

The proteasome regulator PA28αβ is a crucial determinant in graft acceptance

K. Inholz[1], U. Bader[2], M. Basler[1]

1 Kreuzlingen; 2 Konstanz DE

The proteasome generates peptides which can be presented on MHC-I. Those peptides can either origin from pathogenic proteins after infections or from self-proteins, which is crucial for T cell selection. The proteasome regulator PA28αβ was shown to change the cleavage pattern of the proteasome. In particular, several immunogenic peptides have been reported to be PA28αβ dependent. In contrast to those studies, we could not observe a major impact of PA28αβ expression on the generation of several tested immunogenic peptides. There was no difference in the presentation of different lymphocytic choriomeningitis virus (LCMV) or vaccinia virus derived peptides in *in vitro* experiments comparing wildtype and PA28αβ knockout cells, which was consistent with a normal CD8 response and viral clearance in infected PA28αβ knockout mice. However, we observed that the adoptive transfer of wildtype cells into PA28αβ knockout mice, but not vice versa, led to the rejection of the transferred cells. Depletion experiments showed that the observed rejection is mediated by CD8+ cytotoxic T cells. These data indicate that PA28αβ is involved in the development of the CD8+ T cell repertoire in the thymus. Taken together, our data suggest that PA28αβ is a crucial factor determining graft survival.

P10

AhR agonism by tapinarof regulates Th2 and Th17 cell function in human skin

F. Luther[1], O. Steck[1], N. L. Bertschi[1], S. Schärli[1], C. Schlapbach[1], L. Pohlmeier[1], N. Yawalkar[1], D. Simon[1], S. Radonjic-Hoesli[1]

1 Bern

The aryl hydrocarbon receptor (AhR) is a transcription factor for skin homeostasis and barrier function. Tapinarof, a topical AhR agonist, has shown impressive clinical efficacy in psoriasis (PSO) and atopic dermatitis (AD), inducing long-lasting remissions. However, tapinarof's anti-inflammatory mechanism remains unclear. We aimed to investigate tapinarof's effects on T cells in healthy skin, AD, PSO, and allergic contact dermatitis (ACD).

Using a short-term human skin explant model, we cultured skin biopsies from PSO, AD and ACD with tapinarof for 24 hours. We observed elevated cytokine levels in disease-driving populations of T_{RM} (IL-13$^+$CD4$^+$ T_{RM} in AD and IL-17a$^+$CD8$^+$ T_{RM} in PSO), validating our model. Tapinarof significantly reduced IL-13 and IL-17a in the respective diseases and populations. In ACD, tapinarof decreased IL-13 levels in T_{RM} and CD4$^+$ T cells without affecting IFN-g.

Transcriptomic analysis on tapinarof-treated T cells showed reduced metabolic enzymes, T cell activation and a reduction of *IL13* and *IL17A*. Single-cell RNA-seq on tapinarof-treated T cells from AD and PSO biopsies showed similar metabolic impairments. Preliminary mechanistic studies revealed reduced glycolysis and oxidative phosphorylation in resting and activated T cells after tapinarof treatment.

In conclusion, our ex vivo model demonstrated tapinarof's impact on skin T cells, with significant reduction in disease-relevant cytokines in AD, PSO, and ACD. Lastly, tapinarof directly affected T cells and impairs glycolysis and oxidative phosphorylation, revealing a previously unknown mechanism of action.

P11

Probing monoclonal antibody specificity for Alzheimer's vaccine design

A. C. Vogt[1], M. Vogel[1], A. Zeltins[2], M. Bachmann[1]

1 Bern; 2 Riga LV

Alzheimer's disease (AD) is the most common form of dementia and may contribute to 60–70% of cases. Accumulation of extracellular plaques containing amyloid-b peptide characterizes one feature of the neuropathology of AD.

It seems that the disease's pathophysiology develops 10–20 years before AD's clinical manifestation. Therefore, novel therapeutics should aim to delay or prevent the disease at the preclinical stage.

Recently, 2 mAbs have been approved for the treatment of AD, namely Aducanumab and Lecanemab. In the current project, we design vaccines to induce polyclonal antibodies of the same specificity as the approved mAbs. We developed a vaccine based on virus-like particles (VLP) derived from the cucumber mosaic virus ($CuMV_{TT}$) fused with different epitopes of $Abeta_{1-42}$. Our experiments have now shown that genetic fusion of different epitopes of Ab ($Abeta_{3-6}$, $Abeta_{1-6}$ and $Abeta_{1-7}$) to the surface of $CuMV_{TT}$ resulted in three vaccine candidates which are highly immunogenic and induced IgG antibodies against the full-length $Abeta_{1-42}$. Moreover, IgG generated by the $CuMV_{TT}$-$Abeta_{3-6}$ vaccine showed the same recognition profile as approved mAb Aducanumab, preferably binding oligomers. Additionally, the generated antibodies specifically bound Abeta plaques in human brains as well as in mouse brain

tissue. Currently, transgenic mice prone to the disease, are treated with $CuMV_{TT}$-$Abeta_{3-6}$ vaccine, to test its efficacy in inhibiting Abeta plaque formation and deposition in vivo. In addition, we are currently testing $CuMV_{TT}$-$Abeta_{1-16}$ for the induction of Lecanemab-type antibodies.

P12

Recombinant Interferon Gamma restores altered immunometabolism in Chronic Granulomatous Disease

M. Bruno[1], C. Kroger[2], B. Zhang[3], A. Ferreira[1], R. Liu[1], R. J. Röring[1], C. I. van der Made[1], L. Groh[1], M. van Uelft[2], A. Ziogas[1], V. Klück[1], N. A. Janssen[1], D. Rosati[1], S. J. Moorlag[1], P. A. Debisarun[1], J. Saiz[4], O. Gaal[5], H. J. Ferreira[2], S. Warnat- Herresthal[2], L. Holsten[2], S. Müller[2], M. Kraut[2], J. Schulte-Schrepping[2], L. Bonaguro[2], K. Händler[2], J. L. Schultze[2], C. Cunha[6], C. Barbas[4], L. A. Joosten[1], M. G. Netea[1], Y. Li[3], A. C. Aschenbrenner[2], A. Carvalho[6], F. L. van de Veerdonk[1], M. Becker[2]

1 Nijmegen NL; 2 Bonn DE; 3 Hannover DE; 4 Madrid ES; 5 Nijmegen RO; 6 Braga PT

Aims: Chronic granulomatous disease (CGD) is characterized by severe infections and hyperinflammatory complications, resulting from ROS deficiency caused by genetic mutations in the NADPH oxidase complex. While prophylactic recombinant interferon gamma (rIFN-γ) reduces the risk of severe infections, the biological mechanisms are unknown. The aim of this study was to explore the metabolic defects in CGD and the potential rebalancing effects of rIFN-γ.

Methods: We compared the immune and metabolic profile of immune cells from CGD patients with controls using scRNAseq, ATACseq, ELISA, proteomics, seahorse and metabolomics. We also investigated the immunometabolic consequences of *in vitro* and *in vivo* rIFN-γ treatment.

Results: Innate immune cells from CGD patients are epigenetically and functionally reprogrammed to maintain a hyperactivated state, paralleled by impaired *in vitro* induction of trained immunity. Concurrently, CGD monocytes have deficient intracellular amino acids levels and profound glycolytic and mitochondrial defects. *In vitro* treatment with rIFN-γ restored those defects and reduced abnormal fungal-induced IL-1β and IL-6 production in CGD monocytes. *In vivo* rIFN-γ also restored the defective immunometabolism in one CGD patient.

Conclusions: The efficacy of prophylactic rIFN-γ efficacy in CGD likely has a metabolic basis. Learning more about pathological immunometabolism will not only give new insights into disease pathogenesis but, beyond CGD, might open a new avenue of research for efficient and targeted immunotherapy aimed at correcting these defects.

P13

Early life imprinting of immune system by the gut microbiota

Z. Al Nabhani[1], L. Morelli[1], N. Joseph[1], R. Abdoul Ahad[1]

1 Bern

Background: The intestinal microbiota is a key player in the development of the immune system. We have previously shown that the introduction of solid foods at weaning is accompanied by a transient immune response induced by intestinal bacteria (weaning reaction). This reaction is necessary to prevent susceptibility to colitis and colorectal cancer (CRC) in adult mice. However, to which extent the dietary-fiber amounts at weaning affects the microbiota establishment and the susceptibility to develop of intestinal inflammation remains unknown.

Objectives: This project aims to determine how a fiber-based diet in early life influence the generation of the weaning reaction and the long-term immune responses.

Methods: Mice with a complex microbiota were exposed to a high fiber or low fiber diet early in life and their susceptibility to colitis and CRC was assessed in adulthood. Causal relationships between microbiota and disease development were investigated in germ-free (GF) mice.

Results: We showed that the amounts of dietary fiber in early life determine the type of immune response at weaning, with lasting consequences on the severity of intestinal inflammation. T-cell subtypes regulate the weaning reaction in response to Firmicutes bacterial members that are expanded in high-fiber context. Transferring these bacteria to neonatal GF mice, but not adults, was sufficient to prevent colitis and CRC in adults.

Conclusion: The susceptibility of adults to the development of the disease is related to the immune imprint by microbes in early life.

P14

A tetravalent nanovaccine that inhibits growth of HPV-related head and neck carcinoma via dendritic and T cell activation

R. Josi[1], D. E. Speiser[2], S. de Brot[1], A. C. Vogt[1], G. Tolstonog[2], E. M. Sevick-Muraca[3], M. F. Bachmann[1], M. O. Mohsen[1]

1 Bern; 2 Lausanne; 3 Houston, Texas US

Background: The prevalence of head and neck carcinoma (HNC) caused by human papillomavirus (HPV) is constantly rising. HPV's oncogenic proteins E6 and E7 are co-responsible for malignant cell proliferation and are therefore appropriate therapeutic targets.

Methods: The current study aimed at developing therapeutic vaccination against HNC. We produced a tetravalent vaccine (Qb-HPVag) consisting of virus-like nanoparticles (Qb), loaded with a potent TLR9 ligand and coupled to four elongated synthetic HPV peptides by click chemistry. The antitumor efficacy of the Qb-HPVag vaccine was evaluated in the murine mEERL95 HNC model.

Results: Therapeutic vaccination with the tetravalent Qb-HPVag could significantly hinder tumor progression and enhance the infiltration of HPV-specific $CD4^+$ and $CD8^+$ T cells which readily produced interferon gamma (IFN-γ) and tumor-necrosis factor α (TNF-α). The resulting antitumor efficacy was associated with favorable immune-repolarization of the tumor microenvironment through expansion of activated DCs and their different subsets, including cDC1, cDC2, and $CCR7^+$ DCs. Furthermore, Qb-HPVag strongly decreased post-surgical tumor recurrence and prolonged mouse survival.

Conclusion: Monotherapy with the tetravalent Qb-HPVag vaccine shows promising antitumor efficacy in an HNC murine model. Future clinical application using this strategy is readily feasible and practical, as click chemistry peptide coupling to nanoparticles can readily be done under good manufacturing practice (GMP) conditions.

P15

Understand how cDC1-mediated support guides TCF1 populations

S. Vilbois[1], A. Bénéchet[2], F. Alfei[3], T. H. Chang[1], P. C. Hsueh[1], W. Held[1], P. C. Ho[1]

1 Epalinges; 2 Lausanne; 3 Geneva

Aim: Understanding how TCF1 differentiation in CD8+ exhausted T cells is controlled in the TME. Our project focuses on the interaction between TCF1 subsets and conventional dendritic cell type 1 (cDC1) as they localize in the same intratumoral niches.

Methods: We engrafted Yumm1.7-OVA tumors into different transgenic mice models. XCL1 reporter mice were used to assess XCL1 expression in TCF1+ and TCF1- subsets. Mice lacking IL-15 secretion in cDC1 were used to explore its role in TCF1 differentiation. Temporal depletion of cDC1 was done at different time points of tumor development to explore the TCF1 subsets spatial distribution and infiltration capacity in the tumor. We analyzed by flow cytometry and intravital 2-photon microscopy.

Results: We find that progenitor 1 and terminally exhausted-like T cells are the TCF1 subsets responsible for maintaining a sustained interaction with cDC1 in the tumor through the XCL1-XCR1 axis. The cDC1 controls TCF1 differentiation by maintaining the TCF1+ subsets and via IL-15 secretion prevents the terminal differentiation of TCF1- cells. Moreover, cDC1 regulates TCF1 differentiation by limiting the accumulation of tumor-specific CD8+ T cells with higher exhaustion and improves T-cell infiltration by controlling fibrosis in the TME. However, cDC1's capacity to regulate TCF1 differentiation becomes altered during tumor progression, enabling other pro-tumorigenic immune cells to overstimulate T cells and accelerate their exhaustion severity.

Conclusion: cDC1 controls TCF1 differentiation and simultaneously prevents fibrosis in the TME.

P16

The Peptidyl-Prolyl cis-trans Isomerase, Pin1, Associates with and Regulates the Activity of Protein Kinase C θ (PKCθ) in Activated T Cells

N. A. Ponnoor[1], A. Muraleedharan[1], J. Malter[2], C. Keasar[3], A. Braiman[3], A. Altman[4], K. F. Kong[4], N. Isakov[3], J. Shaik[3], E. Livneh[3]

1 CA; 2 Texas US; 3 Beer Sheva IL; 4 San Diego US

Protein kinase C-θ (PKCθ), a member of the novel PKC subfamily is abundantly expressed in T lymphocytes and regulates key cellular functions. Previously, we demonstrated that a proline-rich (PR) region within the V3 regulatory domain of PKCθ is a requisite for PKCθ immunological synapse (IS) localization and function. Herein, we highlight the importance of the Thr^{335}-Pro motif in the PR region, the phosphorylation of which is critical for PKCθ activation, its subsequent IS localization, and interaction with the peptidyl-prolyl *cis-trans* isomerase (PPIase), Pin1, an enzyme that specifically recognizes peptide bonds at phospho-Ser/Thr-Pro motifs. Protein interaction studies revealed that the Pin1-PKCθ association is contingent upon the phosphorylation of the PKCθ-Thr^{335}-Pro motif and the integrity of the Pin1-WW domain. TCR crosslinking promoted a rapid and transient formation of Pin1-PKCθ complexes, which followed a T cell activation-dependent temporal. Confocal imaging analyses revealed that PKCθ and Pin1 were recruited to the T cell membrane, predominantly at the IS, following TCR stimulation. Enzymatically active Pin1 downregulated the catalytic activity of PKCθ *in vitro*, via the isomerization of PKCθ. Furthermore, TCR stimulation of [Lck^{cre} x $Pin1^{lox}$] F_1 mice-derived Pin1-deficient T cells led to augmented phosphorylation of the SPAK kinase, a *bona fide* PKCθ downstream substrate. The results suggest that the Pin1 PPIase is a novel binding partner and regulator of PKCθ and highlights the potential contributions of Pin1 to the fine-tuning of the activated T cells.

P17

CD8+ T cells require signal 3 from non-antigen-presenting cells for optimal clonal expansion

J. Abe[1], P. Germann[2], J. Ripoll[3], J. Sharpe[2], J. V. Stein[1]

1 Fribourg; 2 Barcelona ES; 3 Madrid ES

Naive $CD8^+$ T cells proliferate and differentiate into effector $CD8^+$ T cells as a result of the integration of signals from cognate pMHC (signal 1), costimulatory molecules (signal 2) and cytokines (signal 3). While activated dendritic cells (DCs) act as the primary source of signals 1 and 2, it remained elusive whether DCs supply sufficient levels of signal 3 to naïve $CD8^+$ T cells for effector cell generation. The main technical hurdle to tackle this key aspect has been to determine the precise stoichiometry of naïve $CD8^+$ T cells and DCs required for *in vivo* effector responses. Here, we addressed this using light sheet fluorescence microscopy of intact reactive lymph nodes, combined with flow cytometry and intravital imaging. Shortly after DC vaccination, naïve $CD8^+$ T cells and DCs established hours-long 1 : 1 interactions. However, exponential effector $CD8^+$ T cell expansion required an additional supply of proinflammatory cytokine interleukin-12 (IL-12) secreted by a seven-fold excess of activated yet non-interacting DCs. In this setting, IL-12 primarily acted on host cells rather than antigen-specific $CD8^+$ T cells. Thus, in addition to the integration of signal 1 and 2 via direct DC engagement, reactive T cells sense the proinflammatory milieu of lymphoid tissue to unleash the generation of effector $CD8^+$ T cells. This mechanism may contribute to safeguarding against unwarranted effector $CD8^+$ T cell generation by occasional encounter with activated “rogue” DCs in otherwise resting lymphoid tissue.

P18

IFN-γ Modulated DCsion Making on T-cell Anti-tumor Immunity

T. H. Chang[1], F. Alfei[1], P. C. Ho[1]

1 Epalinges

Resistance to immune checkpoint blockade (ICB) treatments represent a major challenge in cancer therapy. Emerging evidence suggests that interferon-gamma (IFN-γ) plays a crucial role in modulating the response to checkpoint blockade and prolonged exposure to IFN-γ or chronic IFN-γ signaling can lead to the development of resistance. Here, we found that tumors with a loss-of-function on IFN-γ sensing contained a high concentration of IFN-γ in the tumor microenvironment (TME) associating with the increased exhaustion in $CD8^+$ T cells. To elucidate whether the exposure of IFN-γ promote T cell exhaustion, we found that genetic ablation of IFN-γ or antibody blockade robustly increased tumor-specific $CD8^+$ T cells, but did not affect the percentage of $PD\text{-}1^+$ $Tim3^+$ exhaustion and $TCF\text{-}1^+$ progenitor $CD8^+$ T cells. Surprisingly, we further revealed that in response to IFN-γ blockade, cDC2 instructed $CD4^+$ T cells to restrict formation of $TCF\text{-}1^+$ progenitor $CD8^+$ T cells via the IL-4/IL-13 cytokine axis. Altogether, our findings suggest that bypassing IFN-γ signaling, type 2 immune responses orchestrated by cDC2 and IL-4/-13-producing CD4 T cells can cooperatively modulate the differentiation of tumor-specific progenitor CD8 T cells. Our study reveals an intricate correlation of cDC and $CD4^+$ T cells signature in shaping non-IFN-γ-driven

$CD8^+$ T cells expansion that provides the possibility to boost anti-tumor T cell response by blocking IFN-γ as a therapeutic approach in tumors with acquired resistance on sensing IFN-γ.

P19

Regulatory T cells define avidity thresholds for T cell tumor infiltration

M. Mohsen[1], R. Josi[2], P. Krenger[2], S. de Brot[2], D. E Speiser[3], E. Sevick[4], M. F Bachmann[2]

1 bern; 2 Bern; 3 Lausanne; 4 Houston US

Introduction: Regulatory T cells (Tregs) are crucial for peripheral tolerance mostly by maintaining a non-inflammatory environment in healthy tissue.

Aims: In the present study, we extend this function of Tregs to the control of low avidity antigen-specific T cells. We used the low avidity altered peptide ligand A4Y for vaccination with our nano-platform and the aggressive B16F10p33 tumor model. We performed *in vitro* as well as *in vivo* T cell assays. We conducted digital histological assessment to quantify tumor infiltrating lymphocytes (TILs) and their spatial distribution using visual colorization. We studied the survival and the induction of memory T cells in the treated groups via t-distributed stochastic neighbor embedding algorithm.

Results: Using low avidity, altered peptide ligand A4Y for vaccination, we confirm absence of relevant acute lytic activity and anti-tumor protection against the original ligand p33. However, upon depletion of Tregs, A4Y-induced T cells assumed high acute lytic *in vivo* activity and conferred strong protection against p33 tumor growth with similar efficiency as p33-induced T cells. The latter anti-tumor activity was not only due to enhanced acute lytic activity but also due to strongly enhanced tumor infiltration. Our results revealed successful induction of $TCF\text{-}1^+$stem-like and $CD69^+$ tissue-resident specific memory T cells.

Conclusion: These data not only reveal a novel function of Tregs to control activity of low avidity T cells but open up novel pathways to unleash the power of low avidity T cells such as germ-line encoded tumor antigens.

P20

CD8 T cell diversity and differentiation in acute infection

I. Sandu[1], N. Ramirez Comet[1], W. Zheng[1], C. Gerlach[1]

1 Solna SE

Elucidating how cytotoxic CD8 T cell responses to pathogens arise is critical for understanding how infections are cleared. Naïve CD8 T cells have been shown to be heterogeneous, which has an impact on their differentiation upon priming. The goal is to understand how differences observed at the population level are generated and regulated at the clonal level. We aim at characterizing the clonal responses of different naïve CD8 T cell subsets to acute Lymphocytic Choriomeningitis virus by using barcoded transgenic T cell receptor virus-specific cells. Naïve Ly5.1+ OT-I T cells recognizing the SIINFEKL peptide from ovalbumin carrying a unique short DNA sequence will be generated by means of retroviral transduction of thymocytes. This DNA barcode will be present in all progenies derived from a given cell, allowing tracking of individual clones. The pool of barcoded Ly5.1+ OT-I thymocytes will be injected into the thymus of naïve Ly5.2+ specific pathogen-free wild-type mice. After several weeks in the recipient mice to allow generation and maturation of naïve CD8 T cells from the transduced thymocytes, the naïve cells are harvested and adoptively transferred into naïve recipients one day prior LMCV Armstrong infection. After one week, OT-I cells will be sorted into different populations and then sequenced, which will allow us to assess the clonal diversity. Understanding the factors that modulate the clonal diversity of CD8 T cell responses to acute infections has practical implications for vaccine design.

P21

Spatial mapping of cellular microenvironment in inflammed adipose-tissue: Revealing innate immune signatures

R. Ziadlou[1], M. c. Brüggen[1], M. Ameri[1]

1 Zürich

Panniculitis is the inflammation of subcutaneous adipose tissue which has different phenotypes. The pathomechanisms underlying panniculitis remain largely unknown. The aim of our study was to explore the cellular and molecular immune-patterns of two most common panniculitis subtypes, erythema nodosum (EN) and erythema induratum (EI).

We collected paraffin-embedded deep lesional skin biopsies of EN and EI patients (n=16 per group), as well as healthy controls (HC) (n=6). For an immune response-related gene expression analysis, we used bulk-RNA for NanoString. To characterize and map the leukocytic infiltrate, we used imaging mass cytometry (IMC).
IMC analysis revealed distinct cellular profiles of EN and EI and HC. In both conditions, macrophages were the most prominent immune cell type compared to HC. This was accompanied by a highly activated CD8+ T cell population, which clustered with M1 macrophages. These cellular changes were paralleled by an overall strong type 1 immune signature on the mRNA level on both EN and EI. We showed a strong innate immune activation, with upregulation of NLR-, NFkB- and inflammasome-signaling-pathway genes compared to HC. In line with IMC data, type I signature was more pronounced in EI compared with EN.

Our data shift the current paradigm of panniculitis: EN and EI showed a strong type 1 immune response, which is mostly mediated by innate immune cells, namely M1 macrophages. We need to further explore the mechanisms of adipose tissue inflammation and how it could be therapeutically targeted.

P22

Altered neutrophil transcriptional profile and suppressed chemokine release hinder immune cell recruitment in drug-resistant parasitic infections

M. Diaz Varela[1], A. Sanchez Hidalgo[2], S. Calderon Copete[3], V. Tacchini[1], T. Shipley[1], L. G. Ramirez[2], J. Marquis[3], O. L. Fernandez[2], N. Gore Saravia[2], F. Tacchini-Cottier[1]

1 Epalinges; 2 Cali CO; 3 Lausanne

Neutrophils play essential roles in antimicrobial host defense. Drug resistance (DR) to microbes is increasing in the world and the role of neutrophils in modulating antimicrobial resistance is emerging. Here, we investigated the role of neutrophils in drug-resistant cutaneous leishmaniasis.

Patient-derived strains isolated prior to clinical treatment that showed distinct drug susceptibility were used to address the relationship between susceptibility to one widely used anti-leishmanial and the induced neutrophil response. Transcriptomics identified genes specifically induced in human neutrophils exposed to parasites that are susceptible or resistant to the drug. Susceptibility was linked to the induction of inflammatory genes including chemokine genes. These results were validated in human neutrophils using trans-well experiments and in a murine model of infection. In contrast, drug-resistant parasites promoted the upregulation of genes related to cell

detoxification while inducing the downregulation of proinflammatory and chemokine genes.

Neutrophil-derived CCL3 and CXCL8 played a major role in drug susceptibility as blocking these chemokines in the supernatant of neutrophils infected with drug-susceptible parasites resulted in diminished recruitment of monocytes and neutrophils. Moreover, drug-resistant parasites impaired the early recruitment of innate immune cells to the infected murine skin.

Collectively, these findings demonstrate that the interplay between neutrophils and parasite DR modulates the host immune microenvironment, which may open novel avenues to impede DR.

P23

IL-1 protects from fatal systemic candidiasis by inhibition of oxidative phosphorylation and hypoxia

S. Horn[1], M. Kopf[2], M. Schmid[2], I. Berest[2], F. Piattini[2], J. Zhang[2], K. de Bock[2], J. Kisielow[2]

1 Zürich; 2 Zurich

Invasive *C. albicans* infections result in mortality rates of 46-75% in infected populations; understanding the immune mechanisms that promote controlling of the pathogen may contribute to combating such infections. The role of IL-1 cytokines in anti-fungal immune response, is still not well understood. In this study we used mice lacking the global receptor for IL-1 cytokines to study the role of IL-1R in systemic candidiasis. We performed extensive analysis of the infiltrating immune cells, in the first hours and days of infection. Interestingly, we found that, during *C. albicans* infection, only non-hematopoietic IL-1R is playing a crucial role, while hematopoietic IL-1R is dispensable in early anti-fungal response. Single nucleus RNAseq reveals excessive ribosomal activity, glycolysis, redox stress, oxidative phosphorylation and hypoxia across all cell types in the kidney of *Il1r1-/-* mice within a few hours upon infection. By showing that hypoxia promotes fungal growth and pathogenicity, our data suggest that IL-1R-signaling in non-hematopoietic cells is required to prevent fatal candidiasis by inhibiting excessive oxidative phosphorylation and hypoxia in the kidney. Our findings unravel the importance of IL-1 cytokines in the protection of kidney cells against metabolic cataclysm that happens upon systemic *Candidiasis*, and in the inhibition of *C. albicans* growth.

P24

Role of microbial-weaning interplay on long-term autoimmune disease development

L. Morelli[1], R. Abdoul Ahad[1], N. Joseph[1], Z. Al Nabhani[1]

1 Bern

The intestinal microbiota play a key role in the development of the immune system. Weaning is a critical time window early in life during which the expansion of intestinal bacteria provokes a strong immune response termed "weaning reaction". This reaction is necessary to prevent increased susceptibility to gut inflammation in adult mice, a phenomenon called "pathological imprinting". However, the cellular and molecular mechanisms behind the weaning reaction and pathological imprinting remain unclear.

This study aims to define whether dietary and microbial components during weaning affect the generation of the weaning reaction and the susceptibility to develop autoimmune diseases later in life.

We characterized the gut microbiota composition and immune responses in neonatal mice exposed to different ratios of dietary fat and fibers early in life and assessed its long-term consequences using a model of STZ-induced diabetes.

Our results indicate that the diet strongly affected microbiota composition of the weaning mouse and that intestinal CD4+ T cell responses are highly dependent on dietary and microbial components. Adult offspring of mice fed a diet rich in fat early in life develops more severe type 1 diabetes compared to mice fed a fiber-rich diet or normal chow. Transfer of microbial dysbiosis to neonatal germ-free mice, but not to adult, was sufficient to recapitulate the phenotype observed in conventional mice.

This study demonstrates that the composition of gut microbiota imprints the immune system early in life with long-term consequences to develop autoimmune diseases.

P25

Role of type 2 innate lymphoid cells in the regulation of colorectal cancer stem cells

I. Monteiro[1], C. Bachmann[1], P. Naef[1], V. Vu[1], C. Schurch[2], P. Krebs[1], A. Ochsenbein[1], C. Riether[1]

1 Bern; 2 Tübingen DE

Colorectal cancer (CRC) is a common malignancy which contains cancer stem cells (CSCs). CSCs are frequently the cause of treatment resistance and relapse. Type 2 innate lymphoid cells (ILC2s) are a recently described innate immune cell population. Our goal is to study the role of ILC2s upon CRC's stem cells.

We have established a co-culture of mouse colonic ILC2s with organoids isolated from AOM/DSS-treated (azoxymethane / dextran sodium sulfate) Bl/6 mice (CRC organoids) or from healthy Bl/6 mice (healthy organoids). When compared to healthy colons, ILC2s from AOM/DSS CRC colons are comparable in number but produce significantly higher amounts of cytokines. In this setting, ILC2s, or their supernatant, significantly decrease CRC AOM/DSS organoid formation. Surprisingly, when incubating ILC2s with healthy colon organoids, organoid formation is increased. ILC2 depletion (RORaF/F IL7RCre mice or RORawt/wt IL7RCre controls) in AOM/DSS-induced CRC, resulted in significantly more tumor lesions but a lower frequency of CSCs. In contrast, ILC2s lead to a lower stem cell frequency in normal healthy colon of mice. Using a multiplexed immunofluorescence technique (CODEX), ILC2s were identified in the CRCs of 35 patients and associated with a Crohn's-like lymphoid reaction. This type of inflammation is associated with a favorable prognosis for CRC patients.

Overall, ILC2s reduce the stemness of colon cancer stem cells through the secretion of soluble factors, most likely cytokines. Future studies will aim at identifying the specific cytokine(s) and mechanism by which ILC2s influence healthy and cancer colon stem cells.

P27

Investigation of the role of IL-33 for intestinal tumorigenesis

A. Herbst[1], V. Vu[1], M. H. Wasmer[1], S. J. Eugster[1], P. Krebs[1]

1 Bern

Aim: Colorectal cancer (CRC) is one of the leading causes of cancer-related deaths, with chronic inflammation in the intestine being a prominent catalyst. Interleukin-33 (IL-33) signaling plays a controversial role in intestinal inflammation and CRC,

and its exact mode of action in the tumor environment is unclear. Here, we want to investigate the dual role (nuclear/ST2-independent vs soluble) of IL-33 in CRC.

Methods: Intestinal tumors were induced by treating wild-type (WT), *St2*$^{-/-}$, *Il33*$^{-/-}$ and *St2*$^{-/-}$*;Il33*$^{-/-}$ mice with azoxymethane (AOM) and dextran sodium sulfate (DSS). Disease progression was observed over time. MC38 cell lines transduced with different IL-33 isoforms were injected s.c. in wild-type, *St2*$^{-/-}$ and *Il33*$^{-/-}$ mice to observe tumor growth.

Results: *St2*$^{-/-}$ mice treated with AOM/DSS were protected from intestinal tumorigenesis for, whereas *Il33*$^{-/-}$ and *St2*$^{-/-}$*;Il33*$^{-/-}$ mice developed colorectal tumors that were similar in numbers and load to WT controls. In MC38 tumors, nuclear IL-33 showed a pro-tumorigenic effect, while soluble IL-33 acted anti-tumorigenic. Similarly to the AOM/DSS model, *St2*$^{-/-}$ mice injected with MC38 cells showed reduced tumor growth compared to *Il33*$^{-/-}$ and WT mice.

Conclusion: IL-33 has a dual role in the AOM/DSS CRC model: pro-tumorigenic when soluble (ST2-dependent) and anti-tumorigenic in the nucleus (ST2-independent). In a model of s.c. injected MC38 cell lines, nuclear IL-33 has a pro-tumorigenic effect in tumors and an anti-tumorigenic effect in non-tumor tissue.

P28

Mapping of IgG-binding epitopes on major birch pollen allergen Bet v 1 for identification of hypoallergenic peptides with potential therapeutic application

L. Šošić[1], M. Paolucci[2], D. Melillo[2], A. Duda[1], Y. Wäckerle-Men[1], A. Streuli[2], K. Eyer[2], T. Kündig[2], P. Johansen[2]

1 Zürich; 2 Zurich

Aim: Allergy is an important socio-economic health problem currently estimated to affect one billion people worldwide. Allergen immunotherapy (AIT), as the only curative approach, is associated with the stimulation of allergen-specific neutralising IgG4 antibodies and allergen tolerance. While IgE epitope mapping has been important to identify and characterise major allergens, IgG epitope mapping has not yet been a major focus of allergy research. In this project, we aim to define IgG-binding epitopes on the major birch pollen allergen Bet v 1 in order to use this information for the production of hypoallergenic peptides for improved AIT.

Methods: Blood from 30 birch pollen allergic patients and 5 non-allergic control patients was collected. 20 of the allergic patients had received AIT. Serological analysis of allergen-specific IgE and IgG4 was done with ImmunoCAP. Analysis of IgG-secreting B cells was done with ELISpot and by DropMap microfluidics. Linear and conformational IgG epitopes were analysed using CLIPS™ technology (Biosynth).

Results: All allergic patients had Bet v 1 specific IgE, while control patients were negative. Both IgE and IgG4 rose upon SCIT and SLIT. Four specific IgG-binding Bet v 1 epitopes were identified with sera from allergic patients. As a next step peptides based on the epitope sequences will be produced for further testing.

Conclusion: CLIPS™ technology enables linear and conformational mapping of Bet v 1-specific IgG binding sites. It remains to be tested if the epitopes identified stimulate allergen-neutralising antibodies when used in AIT.

P29

Characterization of the obesity-driving bacterial consortia in a highly-standardized gnotobiotic mouse model

M. Scalise[1], L. Brandenburg[1], K. Filipová[1], D. Tandon[1], D. Ring[2], C. Eberl[2], A. Segesser von Brunegg[1], A. Boss[1], S. Hapfelmeier[1], M. L. Balmer[1]

1 Bern; 2 Munich DE

The prevalence of obesity is rapidly rising worldwide due to genetic and environmental factors. The gut microbiota and their metabolites contribute to disease development and germ-free (GF) mice, which are devoid of microbes, are generally more resistant to diet-induced obesity (DIO) than conventional mice.

To understand the mechanism, we aimed to reproduce DIO in a fully standardized gnotobiotic animal model and identify minimal gut microbial consortia that drive HFD-induced obesity.

Gnotobiotic sDMDMm2 mice, colonised for multiple generations with 12 mouse bacterial species representing the bacterial phylum-level diversity of a conventional murine microbiota, showed increased baseline body weight and pronounced DIO, glucose-intolerance and liver steatosis compared to GF mice. In contrast, GF mice, newly colonised with the sDMDMm2 bacteria and studied in the F1 generation showed baseline weights similar to GF and, were almost completely protected from DIO, glucose-intolerance and liver steatosis. However, a reduced gnotobiotic model that we newly colonized with only 5 of 12 sDMDMm2 strains, developed DIO and comorbidities comparable to long-term established sDMDMm2 mice.

In summary, we could successfully reproduce DIO and its complications in a 12-species gnotobiotic animal model. Our data suggests that the bacterial consortium underwent evolution and adaptation within the host over years of breeding, promoting obesity, glucose-intolerance and liver steatosis. Finally, reduction of the model to only 5 bacterial species was sufficient to induce DIO and its comorbidities.

P30

Homeostatic immunity against the skin mycobiome

S. Leibundgut-Landmann[1], F. Ruchti[1], M. Tuor[1]

1 Zürich

The skin is an important barrier protecting the body against environmental stressors. Commensal microbes colonizing the skin are an integral part of homeostatic circuits that contribute to the maintenance of skin homeostasis. In turn, commensals need to be tightly controlled by the immune system to prevent their uncontrolled growth and/or invasion. The fungal community of the skin microbiome is dominated by a single fungal genus, *Malassezia*. Homeostatic immunity against *Malassezia* is mediated by IL-17. Here, we characterised the IL-17-producing cells in the infected skin of experimentally colonized mice and found a major contribution of Vγ4^{+} γδ T cells. γδT17 cells form a long-lasting pool of memory-like cells in the ear-draining lymph nodes, which are protective upon re-challenge. Surprisingly, activation of the γδT17 cell response is independent of the C-type lectin signaling pathway via Card9 and of CD11c^{+} antigen-presenting cells and no T cell receptor signaling is triggered upon stimulation of γδT17 cells with *Malassezia*-pulsed APCs. Also, cytokine stimulation is inefficient at eliciting IL-17A production from *Malassezia*-induced γδ T cell. Instead, conserved soluble structures from *Malassezia* spp., that are not shared with other fungi such as *C. albicans* or *S. cerevisiae*, act directly on γδT17 cells and efficiently trigger IL-17A-production. Together, this study reveals a novel mechanism of homeostatic immunity against the major skin commensal yeast *Malassezia*.

P31

Lymph node dendritic cells harbor inducible replication competent HIV despite years of suppressive ART

R. Banga[1], F. Andrea Procopio[1], E. Lana[1], A. Kauzlaric[1], M. Foglierini[1], O. Alfageme Abello[1], S. H.m. Sluka[2], O. Munoz[1], A. Mastrangelo[1], C. Fenwick[1], Y. Muller[1], M. Cavassini[1], R. Trunfio[1], S. Déglise[1], M. Lichterfeld[3], G. Pantaleo[1], M. Perreau[1], C. Gerald Mkindi[4], C. Daubenberger[4], J. M. Corpataux[1], M. Delorenzi[1]

1 Lausanne; 2 Zurich; 3 Massachusetts US; 4 Basel

Aim: The comprehensive characterization and quantification of the HIV tissue reservoirs is required to design appropriate therapeutic intervention(s) to achieve a cure. While multiple studies demonstrated the preponderant role of CD4 T cells in HIV persistence, the role of dendritic cells (DCs) has long time been neglected, probably because of their short lifespan, their low frequency, the scarcity of human tissues and a strong dogma established from *in vitro* derived DCs. In the present study, we wished to unravel the contribution of the two major lymph node (LN) DC subpopulations (resident *versus* migratory DCs) to HIV persistence.

Methods: To address this issue, we performed 1) an in-depth transcriptomic, phenotypic and functional characterization of LN DCs; 2) assessed the susceptibility of LN DCs to HIV infection *in vitro* and 3) assessed the major virological parameters (viral outgrowth assay, HIV proviral sequencing with integration site analysis and HIV transcription analysis) associated with HIV persistence in LN DCs isolated from viremic and aviremic ART treated HIV-infected subjects.

Results: We showed that LN migratory (Lin-HLADR+CD45+CD11c+CCR7+) and resident (Lin-HLA-DR+CD45+CD11c+CCR7-) DCs were susceptible to HIV infection and supported cycles of *de novo* viral replication *in vitro* ($P<0.05$). In addition, LN resident and migratory DCs isolated from viremic individuals contained intact HIV provirus, were transcriptionally active directly *ex vivo* and were capable of producing HIV RNA/p24 upon TLR7/8 stimulation in vitro ($P<0.05$). Interestingly, both LN DC subpopulations isolated from ART treated HIV-infected individuals contained HIV intact provirus and inducible replication competent and infectious HIV despite the expression of the anti-viral restriction factor SAMHD1. Notably, HIV-1 RNA was detected in culture supernatants of LN DCs from HIV-infected individuals who were treated for up to 14 years of suppressive therapy.

Conclusions: These findings indicate that LN DCs isolated from ART-treated aviremic HIV-infected individuals may represent a yet untapped reservoir of infectious HIV in LN tissues.

P32

Differential regulation of epidermal differentiation and its metabolism by interleukin-13 and interleukin-22 in atopic dermatitis

Y. Mitamura[1], S. M. Duphey[1], N. Stocker[1], P. D'avino[1], M. Li[1], C. A. Akdis[1], M. Sokolowska[1]

1 Davos

Atopic dermatitis (AD) is a chronic skin disease. Interleukin-13 (IL-13) and IL-22 play the central role in the pathogenesis of AD. The influence of these cytokines on the keratinocytes metabolism and metabolic control of skin barrier is still unclear. We investigated the roles of IL-13 and IL-22 on the metabolism of keratinocytes and their subsequent influence on barrier function. Bulk and spatial RNA-seq data of lesional and non-lesional skin of AD patients were analyzed. In addition, we performed bulk RNA sequencing of IL-13-stimulated-reconstructed epidermis (Episkin®). Next, we analysed real-time glycolysis utilization by proliferating and differentiating keratinocytes in response to IL-13 and IL-22 in the seahorse glycolysis stress test. Finally, immunohistochemistry and qPCR experiments in air-liquid interphase cultured keratinocytes and ex-vivo human skin were performed. The expression of glycolysis-related genes was significantly upregulated especially in the epidermis in the lesional skin of AD patients and in the IL-13-treated Episkin together with downregulation of several barrier molecules. In the seahorse experiments, IL-13 increased the early glycolytic capacity of proliferating and differentiating keratinocytes whereas IL-22 decreased the former. The functional blocking of glycolysis by 2-deoxy-d-glucose influenced the expression of some of the keratinocyte-specific molecules. Our study suggests that IL-13 and IL-22 may regulate the glycolytic capacity of keratinocytes, which might further affect skin proliferation, differentiation, and barrier in AD.

P33

Study of a potential regulator of tumor-induced T cell exhaustion

M. Ongaro[1], D. Cropp[2], L. Tillé[2], N. Maillard[2], G. Verdeil[2]

1 Epalinges; 2 Lausanne

Persistent antigen exposure in chronic infection and cancer drives CD8 T cells towards an exhausted state of differentiation. We found that IRF8, an Interferon Regulatory Factor family member and homolog of IRF4, was overexpressed in tumor infiltrating lymphocytes (TILs) from murine and human tumors. Our hypothesis is that IRF8 is part of the regulatory network of transcription factors driving TILs towards exhaustion.

We adoptively transferred CRISPR/Cas9-manipulated tumor-specific CD8 T cells in melanoma-bearing mice to investigate the role of IRF8 in TILs. Specifically, we assessed the capacity of IRF8 knockout (KO) CD8 T cells to control tumor growth and we investigated the expression of inhibitory receptors and production of cytokines by flow cytometric analysis.

We observed a TCR-dependent upregulation of IRF8 in tumor-specific CD8 T cells. Moreover, expression of IRF8 in CD8 T cells was maintained in TILs but not in virus-specific CD8 T cells in settings of chronic infection. CRISPR/Cas9-mediated knockout (KO) of IRF8 could increase IFNγ and GrzB production of TILs compared to the WT counterpart in a melanoma mouse model. The exhaustion-associated transcription factor TOX was also decreased in IRF8 KO TILs. Further, preliminary data indicated that adoptively transferred IRF8 KO T cells enhanced tumor control compared to WT transferred T cells.

Together, our data suggest that IRF8 might play a role in regulating CD8 T cell exhaustion in the context of cancer.

P34

Infectious avatars entering the peripersonal space trigger immune responses

S. Trabanelli[1], J. Fellrath[2], G. Vanoni[3], M. Akselrod[2], T. Bertoni[2], S. Serino[2], G. Ercolano[3], G. Ellena[2], A. Cornu[1], G. Mastria[2], H. Gallart-Ayala[2], J. Ivanisevic[2], P. Grivaz[2], M. P. Paladino[4], C. Jandus[1], A. Serino[2]

1 Geneva; 2 Lausanne; 3 Epalinges; 4 Trento IT

Aim(s) or purpose: Predicting potential contact with pathogens is critical for survival. In primates, a neural mechanism is triggered to predict contact with potential harm by integrating tactile stimuli on the body with external sensory information close to the body (i.e., the peripersonal space (PPS)). Here, we asked whether and how this mechanism anticipates potential contacts

with infection threats and cooperates with the immune system to trigger an anticipatory response.

Design & methods: We exposed healthy participants to potential infection threats in the form of infectious avatars entering their PPS in virtual reality (VR) and measured behavioral, neural, and immune responses, in comparison to the ones evoked by control stimuli or actual contact with pathogens (i.e., flu vaccine).

Results: We show that exposure to infectious avatars is anticipated by multisensory-motor areas, activates the salience network, and modulates innate lymphoid cell frequency and activation. Changes in connectivity between virtual infection-activated brain areas and the hypothalamus, together with a concurrent modulation of neural mediators, link these neural-immune effects to the hypothalamic-pituitary-adrenal axis.

Conclusions: These findings suggest that the brain and the immune system mount an integrated neuro-immune response to infection threats not only once in contact with the body, but already when they overcome a primary functional boundary of body-environment interaction, i.e., the PPS.

P35

The cellular metabolism of SLE NK cells is primarily altered at the level of mitochondrial homeostasis

N. Fluder[1], M. Humbel[1], C. Ribi[1], D. Comte[1]

1 Lausanne

Aim: Systemic lupus erythematosus (SLE) is a prototype autoimmune disease. NK cells from SLE patients are decreased in number, exhibit reduced cytotoxicity and impaired cytokine production, compared to NK cells from healthy controls (HC). To understand the molecular alterations that lead to NK cell dysfunction, we examined the cellular metabolism of peripheral blood SLE compared HC NK cells.

Methods and results: Real-time metabolic assay showed that SLE NK cells mitochondrial respiration (OXPHOS) is significantly increased. Accordingly, we examined the mitochondrial fitness of SLE NK cells by flow cytometry, qPCR and transmission electron microscopy (TEM). Our data indicate that mitochondrial number is similar between SLE and HC NK cells, but mitochondrial mass is increased in SLE, while mitochondrial activity is decreased. TEM examination of SLE NK cells showed mitochondrial cristae disorganization. These data suggest an accumulation of large dysfunctional in SLE NK cells. Quantitative proteomic analysis revealed that SLE NK cells express high levels of proteins associated with mitochondrial assembly. We also observed a reduction in proteins that play a key role in mitochondrial clearance (RNF181, MARCHF5), and lysosomal acidification (TMEM9). Consistently, we demonstrated that the number of lysosomes in SLE NK cells is normal whereas their pH is more alkaline than in HC.

Conclusion: These results suggest an alteration of mitophagy in SLE NK cells and indicate that impaired mitochondrial homeostasis represents a major feature of SLE pathogenesis.

P36

Targeting tumor-associated macrophages to improve immunotherapeutic management of bladder cancer

E. Desponds[1], M. Leblond[1], H. Zdimerova[1], G. Verdeil[1]

1 Lausanne

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. Understanding why MIBC do not respond to these treatments and developing new therapeutic strategies are urgently needed.

Using an anti-PD1-resistant mouse model of BC already developed in the lab, we identified CXCR4-CXCL12 pathway as a promising target to prevent pro-tumor macrophages accumulation. We observed an increase of the expression of the receptor CXCR4 at advanced stages of the disease, specifically on MHCII^{low}/pro-tumor macrophages. Preliminary human immunohistochemistry data confirmed the expression of CXCR4 in advanced human BC. In parallel, the concentration of CXCR4's ligand, CXCL12, was strongly increased in tumor colonized bladder, serum and urine of mice at the MIBC stage. Interestingly, upon the administration of a small CXCR4 inhibitor, we strongly reduced MHCII^{low} macrophage number within the tumor and significantly prolonged mice survival.

Altogether, through the use of this pre-clinical model recapitulating the human disease and the analysis of patient samples, we have identified the CXCR4/CXCL12 pathway as a promising target to decrease pro-tumor macrophages in MIBC. In the future, we aim to translate this knowledge to clinical settings by exploring combination therapies.

P37

Time-of-Day-Dependent Dynamics of Barrier-Tissue Leukocytes

L. Lutes[1], Q. Zeng[1], S. Jemelin[1], V. Oliva[1], F. de Virgiliis[1], C. Scheiermann[1]

1 Genève

Aim: An organism's first defense is the epithelial barrier dividing it from the outside world. Leukocytes in barrier tissues must defend against external threats, however, the likelihood of encountering such threats varies throughout the day. While it is becoming clear that systemic immune responses are time-of-day dependent, it is completely unknown if homeostatic oscillations at initial sites of invasion exist and how they may shape immune responses to external threats.

Methods: We use a combination of flow cytometry and scRNAseq to investigate diurnal leukocyte in the three largest epithelial barrier tissues: the skin, the lung, and the intestine.

Results: We found that each of these organs has its own circadian immune signature at steady state. Lung leukocytes are highly rhythmic and peak during the behavioral rest. While skin leukocytes are less numerically rhythmic than those in the lung, skin macrophages exhibit oscillatory changes in gene expression at the RNA level, suggesting circadian changes in their immune phenotype. Finally, our data for the small intestine suggest that leukocytes may oscillate between the lamina propria and the peyer's patches.

Conclusions: Our findings provide the first ever comprehensive examination of the circadian immune landscape in barrier tissues and shed light on how steady-state circadian changes may impact immune responses. The data generated in this study will create a temporal atlas of steady-state, barrier-tissue immunology that will aid researchers in harnessing natural circadian rhythms to improve barrier tissue immunotherapies.

P38

Epithelial RIG-I inflammasome and ACE2 isoforms in type 2 inflammation, asthma and COVID-19

M. Sokolowska[1], U. Radzikowska[1], A. Eljaszewicz[1], G. Tan[1], N. Stocker[1], A. Heider[1], P. Westermann[1], S. Steiner[2], A. Dreher[1], P. Wawrzyniak[1], B. Ruckert[1], J. Rodriguez-Coira[1], D. Zhakparov[1], M. Huang[1], B. Jakiela[3], M. Sanak[3], M. Moniuszko[4], L. O'mahony[5], M. Jutel[6], T. Kebadze[7], D. Jackson[7], M. Edwards[8], V. Thiel[2], S. Johnston[7], C. Akdis[1], M. Ding[1]

1 Davos; 2 Bern; 3 Cracow PL; 4 Bialystok PL; 5 Cork IE; 6 Wroclaw PL; 7 London GB; 8 London

Rhinoviruses and allergens, such as house dust mite are major agents responsible for asthma exacerbations. The influence of pre-existing airway inflammation on the infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is largely unknown. We analyzed mechanisms of response to viral infection in experimental in vivo rhinovirus infection in healthy controls and patients with asthma, and in in vitro experiments with house dust mite, rhinovirus and SARS-CoV-2 in human primary airway epithelium. Here, we show that rhinovirus infection in patients with asthma led to an excessive RIG-I inflammasome activation, which diminished its accessibility for type I/III interferon responses, leading to their early functional impairment, delayed resolution, prolonged viral clearance and unresolved inflammation in vitro and in vivo. Pre-exposure to house dust mite augmented this phenomenon by inflammasome priming and auxiliary inhibition of early type I/III interferon responses. Prior infection with rhinovirus followed by SARS-CoV-2 infection augmented RIG-I inflammasome activation and epithelial inflammation. Interleukin-13, the main type 2 cytokine, decreased expression of long ACE2 mRNA and reduced glycosylation of full-length ACE2 protein via alteration of N-linked glycosylation process, limiting its availability on the apical side of ciliated cells. House dust mite did not affect the expression of ACE2. Rhinovirus infection increased short ACE2 mRNA, but it did not influence its protein expression. Timely inhibition of the epithelial RIG-I inflammasome and type 2 inflammation may lower the burden of rhinovirus and SARS-CoV-2 infections.

P39

Molecular mechanisms of food emulsifier, polysorbates and professional dishwasher detergents on epithelial barrier disruption

I. Ogulur[1], D. Yazici[2], Y. Pat[2], H. Babayev[2], S. Ardicli[2], B. Rückert[2], A. Heider[2], R. Dhir[3], M. Akdis[2], K. Nadeau[4], C. Akdis[2]

1 Davos Wolfgang; 2 Davos; 3 Los Angeles US; 4 Boston US

Aim: We investigated the effects of food emulsifiers and professional dishwasher detergents on cytotoxicity, barrier function, transcriptome alterations, and protein expression in gastrointestinal epithelial cells.

Design & Methods: Human intestinal organoids, organoid organ-on-a-chips, and liquid-liquid interface cells were cultured in the presence of two emulsifiers, polysorbate 20 (P20) and polysorbate 80 (P80), and dishwasher detergent and rinse aid.

Results: Cells showed lysis in response to P20 and P80 exposure starting at a 0.1% concentration and to rinse aid at 1:20,000 dilution. Epithelial barrier disruption correlated with decreased TEER, increased paracellular-flux. Alcohol ethoxylates found in rinse aid were identified as the culprit with a strong cell toxic and epithelial barrier damaging effect. RNA-seq and targeted-proteomics analyses demonstrated upregulation of pathways related to cell development, proliferation, apoptosis, inflammatory response and response to stress at 0.05% concentration. In addition, immune and inflammatory responses, cell death, migration, epithelial development, proliferation, were significantly upregulated in response to rinse aid. Epithelial cells incubated with residue from professional dishwashers demonstrated the presence of a significant amount of cytotoxic detergents remaining on ready to use dishware.

Conclusions: Our studies provide evidence on the detrimental effects of food emulsifiers and rinse aid on gut epithelial cell integrity. Even at concentrations lower than the toxic doses, they induced proinflammatory responses.

P40

Role of the sympathetic nervous system in leukocyte dynamics in the skin

V. Oliva[1], C. Scheiermann[1], C. Wang[1], F. de Virgiliis[1], L. Lutes[1], C. Barnoud[1]

1 Genève

A crosstalk exists between the nervous system and the immune system, with important implications for homeostasis and disease. All tissues are innervated by the sympathetic nervous system (SNS), with nerve fibers locally releasing the neurotransmitter noradrenaline. The skin represents one of the first lines of defense of an organism against pathogens. It harbors numerous immune cells, but it is also densely innervated by the SNS. Immune cells in the skin act as sentinels, reacting to physical injuries or pathogens by mounting a robust inflammatory response and migration to the draining lymph node. Dendritic cells (DCs) can recognize and expose external pathogens on their surface to activate T cells upon migrating to lymph nodes. The migration of DCs is therefore crucial to achieve an efficient immune response. Here, we show, via both *ex vivo* and *in vivo* approaches using B2-adrenergic receptor KO mice, that noradrenergic signaling via the B2-adrenergic receptor reduces trafficking of DCs into afferent lymphatic vessels and the draining lymph node. This study provides mechanistic insights into how the immune system is tuned by the SNS, which could be used to optimize vaccination regimes.

P41

Regulation of gut epithelial barrier by microbiome metabolites

Y. Pat[1], D. Yazici[1], I. Ogulur[1], S. Ardicli[1], S. Simmons[2], A. Almada[2], C. Avena[2], T. Jensen[2], M. Li[1], Y. Mitamura[1], H. Babayev[1], A. Heider[1], R. Dhir[2], M. Akdis[1], K. Nadeau[3], C. Akdis[1]

1 Davos; 2 California US; 3 Boston US

It is known that healthy gut microbiota-related metabolites promote epithelial homeostasis. However, the increased exposure to food additives such as emulsifiers and food colorants in processed food poses a significant threat to the gastrointestinal epithelial barrier. In this context, the microbiota and their metabolites could be the key to rescuing the damaged gastrointestinal barrier. With this perspective, we identified four different microbiome metabolites that elicit rescue activity against the epithelial barrier damage of sunflower-derived lecithin using a Caco-2 microfluidic organochip. Our results revealed that a prototype, compound X alone increased the TER value during the development phase of the gut barrier.Furthermore, when the gut barrier developed in the presence of compound X, it exhibited a preventive effect against the damage caused by sunflower-derived lecithin, as demonstrated by TER, paracellular permeability assays, and immunofluorescence staining. The expression of claudin 1 and 4 was also increased with compound X treatment, compared to the control and sunflower-derived

lecithin-treated conditions. Compound X displayed anti-inflammatory effects, as detected by targeted proteomics. Immunofluorescence staining of occludin and ZO-1 on a gut-on-a-chip model indicated irregular and heterogeneous protein localization after exposure to lecithin. In conclusion, our current data highlight compound X, a microbiome metabolite, as a promising agent for rescuing and treating the gut epithelial barrier disruption and inflammation caused by food additives.

P42

Epithelitis: barrier disruption and activation of pro-inflammatory pathways in gut epithelial cells by food additives

D. Yazici[1], Y. Pat[1], S. Ardicli[1], I. Ogulur[1], S. Simmons[2], A. Almada[2], C. Avena[2], T. Jensen[2], M. Li[1], Y. Mitamura[1], H. Babayev[1], A. Heider[1], B. Buyuktiryaki[3], C. Sackesen[3], R. Djir[2], M. Akdis[1], K. Nadeau[4], C. A. Akdis[1]

1 Davos Wolfgang; 2 California US; 3 Istanbul TR; 4 Boston US

The barrier function of the epithelia is crucial for maintaining homeostasis. Environmental exposures, such as food additives may alter the epithelial barrier integrity and influence development of diseases. In the present study, a microfluidic-titer plate was used to enable the formation of an epithelial barrier by Caco-2 cells to examine the effects of commonly used food additives. Several commonly used food additives, such as sunflower-derived lecithin (SunLec), DATEM, sodium saccharin (NaSa) and carboxymethylcellulose (CMC), were examined at consumer-relevant doses using transepithelial-electrical resistance (TER) and targeted proteomics. Results showed that DATEM, SunLec and NaSa exhibited a dose-dependent cytotoxicity exceeding 40% when exposed to 0.25 mg/ml of DATEM, 0.39 mg/ml of SunLec and over 20% when exposed to NaSa above 1.56 mg/ml. However, CMC did not have any cytotoxic effect. DATEM, SunLec and NaSa elicited a dose- and time-dependent decrease of relative TER. Although CMC did not exhibit any effects on cytotoxicity and barrier integrity, it induced the production of pro-inflammatory molecules, as observed with DATEM, SunLec, and NaSa. SunLec and DATEM specifically upregulated cytokine-mediated signaling pathways, such as NOD-like receptor and MAP kinase pathways. Conversely, CMC resulted in upregulation of proinflammatory and profibrotic mediators. NaSa upregulated chemokines associated with granulocyte chemotaxis and response to IL-1β. In conclusion, current data show that commonly used food additives cause epithelitis and disrupt gut barrier integrity.

P43

JAK-inhibitor treatment for inborn errors of JAK/STAT signalling: An ESID and EBMT IEWP retrospective study

M. Fischer[1], P. Olbrich[2], J. Hadjadj[3], V. Aumann[4], S. Bakhtiar[5], V. Barlogis[6], P. von Bismarck[7], M. Bloomfield[8], C. Booth[9], E. Buddingh[10], D. Cagdas[11], M. Castelle[3], A. Y. Chan[12], S. Chandrakasan[13], K. Chetty[9], P. Cougoul[14], E. Crickx[15], A. Deyà-Martínez[16], S. Farmand[17], R. Formankova[8], A. R. Gennery[18], L. I. Gonzalez-Granado[19], D. Hagin[20], L. G. Hanitsch[21], J. Hanzlikovà[22], F. Hauck[23], J. Ivorra Cortés[24], K. Kisand[25], A. Kiykim[26], J. Körholz[27], T. R. Leahy[28], J. van Montfrans[29], Z. Nademi[18], B. Nelken[30], S. Parikh[13], S. Plado[31], J. Ramakers[32], A. Redlich[7], J. Rivière[16], Y. Rodina[33], P. Roxo Júnior[34], S. Salou[35], C. Schütz[27], A. Shcherbina[33], F. Touzot[36], E. Unal[37], S. Burns[9], M. R. Seppänen[38], O. Neth[2], M. H. Albert[23], S. Ehl[35], B. Neven[3], C. Speckmann[35]

1 Zurich; 2 Sevillla ES; 3 Paris FR; 4 Magdeburg DE; 5 Frankfurt am Main DE; 6 Marseille FR; 7 Kiel DE; 8 Prague CZ; 9 London GB; 10 Leiden NL; 11 Ankara TR; 12 San Francisco US; 13 Atlanta US; 14 Toulouse FR; 15 Créteil FR; 16 Barcelona ES; 17 Hamburg DE; 18 Newcastle upon Tyne GB; 19 Madrid ES; 20 Tel-Aviv IL; 21 Berlin DE; 22 Pilsen CZ; 23 Munich DE; 24 Valencia ES; 25 Tartu EE; 26 Istanbul TR; 27 Dresden DE; 28 Dublin IE; 29 Utrecht NL; 30 Lille FR; 31 Tallinn EE; 32 Palma ES; 33 Moscow RU; 34 São Paulo BR; 35 Freiburg im Breisgau DE; 36 Québec CA; 37 Kayseri TR; 38 Helsinki FI

Aims: Inborn errors of immunity (IEI) with dysregulated JAK/STAT signalling can present with variable manifestations of severe immune dysregulation. Hematopoietic stem cell transplantation (HSCT) is potentially curative, however reported treatment related mortality of JAK/STAT patients is significant. Targeted therapies with JAK Inhibitors (JAKi) offer a promising alternative treatment option. However, data on the current prescribing practice, treatment efficacy and adverse events (AE) are limited. We sought to evaluate the current off-label JAKi treatment experience for JAK/STAT IEI among ESID/EBMT-IEWP centers.

Methods: Multicenter retrospective study on patients with pathogenic variants in a JAK/STAT IEI-gene, who received JAKi treatment for at least 3 months.

Results: 70 patients (73% children) were included (45 STAT1-GOF, 21 STAT3-GOF, 1 STAT1-LOF, 1 STAT5B, 1 SOCS1, 1 JAK1-GOF). Ruxolitinib was the predominantly prescribed JAKi (81%). Improvement of general well-being was observed in 91%. However, therapeutic responses varied among underlying diseases and manifestations. Moreover, we documented the use of very heterogeneous dosing and monitoring regimens. AE (i.e. infections and weight gain) were frequent (39%) but mild and transient in most patients. Currently, 74% of patients are maintained on JAKi while 16% patients have received HSCT.

Conclusions: Our study suggest that JAKi are effective to treat symptomatic JAK/STAT-IEI patients. Prospective randomized studies to define optimal JAKi dosing for the variable clinical presentations and age ranges should be pursued.

P44

Safety and immunogenicity of the mRNA-1273 vaccine for SARS-CoV-2 in solid organ transplant recipients

A. L. Figueroa[1], F. Priddy[1], D. Stolman[1], U. Siangphoe[1], I. L. Lasso[1], E. de Windt[1], B. Girard[1], J. Azzi[2], B. Eghtesad[3], H. Zhou[1], J. M. Miller[1], R. Das[1]

1 Cambridge US; 2 Boston US; 3 Cleveland US

Aim: Immunosuppressed persons remain at high-risk for severe COVID-19.

Methods: Study P304 is an open-label, phase 3b trial evaluating mRNA-1273 safety and immunogenicity in 137 adult kidney and 77 liver solid organ transplant recipients (SOTR) and 20 healthy participants. In Part A, SOTR received ≤3 doses of mRNA-1273; healthy participants received 2 doses. In Part B, a booster dose (BD) was offered to participants ≥4 months from the primary series. Neutralising antibody (nAb) geometric mean concentrations (GMCs) vs D614G spike protein and binding antibody (bAb) GMCs vs D614G and variants of interest were assessed.

Results: In baseline-SARS-CoV-2-negative SOTR, mRNA-1273 elicited modest nAbs 1 month post-dose 2. At 1 month post-dose 3, mRNA-1273 enhanced antibody responses; liver SOTR nAb was comparable to post-dose 2 responses in healthy participants. At 1 month post-BD, mRNA-1273 boosted nAbs vs pre-BD regardless of primary series vaccination. Post-boost enhancement vs post-dose 2 in liver SOTR was ~3-fold but lower than healthy participants. Reduced antibody responses were observed in kidney vs liver SOTR; most were on multiple immunosuppressants. bAbs were lower against omicron vs other variants. mRNA-1273 was well-tolerated in SOTR. Four vaccine-related SAEs reported by the investigator may have been related to pre-existing comorbidities. No vaccine-related biopsy-proven organ rejection or death was reported.

Conclusions: mRNA-1273 enhanced immune responses in liver and kidney SOTR and was safe and well-tolerated.

P46

Impact of the COVID-19 Pandemic on Rates of Cytomegalovirus Testing and Diagnosis Among Immunocompromised Children in the United States

P. Jain[1], S. Basnet[2], S. Syed[1], B. Arakaki[1], K. E. Mues[1], Z. Marcum[1], J. Diaz-Decaro[2]

1 New York US; 2 Cambridge US

Aim: Cytomegalovirus (CMV) is a latent virus that may result in severe complications and worsening prognosis among immunocompromised individuals. No studies have examined how the COVID-19 pandemic may have impacted CMV testing and diagnosis among immunocompromised children in the US. In this study, we evaluated changes in CMV testing and diagnosis rates among immunocompromised children (aged <1-11 years) during the COVID-19 pandemic in the US.

Methods: This was an observational retrospective cohort study using HealthVerity claims data from December 2017 to May 2022. A quasi-Poisson interrupted time series model accounting for seasonality was used to determine if underlying CMV testing and diagnosis trends were associated with COVID-19-related interruption time points. Three distinct time periods were defined: pre-COVID-19 pandemic, 6/1/2018-3/31/2020; pre-COVID-19 vaccine, 4/1/2020-12/31/2020; and post-COVID-19 vaccine, 1/1/2021-5/2022.

Results: Comparing the observed rates of CMV testing in the pre- and post- vaccine periods to the rate that would have been observed based on the trends observed in the pre- pandemic period (ie, the counterfactual), the rate of CMV testing slightly decreased in the pre- vaccine (event rate ratio [ERR], 0.98; 95% CI, 0.964-0.995) and post- vaccine periods (ERR, 0.99, 95% CI, 0.979-0.992). The results were consistent for CMV diagnosis.

Conclusions: Declaration of COVID-19 as a pandemic and the initial EUA for a COVID-19 vaccine were both associated with a slight decrease in CMV testing and diagnosis rates among immunocompromised children.

P47

SHAECS: The Swiss Hereditary Angioedema Cohort Study

I. Morales[1], W. A. Wuillemin[2], S. Zeerleder[2], L. M. Bachmann[3], L. Jörg[1], B. Ballmer-Weber[4], P. Schmid-Grendelmeier[3], C. Weber[3], H. Hengartner[4], U. C. Steiner[1]

1 Bern; 2 Lucerne; 3 Zurich; 4 St.Gallen

Aims: New medications have improved the treatment options for patients affected by hereditary angioedema (HAE). However there is limited knowledge about the long-term impact of these therapies on the quality of life and their influence on therapeutic decision-making. These questions are addressed in a longitudinal cohort study.

Designs and methods: A prospective, longitudinal, multi-center cohort study in Switzerland. Participating centers are Bern, Lucerne, Zurich and St. Gallen. Centers in the French- and Italian speaking part will be included later. Patients diagnosed with HAE Type 1, Type 2, HAE with normal C1-Inhibitor and acquired angioedemas are included. Clinical data including HAE attack-rate, medication use, comorbidities and quality of life (Angioedema Control Test, Angioedeme Quality of Life) are collected at inclusion and at annual follow-ups. Blood- and urine samples will be obtained at inclusion, at annual follow-ups and if possible during HAE attacks. Biosamples are stored in a central biobank at the Inselspital Bern. The study is planned for the duration of 15 years.

Results: Data and sample collection is installed. The first 5 patients have already been included. We aim to include the majority of the 150-180 HAE patients in Switzerland into the SHAECS.

Conclusions: This study will provide answers to pertinent questions concerning treatment influence on quality of life and treatment decision making. The study will provide knowledge about clinical characteristics, disease burden, treatment patterns, patient reported outcomes and disease pathophysiology of HAE.

P48

Dupilumab-induced eosinophilia in patients with Diffuse Type 2 Chronic Rhinosinusitis

F. Ryser[1], A. Yalamanoglu[2], A. Valaperti[2], C. Brühlmann[2], T. Mauthe[2], S. Traidl[2], M. B. Soyka[2], U. C. Steiner[1]

1 Bern; 2 Zürich

Aim: Dupilumab, an anti-IL-4Ra antibody, is approved for several type 2 inflammatory diseases like asthma, atopic dermatitis, and diffuse type 2 chronic rhinosinusitis (CRS). Clinical studies had reported a transient increase in blood eosinophils during dupilumab therapy. This study aimed to assess the impact of elevated blood eosinophils on clinical outcomes and to investigate the cause of high blood eosinophil levels under dupilumab therapy.

Methods: Patients suffering from diffuse type 2 CRS treated with dupilumab were examined on days 0, 28, 90, and 180 after therapy start. Sino-Nasal-Outcome-Test Score (SNOT-22), Total Nasal Polyp Score (TNPS), and blood samples were collected. Cytokine measurements, proteomics, and flow cytometry analysis were conducted.

Results: 68 patients were included. Baseline eosinophilia ≥0.3G/L was observed in 63.2% of patients, and in 30.9% of patients, eosinophils increased by ≥0.5G/L under dupilumab. Eosinophil elevation during dupilumab therapy had no impact on clinical scores. The eosinophil adhesion molecule VCAM-1 decreased significantly during therapy in all patients. The chemokine receptor CXCR4 was significantly down- and IL-4 upregulated in subjects with eosinophil increase.

Conclusion: Our findings suggest that increased eosinophils in type 2 CRS are associated with a good clinical response to dupilumab. Patients with elevated IL-4 at baseline developed transient eosinophilia. We identified the downregulation of VCAM-1 and surface markers CD49d and CXCR4 on eosinophils as possible explanations of dupilumab-induced eosinophilia.

P49

CD4+ T Cells reduce the Population of Leukemic Stem Cells in Acute Myeloid Leukemia

N. Schnüriger[1], W. Gharib[1], I. Keller[1], L. Taylor[1], R. Bruggmann[1], A. Ochsenbein[1], C. Riether[1]

1 Bern

Leukemic stem cells (LSCs) are the origin of acute myeloid leukemia (AML). LSCs are frequently resistant to current therapies and elimination by cells of the immune system.

Our major goal is to study the role of $CD4^+$ T cells in the regulation of LSCs in AML.

LSCs and T cells were isolated from bone marrow (BM) and blood of AML patients. $CD4^+$ and $CD8^+$ T cells were analyzed

by scRNA-sequencing. The interactions between LSCs and T cells were studied by co-culture experiments and functional readouts such as colony formation, flow cytometry and FluoroSpot. RNA-sequencing was performed to understand how CD4⁺ T cells affect LSCs on a transcriptional level.

Co-culture with autologous BM CD4⁺ T cells reduced clonogenicity of LSCs but not normal hematopoietic stem cells. ScRNA-sequencing identified a subpopulation of CD4⁺ T cells expressing Granzymes and other cytotoxicity genes enriched in the BM of AML patients compared to healthy controls. We show that CD4⁺ T cells can release Granzyme B in the presence of LSCs and that blockade of Granzyme B partially restored colony formation in co-culture experiments, suggesting that cytotoxic killing is just one mechanism how CD4⁺ T cells affect LSCs. Furthermore, CD4⁺ T cells additionally trigger signaling pathways in LSCs involved in cell cycle stimulation and differentiation

Our data shows that CD4⁺ T cells reduce LSCs in AML by various mechanisms, including direct killing and induction of cell cycle or differentiation. Subsequent experiments will reveal the signaling cascades that are triggered in LSCs after interaction with CD4⁺ T cells.

P50

Dupilumab normalizes correlates of lysosomal function in atopic dermatitis

C. Bürgler[1], D. Simon[1], M. Lehmann[1], D. Dutoit[1], S. Radonjic-Hoesli[1], K. Thormann[1], H. U. Simon[1], S. Yousefi[1]

1 Bern

Aim: Atopic dermatitis (AD) is characterized by skin barrier dysfunction and immune dysregulation. Autophagy, important for the epidermal differentiation, is impaired in AD. Treatment with dupilumab, an IL-4/IL-13 receptor blocker, can reduce skin inflammation and restore the skin barrier. We investigated the effect of dupilumab on the expression of key proteins involved in autophagy and lysosomal degradation.

Methods: We performed immunofluorescence staining and microscopic analyses of skin of AD patients before and under therapy with dupilumab to investigate the expression of autophagy-related (ATG)5 and ATG7 proteins, beclin-1, microtubule-associated protein light chain 3 (LC3B), sequestosome-1 (p62), lysosomal proteases (cathepsins B, D, L), serine protease inhibitors (SERPINB3, SERPINB4), interleukin (IL)-33 and thymic stromal lymphopoietin (TSLP).

Results: The expression of LC3B, p62 and SERPINB3, SERPINB4 was highly increased in untreated AD skin compared to non-lesional and normal skin and decreased upon dupilumab therapy. In contrast, the expression of ATG5 and ATG7 further increased under therapy. Before therapy, cathepsin D and L expression levels were significantly lower compared to normal skin, but increased under dupilumab therapy. The increased expression of IL-33 and TSLP in the epidermis of AD patients correlated with that of LC3B and p62.

Conclusions: Our study provides further evidence that autophagy is inhibited in lesional AD skin owing to lysosomal dysfunction. Upon dupilumab therapy, a restoration of dysregulated key players of autophagy is observed.

P51

Detection of TPSAB1 copy number variation for the diagnosis of hereditary alpha-tryptasemia by qPCR is a valuable alternative method to ddPCR.

D. Spoerl[1], M. Docquier[1], T. Nouspikel[1], M. Abramowicz[1], J. L. Blouin[1], J. Seebach[1]

1 Geneva

Aim: Hereditary alpha-tryptasemia (HAT) is a recently described inherited condition associated with elevated basal tryptase levels, characterized by extra copies of the alpha tryptase encoding gene *TPSAB1.* The diagnosis of HAT can be challenging and requires a careful analysis of the *TPSAB1* and *TPSB2* copy number variation (CNV). The gold-standard method of CNV analysis is digital droplet PCR (ddPCR). Here we investigated whether quantitative PCR (qPCR) could be used instead of ddPCR to correctly identify individuals with HAT.

Methods: A total of 25 patients with elevated basal tryptase levels of unknown origin (except for one with known mastocytosis) were recruited in our outpatient unit. Additionally, 29 randomly selected DNA samples obtained for other genetical tests were used as controls. *TPSAB1* CNV were tested in parallel by qPCR on blood DNA and ddPCR on mucosal swab DNA.

Results: One positive qPCR was a false positive result. The two methods were compared, showing a 96% consistency. Supposing a normal number of *TPSAB1* copies in the randomly selected controls, qPCR specificity was 97% and sensitivity was 100%.

Conclusions: qPCR shows good results in discriminating patients with HAT from healthy controls. qPCR could be used in laboratory facilities where ddPCR is not available. We hope that our findings can help to spread the availability to identify patients with HAT, possibly avoiding unnecessary bone marrow biopsies for suspected mastocytosis. Positive results by qPCR close to the cutoff should be verified by ddPCR if available.

P52

Investigation Bio-Layer interferometry as a quantification method for IL-1b

J. Jorge Da Costa[1], M. Bachmann[1], M. Vogel[1]

1 Bern

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). Therapeutic strategies with anti-IL-1b monoclonal antibodies and other inhibitors decrease the action of IL-1β, and although effective, relapses are still frequent. Monitoring IL-1β in patients under treatment, can impact decision about dose regulation and frequency, and better control disease progression.

The presence of IL-1b levels in human serum, around picogram/mL, makes it challenging for detection with ELISA assays or similar, so herein we present biolayer interferometry (BLI) based assay to overcome this difficulty. Our main goal using this technology is to reach physiological detection of IL-1b at a concentration around 1pg/mL and be able to discriminate signal for concentrations ranging between 0.1pg/mL and 10 pg/mL, accounting in this way for dilution factor. To achieve this a sandwich-based assay was built up in which Canakinumab was loaded on biosensor, followed by incubation first with IL-1b at different concentration and then with a second anti-IL1b antibody, Gevokizumab. Real-time detection was monitored using 3,3'-diaminobenzidine (DAB), which amplifies the signal. The results showed a linear response and sensitivity for IL-1β detection at a concentration of 10 fg/mL. This sensitivity which

is 1000 times higher with that obtained with a conventional ELISA brings BLI assay to the light as a quantification tool.

P53

Exploring the Clinical Potential of Siglec-1 Immunoassay in Autoimmune and Inflammatory Diseases.

J. Iype[1], M. Fux[1], J. Hogervorst[1], U. Wirthmüller[1], M. Horn[1], U. Steiner[1]

1 Bern

Aim: To evaluate sialic acid binding Ig-like lectin 1 (Siglec-1) expression on circulating monocytes by flow cytometry as type I interferon biomarker in autoimmune and inflammatory diseases.

Methods: We developed an immunoassay that enables quantification of Siglec-1 molecule expression on monocytes per cell. We evaluated preanalytical handling, established reference values, and assessed the impact of flow cytometer variations of the assay. Additionally, we investigated Siglec-1 in patients with autoimmune and inflammatory diseases to explore its potential association with disease status.

Results: Staining within 8 hours post-blood collection yielded comparable results to analysis within 4 hours. Different flow cytometers exhibited CVs <5%, indicating standardized testing independent of variables. The reference range for Siglec-1 was determined as 450 molecules per monocyte. In a Lupus-patient with elevated Siglec-1, administration of interferonα-receptor blocker therapy resulted in a significant reduction in Siglec-1.

Conclusions: As the first lab in Switzerland to implement the Siglec-1 assay in a diagnostic setup, we successfully established the methodology and validated its performance. Preliminary findings suggest Siglec-1's potential as a biomarker for SLE, with distinct expression patterns at disease onset and during therapy. Further research is warranted to explore its value as a biomarker and the broader applicability in autoimmune and inflammatory diseases.

P54

"Cytokinergic" IgE clone SPE-7 contains functional IgG-IgE complexes

M. Sypka[1], M. Zwicker[1], A. C. Uldry[1], M. F. Bachmann[1], M. Vogel[1], P. Engeroff[1]

1 Bern

IgE plays a vital role in type 2 immune responses. IgE binds to two principal receptors, the high-affinity IgE receptor FcεRI and the low-affinity IgE receptor FcεRII CD23, the former being abundantly expressed by mast cells and basophils, whereas the latter is constitutively expressed in B cells. If an allergen binds to the already formed IgE-FcεRI complex, the mast cell/basophil degranulation and cytokine synthesis take place, leading to both immediate and late-phase allergic reactions. The mouse IgE anti-DNP clone SPE-7 has been shown to induce mast cell degranulation and cytokine release in the absence of the allergen. Therefore, it was designated as a "cytokinergic" molecule, highlighting its unique attributes. Here, we compared this clone to a number of other IgE clones in a variety of immunoassays, proteomic analysis, and its functional effects on FcεRI expressing bone-marrow derived mast cells and CD23 expressing B cells. Surprisingly, we found that IgE SPE-7 contains IgG-IgE complexes. The removal of those IgG-IgE complexes from IgE SPE-7 reversed a number of its unique functional effects including mast cell degranulation, mast cell interleukin-6 release, mast cell survival and CD23 binding. Our findings suggest that "cytokinergic" IgE features can occur when IgE is pre-complexed with IgG molecules.

P55

The transcriptional regulator IκBζ controls the effector functions of group 2 innate lymphoid cells

I. Iten[1], X. Feng[2], M. Matsushita[1], C. Schneider[2], M. Kopf[1]

1 IMHS, ETH Zurich, Zurich; 2 Institute of Physiology, University of Zurich, Zurich

Group 2 innate lymphoid cells (ILC2s) are dispersed across various organs, where they contribute to control nematode infections and regulate tissue homeostasis. Following activation by epithelial cell-derived factors such as IL-33 and IL-25 as well as other stimuli including neuropeptides and lipid mediators, ILC2s expand and secrete large amounts of IL-5 and IL-13. However, the regulation of the transcriptional program governing ILC2 functions remains incompletely understood. Here, we show that the transcriptional regulator IκBζ, an atypical member of the IκB family, is essential for ILC2 functions at steady state as well as after cytokine- and helminth-induced activation. *Nfkbiz* (encoding for IκBζ) is constitutively expressed in peripheral ILC2s and is further induced after stimulation by IL-33 or IL-25 in an NF-κB–dependent manner. In the absence of IκBζ, ILC2s developed normally but exhibited reduced capacity to proliferate and failed to secrete IL-5 and IL-13 upon activation. Due to the impaired type 2 effector response of IκBζ-deficient ILC2s, *Nfkbiz*$^{fl/fl}$/Vav1-Cre had significantly higher worm burden at day 9 after infection with *Nippostrongylus brasiliensis* compared to *Nfkbiz*$^{fl/fl}$ controls but are protected from papain-induced allergic lung inflammation. Taken together, we demonstrate that IκBζ is essential for induction of the ILC2 effector program involved in nematode expulsion as well as type 2 allergic lung inflammation.

P56

SARS-CoV-2 entry and replication is inhibited by neutrophil serine proteases via degradation of the spike protein in vitro and in vivo

C. Benarafa[1], N. Kozarac[1], N. Leborgne[1], C. Devisme[1], A. Taddeo[1], I. Berenguer Veiga[2], N. Ebert[2], A. Godel[1], L. Grau Roma[3], M. Scherrer[4], P. Plattet[4], V. Thiel[2], G. Zimmer[1]

1 Institute of Virology and Immunology, Mittelhäusern; 2 Institute of Virology and Immunology, Bern; 3 COMPATH, Bern; 4 Division of Neurological Sciences, Bern

Aim: Serine proteases TMPRSS2 and furin, or cathepsins L and B are critical for SARS-CoV-2 entry by proteolytic processing of the spike protein (S). Severe COVID-19 disease is associated with a massive influx of neutrophils in the lungs and these inflammatory cells are known to release potent serine proteases (neutrophil elastase (NE), cathepsin G (CatG), and proteinase-3 (PR3)) as a host defense mechanism against the virus. Little is known about the role of neutrophil serine proteases (NSPs) in SARS-CoV-2 replication and pathogenicity.

Methods: We used purified NSPs to test spike degradation in vitro and to determine the effect of NSPs on virus entry using chimeric VSV virus and SARS-CoV-2 replication in Vero/TMPRSS2 cells. NSP-deficient mice were used to determine the function of each protease on virus replication, inflammation and pathology in vivo.

Results: We identify that all three NSPs degrade the S protein of the original pandemic virus protein or the mouse-adapted S (MA10) protein and prevent virus entry in vitro. In NSPs knockout mice infected with mouse-adapted SARS-CoV-2 virus, we demonstrate that deletion of CatG, but not of NE nor PR3, is associated with higher virus titers in the lung. Importantly, we show that lung cytokine and chemokine expression, and pulmonary pathology were increased in *NE.CatG*$^{-/-}$ double-deficient mice compared to wild-type mice.

Conclusions: These findings suggest that NSPs contribute to the innate immune response against SARS-CoV-2 infection via proteolytic inactivation of the S protein and by limiting pulmonary inflammation.

P57

Emergence and fate of stem cell-like Tcf7+ CD8+ T cells during a primary immune response to viral infection

A. Dumez[1], D. Pais Ferreira[2], J. Gomes Silva[3], T. Wyss[4], R. Veber[1], M. Danilo[5], D. Pinschewer[6], M. Charmoy[1], W. Held[1]

1 Department of Oncology, University of Lausanne, Lausanne; 2 Ichnos Sciences Biotherapeutics SA, Epalinges; 3 Lausanne; 4 SIB Swiss Institute of Bioinformatics, Bioinformatics Core Facility, Lausanne; 5 Department of Oncology, Centre Hospitalier Universitaire Vaudois, Lausanne; 6 Department of Biomedicine, Division of Experimental Virology, University of Basel, Basel

In response to infection naïve $CD8^+$ T cells (T_N) yield a large pool of short-lived terminal effector (T_{TE}) cells that eliminate infected cells and a minor population of stem cell-like central memory (T_{CM}) cells that maintain immunity following pathogen clearance. It has remained unclear whether stem-like T_{CM} cells arise by de-differentiation from a subset of cytolytic T_{TE} cells or whether priming generates stem-like cells that will seed the T_{CM} compartment and, if so, when cytolytic T_{TE} cells would branch off. Here we show that $CD8^+$ T cells with stem-like properties, which are identified by Tcf1 expression, are present across the primary response to infection. Priming programs T_N cells to undergo multiple cell divisions, over the course of which Tcf1 expression is maintained. These $Tcf1^+$ cells further expand relatively independently of systemic inflammation, antigen dose or affinity and they quantitatively yield $Tcf1^+$ T_{CM} cells following pathogen clearance. Early divided $Tcf1^+$ cells can differentiate into $Tcf1^-$ cells in response to inflammatory signals, which suppress Tcf1 expression. Tcf1 downregulation is associated with the irreversible loss of self-renewal capacity and the silencing of stem/memory genes, which precedes the stable acquisition of a T_{TE} state. Tcf1 expression restrains cell cycling, explaining in part the limited expansion of $Tcf1^+$ relative to $Tcf1^-$ cells during the primary response. Thus, terminal effector differentiation is a step wise process that is initiated by inflammation in primed stem-like cells, which by default become central memory cells.

P58

Photochemical internalization of a tuberculosis vaccine for stimulation of T-cell responses: a mouse study

P. Johansen[1], T. Kündig[1], Z. Kotkowska[2], P. Sander[3], A. Høgset[4], Y. Wäckerle-Men[3]

1 University Hospital Zurich, Zürich; 2 University of Zurich, Zürich; 3 University of Zurich, Zurich; 4 PCI Biotech, Oslo NO

Aim: Intracellular pathogens are not treated by vaccination due to inaccessibility for antibodies and poor stimulation of cytotoxic T-cell responses. Photochemical internalization (PCI) is a technology that can facilitate cytosolic antigen delivery to APCs, thereby stimulating MHC class I-restricted responses in CD8 T cells. The study aim was to apply PCI with *M. bovis* BCG to trigger CD8 T cells with potential anti-tuberculosis effects.

Methods: Mice received intradermal injections of live *M. bovis* BCG and photosensitizer TPCS2a. TPCS2a was activated by administration of light 18 hours later. *M. bovis* BCG-specific CD4 and CD8 T-cell responses were measured in blood, spleen and early inflammatory reactions in the skin.

Results: PCI improved BCG-specific response of CD4 and CD8 T-cells, characterized by T-cell proliferation and production of IFN-ɣ, TNF-α, IL-2, and IL-17. Light-activation of TPCS2a was necessary for the effect, and PCI improved antigen presentation in part by causing upregulation of MHC and costimulatory molecules on APCs. The skin inflammation suggested strong involvement of neutrophils.

Conclusions: PCI-based vaccination can be applied to live bacteria for targeted delivery of antigen to the cytosol of APCs. PCI enabled cross-presentation of the antigens for stimulation of antigen-specific CD8 T-cells improving the overall immunogenicity of *M. bovis* BCG. PCI may be important for the prevention of intracellular pathogens, and potentially also applied as immunotherapy of infectious diseases such as tuberculosis.

.

AUTHOR INDEX

The numbers refer to the numbers of the abstracts.

SWISS MEDICAL WEEKLY

Editors in chief:
Prof. Adriano Aguzzi
Prof. Gérard Waeber
Academic editors: see www.smw.ch

Managing editors:
Natalie Marty, MD
Susanne Redle, MD
Jan Roth, MD

ISSN online supplement: 2504-1622

Guidelines for authors and online submission:
www.smw.ch

Cover image:
© Saiko3p / Dreamstime.com