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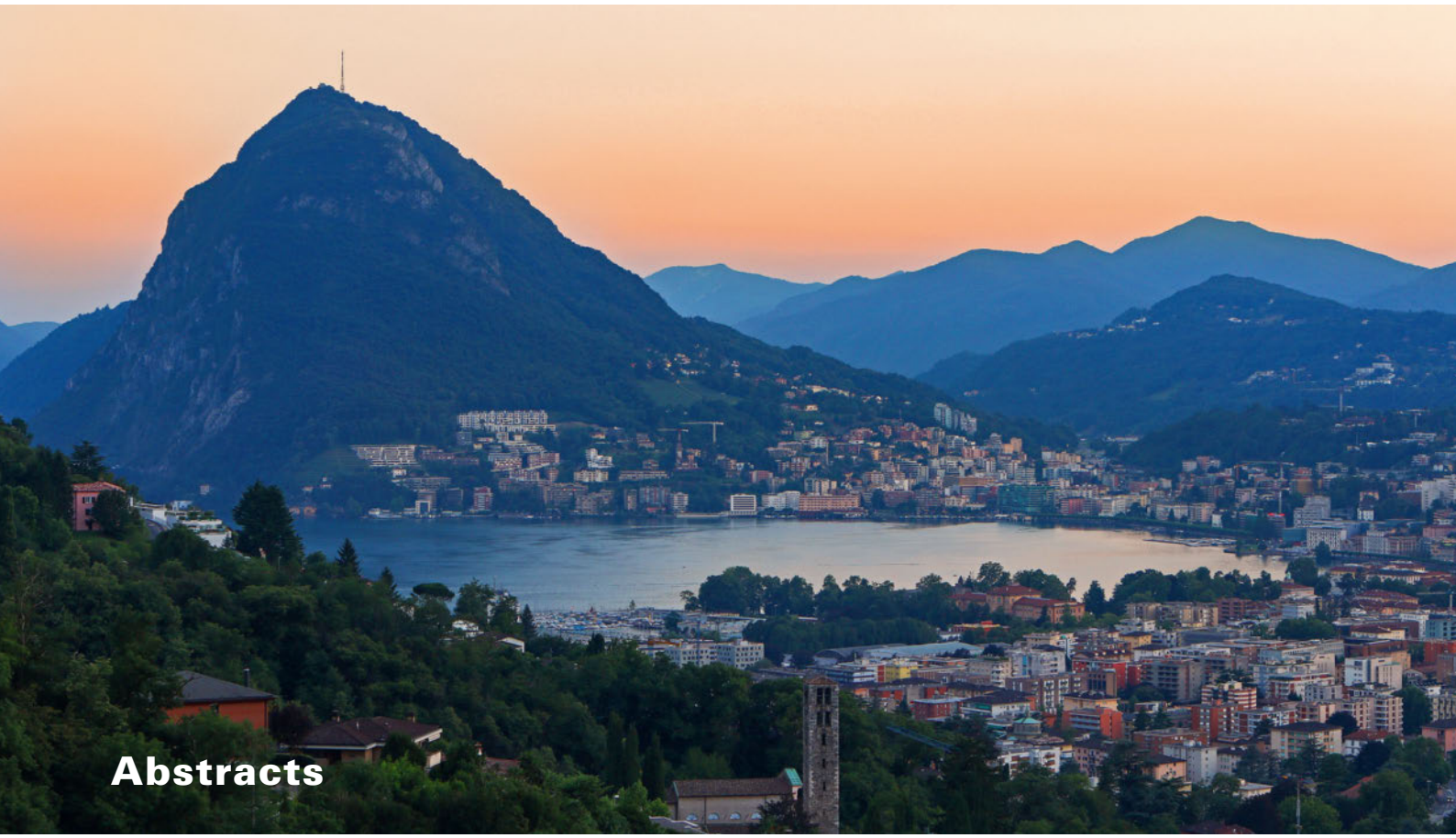

Abstracts

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LUGANO (SWITZERLAND), SEPTEMBER 5–7, 2019

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FREE COMMUNICATIONS, WORKSHOP 1: BASIC IMMUNOLOGY

BI-1

Tissue-resident group 2 innate lymphoid cells differentiate by layered ontogeny and in situ perinatal priming

C. Schneider[1], J. Lee[2], S. Koga[2], R.R. Ricardo-Gonzalez[2], J.C. Nussbaum[2], L.K. Smith[2], S.A. Villeda[2], H.E. Liang[2], R.M. Locksley[2]

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The perinatal period is a critical window for distribution of innate tissue-resident immune cells within developing organs. Despite epidemiologic evidence implicating the early-life environment in the risk for allergy, temporally controlled lineage tracing of group 2 innate lymphoid cells (ILC2s) during this period remains unstudied. Using complementary fate-mapping approaches and reporters for ILC2 activation, we show that ILC2s appeared in multiple organs during late gestation like tissue macrophages, but, unlike the latter, a majority of peripheral ILC2 pools were generated de novo during the postnatal window. This period was accompanied by systemic ILC2 priming and acquisition of tissue-specific transcriptomes. Although perinatal ILC2s were variably replaced across tissues with age, the dramatic increases in tissue ILC2s following helminth infection were mediated through local expansion independent of de novo generation by bone marrow hematopoiesis. Our studies also uncover a process by which local innate responses transition to systemic type 2 responses by niche extrusion of activated sentinel ILC2s from tissue into the circulation. Overall, we provide comprehensive temporally controlled fate mapping of an innate lymphocyte subset with notable nuances as compared to tissue macrophage ontogeny.

BI-2

MR1T cells recognising self-antigens are adaptive-like T cells

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MR1-restricted T cells constitute two distinct populations of αβ T cells characterised by the recognition of antigens presented by the MHC class I-related (MR1) molecule. These antigens are neither peptides nor lipids. The first, Mucosal Associated T cells (MAIT), recognise antigens of bacterial origin and express a semi-invariant TCR, whereas autoreactive MR1-restricted T cells (MR1T) are a heterogeneous subset of T cells that recognise tumour cells with polyclonal TCR. We isolated MR1T cells that differentially respond to tumour cell lines from multiple origins, suggesting the presence of shared MR1-presented antigens, some of which were successfully refolded into soluble human MR1 protein. The antigen-loaded monomers that stimulated MR1T cells were used to form tetramers enabling ex vivo tetramer staining. This approach revealed phenotypically heterogenous cell populations in PBMCs derived from healthy donors. The majority of tetramer-positive cells displayed central- or effector-memory phenotypes while others expressed markers typical of naïve cells. MR1T cells also differentially expressed the activation markers CD150 or CD38, as well as inhibitory markers PD-1 and TIGIT, consistent with prior antigen experience. Tetramer-positive cells were sorted and isolated T cell clones retained antigen specificity and MR1 restriction. Further characterisation revealed differential cytokine release, indicating functional diversity. Together, these findings show that MR1T cells represent a novel population of adaptive-like T cells with diverse self-antigen specificities and have the capacity to kill tumour targets.

BI-3

CD36-PPARδ signaling supports metabolic adaptation of Tregs for survival and functional fitness in tumors

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Regulatory T cells (Tregs) play an indispensable role in maintaining peripheral tolerance and preventing autoimmune disease. In addition to modulating tissue homeostasis, the suppressive properties of Tregs can also be harnessed by cancers to evade immunosurveillance. Therefore, depleting Tregs has been shown to unleash antitumor immunity and interrupt formation of an immunosuppressive tumor microenvironment (TME). However, systemic loss of Tregs due to Treg depletion also leads to severe autoimmunity. Therefore, the identification of novel approaches that specifically target intratumoral Tregs is direly needed for unleashing antitumor immunity and cancer immunotherapy. Here we show that intratumoral Tregs increase lipid uptake and content and elevated expression of CD36, a fatty acid translocase, as compared to Tregs in circulation and other normal tissues, in several cancer types. By using the transgenic mice model, we found that Treg-specific ablation of CD36 reduces accumulation of intratumoral Treg and suppresses tumor growth. Importantly, Treg-specific CD36 deficiency does not lead to autoimmunity in aged mice and CD36-deficient Tregs remain their suppressive activity on restraining CD4 T cell-induced inflammatory bowl disease. Mechanistically, CD36 expression supports survival of intratumoral Tregs by fine-tuning their mitochondrial fitness via PPAR signaling. Thus, high expression of CD36 in intratumoral Tregs orchestrates Treg metabolic adaptation in tumors by intervening metabolic regulations, further promotes tumor growth by suppressing the anti-tumor immune responses. Ultimately, anti-PD-1 blockade treatment elicits therapeutic benefits in mice with Treg-specific ablation of CD36. Altogether, our study suggests that CD36 might be a potential target for specifically waning down intratumoral Tregs and provide proof-of-concept evidence that targeting CD36 in tumors could unleash anti-tumor immunity and synergize with checkpoint blockade treatment.

BI-4

The IL-33/ST2 pathway shapes the regulatory T cell phenotype to promote intestinal cancer

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The composition of immune infiltrates strongly affects the prognosis of patients with colorectal cancer (CRC). Interleukin (IL)-33 and regulatory T cells (Tregs) in the tumor microenvironment have been separately implicated in CRC; however their contribution to intestinal carcinogenesis is still controversial. Here, we reveal that IL-33 signaling promotes CRC by changing the phenotype of Tregs. In mice with CRC, tumor-infiltrating Tregs preferentially upregulate IL-33 receptor (ST2), and IL-33/ST2 signaling positively correlates with tumor number and size. Transcriptomic and flow cytometry analyses demonstrate that ST2 expression induces a more activated and migratory phenotype in FOXP3+ Tregs, which favors their accumulation in the tumor environment. Consequently, genetic ablation of St2 reduces Treg infiltration and concomitantly enhances the frequencies of effector CD8+ T cells, thereby restraining CRC. Mechanistically, IL-33 curtails IL-17 production by FOXP3+ Tregs and inhibits Th17 differentiation. In humans, numbers of activated ST2-expressing Tregs are increased in blood and tumor lesions of CRC patients, suggesting a similar mode of regulation.
Together, these data indicate a central role of IL-33/ST2 signaling in shaping an immunosuppressive environment during intestinal tumorigenesis. Blockade of this pathway may provide a strategy to modulate the composition of CRC immune infiltrates.

BI-5

A single T cell epitope drives the neutralizing anti-idiotypic antibody response to natalizumab in patients with multiple sclerosis

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Natalizumab (NZM), a humanized monoclonal IgG4 antibody to α4 integrins, is used to treat patients with relapsing-remitting multiple sclerosis (MS), but in about 9% of the cases neutralizing anti-drug antibodies (ADAs) are induced leading to therapy discontinuation. To understand the basis of the ADA response and the mechanism of ADA-mediated neutralization, we performed an in-depth analysis of the B and T cell response in two patients. By characterizing a large panel of NZM-specific monoclonal antibodies, we found that, in both patients, the response was polyclonal and targeted different epitopes of the NZM variable region. The neutralizing activity was acquired through somatic mutations and correlated with a slow dissociation rate, a finding that was supported by structural data. Interestingly, in both patients, the analysis of the CD4+ T cell response, combined with mass spectrometry-based peptidomics, revealed a single immunodominant T cell epitope in the NZM variable region. Collectively, our study identifies the basis of T-B collaboration that drives neutralizing anti-idiotypic antibodies to NZM.

FREE COMMUNICATIONS, WORKSHOP 2: ALLERGOLOGY AND CLINICAL IMMUNOLOGY

AC-1

Single cell mass cytometry multiparametric immunophenotyping of peripheral blood cells in systemic lupus erythematosus

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Systemic lupus erythematosus (SLE) is a multisystemic autoimmune disease characterized by a dysregulation of the innate and adaptive immune system leading to the development of autoreactive T cells and autoantibody production. Its etiology remains largely unknown, despite recent advances in understanding the immunopathogenesis of SLE. In this context, phenotypic analysis of immune cells is essential for a better understanding of the disease.
In this study, we used multiparametric single cell mass cytometry (CyTOF), a high-throughput high dimensional technology, to perform a comprehensive analysis of the peripheral blood mononuclear cells of 24 SLE patients that were compared to gender-, age- and ethnicity matched healthy controls (HC). The use of a panel comprising 40 antibodies allowed the exploration of cell surface receptors and intracellular markers, expressed by CD4+ T cells, CD8+ T cells, Natural Killer (NK) cells, invariant NKT cells, B cells, monocytes and dendritic cells.
Among other markers, we examined the expression of Signaling Lymphocytic Activation Molecule Family (SLAMF), a group of immunoregulatory receptors predominantly expressed on immune cells, which are potentially involved in SLE pathogenesis. Cells were analyzed prior and after stimulation with inflammatory cytokines or phorbol 12-myristate-13-acetate and ionomycin. We hypothesised that the SLAMF expression pattern depicts a specific immune signature for SLE that could represent a powerful diagnosis biomarker.
This comprehensive analysis identified major differences in the distribution of immune cell populations and subpopulations in the peripheral blood of SLE patients. Alterations in the expression of activation markers, regulatory receptors (including SLAMF) and cytokine production were also identified.
Overall, our data underscore the importance of multiparametric single cell mass cytometry in deciphering the complexity of SLE pathogenesis by characterizing complex cell surface receptors involved in the activation of immune cells. Furthermore, identifying a specific immune signature for SLE may become a future diagnostic tool.

AC-2

Urticaria is frequent in hypersensitivity reactions to intravenous iron infusions

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Background: Iron deficiency is a common cause of anemia, which affects over 30% of the population. If oral substitution therapy is not efficient or contraindicated, intravenous iron infusions are a useful therapeutic option. Hypersensitivity reactions to iron are rare, but can be serious. The evidence surrounding predisposing factors is limited. Atopy and previous hypersensitivity reactions to medications are considered possible risk factors.
Methods: We analysed clinical data of patients, who presented to the outpatient allergy division between 2007-2019, following hypersensitivity reactions to iron infusions (iron sucrose (Venofer®) or ferric carboxymaltose (Ferinject®)). We obtained a detailed history and recorded possible risk factors such as atopy and previous drug hypersensitivity reactions.
Results: Sixty-one patients were evaluated of which most were female (n=58, 95%). Medium age was 36 years (range 18–77 years). Indication for the iron substitution therapy were iron deficiency with or without anemia (n=43), fatigue (n=2) and other diagnoses (n=16). In this cohort, 18 patients had received iron sucrose, 36 patients ferric carboxymaltose, 1 patient iron sucrose and ferric carboxymaltose and 6 patients an unknown iron product. Twenty-nine patients experienced a mild reaction manifesting with isolated urticaria and/or pruritus (47.5%). Moderate to severe hypersensitivity reactions including symptoms such as angioedema, nausea, dizziness, arthralgia and hypotension occurred in 32 patients (52.5%). Atopy was present in 22 patients (36.1%). Twenty-three patients (37.7%) reported previous hypersensitivity reactions to medications. More than half reported having had a previous episode of urticaria (n=38, 62.3%). With respect to urticaria subtypes, acute urticaria associated with previous medication intake was most frequent (n=20, 52.6%). Less frequently encountered were chronic spontaneous urticaria (n=3, 7.9%), physically induced urticaria or urticaria factitia (n=5, 13.2%), urticaria to another allergen (hymenoptera venom and food) (n=4, 10.5%) and other types of urticaria (n=3, 7.9%). Three patients reported more than one subtype of urticaria (n=3, 7.9%). Severity grade was independent of previously reported urticaria, atopy and previous drug hypersensitivity.
Conclusion: Past medical history of episodes of urticaria was elicited in more than half of our patient cohort who presented with hypersensitivity reactions to iron infusions. Atopy and previous drug hypersensitivity reactions are well-known risk factors. However, in comparison to past medical history of previous episodes of urticaria (all subtypes included), these were less frequent.

AC-3

Apremilast in treatment-refractory recurrent aphthous stomatitis

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Recurrent aphthous stomatitis (RAS) is a common chronic, recurrent, painful ulcerative disease of the oral mucosa where treatment can be challenging in cases of recalcitrant disease. We report on 5 patients with RAS successfully treated with apremilast after being refractory to topical steroids and systemic colchicine therapy. Four patients had a complete response (Physician global assessment, PGA 0) while one patient had a partial response (PGA 1) within 2-6 weeks, with long-term persistent response. Treatment was generally well tolerated with transient gastrointestinal side effects in 4 patients. Apremilast was effective and safe in our patient cohort with treatment-refractory RAS.

AC-4

Establishment of a specialised clinic for mastocytosis patients at the University of Basel

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Mastocytosis is a rare disease characterised by pathological infiltration of mast cells in various tissues. Quality of life in patients with mastocytosis is significantly impaired and treatment options are limited.

We initiated a multidisciplinary mastocytosis service at the University Hospital in Basel (USB), aiming to provide improved and coordinated clinical care of patients with this rare disease in the Basel region.

Within the first few months, approximately 40 patients with mastocytosis have been identified in Basel and the neighbouring region, with numbers steadily rising. The setting for the consultation is the outpatient clinic at the USB Division of Allergy, which has a close link with dermatologists, haematologists, pathologists, and paediatricians. The clinicians caring for patients with mastocytosis discuss specific issues with the other specialities in regular structured meetings, ensuring ongoing communication and shared clinical decision making. Patients are being identified through a variety of routes: Those presenting to the allergy services with anaphylaxis and an elevated baseline tryptase, those with typical skin lesions presenting to the dermatological services and those with characteristic symptoms and laboratory results presenting to the haematology services are screened if the clinical suspicion is sufficiently high. In addition, the pathology departmental database is searched for patients with a histologically confirmed diagnosis. At the 45-minute consultation any current issues are addressed, and the clinical status quo reviewed. Symptoms and signs of possible complications of the disease are assessed, aided by routine laboratory investigations. The possibility of systemic involvement in patients with elevated tryptase levels or typical skin lesions is considered. Further testing is guided by symptoms and signs as well as stage of the disease. Informed consent is obtained from patients to enter their clinical data into the registry of the European Competence Network on Mastocytosis and for donation of biomaterial for future exploratory studies. Ultimately, once a cohort of patients with a diagnosis of mastocytosis is identified in the region, they could be considered for interventional clinical trials.

With the establishment of a specialised multidisciplinary mastocytosis clinic, we aim to provide optimal patient care and contribute to the development of novel diagnostic and therapeutic approaches for this rare disease.

SYMPOSIUM: LABORATORY DIAGNOSTICS

LD-1

Case report: IgE monoclonal gammopathy

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IgE monoclonal gammopathy is a rare immuno-hematological disorder accounting for about 0.1% of all multiple myelomas. Here we report a new case of IgE monoclonal gammopathy.
A 49-year-old male was referred to clinical neurologist after appearance of lower body irradiating pain and paresthesia following surgery for spondylolysis. A peripheral polyneuropathy (pain and dysautonomia) was then investigating and laboratory tests showed a proteinuria at 4.8g/l and a slight monoclonal peak by serum protein electrophoresis. Immunosubstraction by capillary electrophoresis and immunofixation electrophoresis (IFE) using anti-IgG, anti-IgA, anti-IgM, anti-κ and anti-λ antisera showed an abnormal monoclonal band in the λ light chain but none in heavy chains. The patient was addressed to the nephrologist in the context of proteinuria and suspicion of primary kidney amyloidosis. A renal biopsy was performed confirming the diagnostic of light-chain amyloidosis with λ light chains at 53.9 g/l and incomplete nephrotic syndrome without edema. The biopsy also demonstrated kidney and vascular amyloidosis deposits due to large amount of λ light chains. The primary amyloidosis also affecting peripheral nervous system was responsible for his neurological symptoms.
Low concentration of λ light chains for a light chain multiple myeloma and hypogammaglobulineamia led the laboratory to investigate further using IFE with specific anti-IgD, anti-IgE, κ and λ free light chain antisera. This IFE exhibited a band in the IgE and λ light chain, confirming finally an IgE-λ monoclonal gammopathy. In parallel, quantitative analysis of total IgE using UniCAP System immunoassay (Phadia, Thermofisher) showed an IgE concentration at 1'258'000 kU/L that corresponds to 3,02 g/L. The follow up of this patient over a period of 4 years shows a slight decrease of the total IgE level (around 1 g/L) without loss of the monoclonal IgE-λ gammopathy.
This case demonstrates the importance to always screen for D or E heavy chains in presence of slight light chain gammopathy. As knowledge are missing about this multiple myeloma subtype, the objective of this case report is to share one more clinical manifestation of IgE monoclonal gammopathy to then better understand the biology of rare myelomas and improve the outcomes for patient.

LD-2

Analysis of B-Cell Subpopulations by flow cytometry for diagnosing Immunodeficiency: Differences between laboratories in Switzerland

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Introduction: Multi-colour flow cytometry has been proven to be a valid tool to detect abnormalities in the composition of B cell subpopulations in the process of diagnosing immunodeficiency. However, immunophenotyping of B cells by flow cytometry is not standardized. We aim at comparing the results of B-cell subpopulation measurements performed by different laboratories in Switzerland.
Methods: A de-identified EDTA blood sample from an adult individual was sent to four different flow cytometry centres of Switzerland. Each centre determined the relative and absolute numbers of T-, B- and NK-cells, and performed phenotyping of B-cell subpopulations. The B-cell subtyping included transitional, naïve, non-switched/marginal zone, switched memory, and $CD21^{low}$ B cells as well as plasmablasts, based on expression patterns of IgD, IgM, CD19, CD21, CD24, CD27 and CD38. Centre A and B used an in-house established 8-color flow cytometry assay, while centre C and D characterized B-cells according to the recommendation of the EUROclass trial.
Result: The absolute counts of total B-cells were comparable between the four different centres. Interestingly, the percentages of B-cell subpopulations were also comparable, except for the naïve B cells. Centres C and D reported a percentage of naïve B-cells up to twice as high as compared to centres A and B. The difference was due to different gating strategies. Namely, centres C and D classified naïve B cells as $IgD^{pos}CD27^{neg}$, whereas centres A and B determined naïve B cells based on $IgD^{pos}CD27^{neg}CD24^{pos}$ and $IgD^{pos}CD27^{neg}CD21^{pos}$, respectively. It is noteworthy that the gating strategy for naïve B-cells of centres C and D included the transitional B-cells ($IgD^{pos}CD27^{neg}$), whereas centres A and B excluded the transitional B cells from the naïve B cells.
Discussion: The absolute and relative numbers of B-cell subpopulations seem to be comparable between different laboratories in Switzerland, with the exception of naïve B-cells which are differently measured. A working group of representatives from different flow cytometry centres in Switzerland will be formed aiming at further standardization of B cell subtyping.
Centre A: Zürich
Centre B: Bern
Centre C: Lausanne
Centre D: Basel

LD-3

Clinical implications of nucleolar staining of antinuclear antibodies

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Introduction: The presence of antinuclear auto-antibodies (ANA) has been linked to cancer in many studies. Different nuclear antigens (NA) are associated with different ANA patterns in indirect immunofluorescence (IIF). Some specific NA have been associated with the presence of cancer, while other are more typically seen in different autoimmune diseases. This study aims to investigate the association between ANA and cancer, focusing on patients with ANA with a nucleolar IIF pattern.
Methods: Data of ANA patterns and antibodies against NA, in particular those responsible for a nucleolar ANA pattern or associated with systemic sclerosis (CENP-A/B, fibrillarin, Ku, NOR-90, PM/Scl-100, PM/Scl-75, RNAP-III, Scl-70, SSA-52 and Th/To), were collected from the clinical immunology and allergy laboratory of the University Hospital of Geneva between 2010 and 2016. Results were compared to an internal database of ICD-10 codes focusing on the diagnosis of cancer.
Results: The study included 2'903 patients who had an ANA analysis, 240 patients with a nucleolar pattern, 386 patients who had NA analysis by immunodot and 15'701 patients diagnosed with cancer. The presence of ANA with a homogeneous & speckled (HS) pattern was significantly associated with the absence of cancer ($p < 0.01$). In patients with positive ANA, those with a HS pattern were found to have a lower relative risk (RR 0.7, 95%CI 0.5-0.9) of having cancer compared to those with other patterns. The nucleolar pattern conversely showed an increased relative risk (RR 1.5, 95%CI 1.03-2.3). Anti-Scl70 and anti-RNAP-III were associated with the presence of cancer in 15% and 14% respectively, however no specific NA was significantly linked to cancer, probably because of insufficient numbers of patients.
Conclusions: The HS pattern was associated with a significantly lower prevalence of cancer. The presence of ANA with a nucleolar pattern was associated with a significantly increased relative risk of cancer compared to other patterns. Further studies are needed to investigate which particular NA is responsible for the latter association, but anti-Scl70 and anti-RNAP-III, which is frequently associated with the presence of anti-RNAP-I, are good candidates.

LD-4

Are antibodies against La (SSB) no longer useful for the diagnosis of Sjögren's syndrome?

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Introduction: The 2016 American College of Rheumatology (ACR)-European League Against Rheumatism (EULAR) classification criteria

for primary Sjogren's syndrome (SS) do not take into account the presence of anti-La(SSB) antibodies.

Methods: This study retrospectively assessed the value of anti-La(SSB) for the diagnosis of SS between 2010 and 2016.

Results: 2011 anti-Ro(SSA) and 1970 anti-La(SSB) antibody determinations were performed for 807 patients. Thirty-six (4.5%) patients resulted anti-Ro(SSA)-/anti-La(SSB)+. None had a previous diagnosis of SS. During follow-up throughout June 2018, two patients were diagnosed with primary SS, two with secondary SS to systemic lupus erythematosus. Among seventeen retested, two (11.8%) showed negative anti-La(SSB). None developed anti-Ro(SSA).

The prevalence of SS among anti-Ro(SSA)-/anti-La(SSB)+ patients was compared to an anti-Ro(SSA)-/anti-La(SSB)- control group, matched 1:4 according to gender and age. The analysis was aborted after finding four patients with newly diagnosed SS among 105 patients in the control group, according to pre-established criteria for rejecting the null-hypothesis (two-tailed Fisher's test; 5% significance level). The prevalence of SS was 11% (95%CI:0.85%-21.38%) in anti-Ro(SSA)-/anti-La(SSB)+ patients and 4% (95%CI:0.15%-7.47%) in anti-Ro(SSA)-/anti-La(SSB)- patients, the difference being not statistically different.

ANA positivity was compared among different serological subgroups. A significant difference was found between anti-La(SSB)- and anti-La(SSB)+ patients, irrespective of anti-Ro(SSA) antibody status (positivity in 59% and 89% respectively, $p<.0001$), and between anti-Ro(SSA)+/anti-La(SSB)- and anti-Ro(SSA)+/anti-La(SSB)+ patients (67% and 96% respectively, $p<.0001$). No difference was found comparing anti-Ro(SSA)-/anti-La(SSB)- and anti-Ro(SSA)-/anti-La(SSB)+ patients (55% and 58% respectively, $p=.86$).

Conclusion: Four anti-Ro(SSA)-/anti-La(SSB)+ patients have been diagnosed for SS during follow-up. Whether this is worth performing approximately 2000 anti-La(SSB) analysis, is debatable and supports previous studies indicating the low value of anti-La(SSB) testing. However, this study shows that anti-La(SSB) positivity might indicate a higher risk of an underlying auto-immune disease. Further, this study shows that only a minority of anti-Ro(SSA)-/anti-La(SSB)+ patients loose anti-La(SSB) positivity and that development of anti-Ro(SSA) during follow-up is rare.

SYMPOSIUM: ADAPTIVE IMMUNITY IN VIRAL INFECTIONS

S1

Deciphering CD4+ T cell immunodominance to Influenza virus

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Influenza viruses represent a public health concern due to their pandemic potential and to the sporadic spread of highly pathogenic strains from zoonotic hosts to humans. Several recent studies on the clonal composition of the human antibody repertoire against hemagglutinin (HA) – a main target of neutralizing antibodies against Influenza virus – have revealed that broadly neutralizing antibodies against conserved epitopes in the HA stem region can develop in the course of an immune response to infection or vaccination. Surprisingly, the repertoire of human CD4+ T helper (Th) cells against HA remains poorly defined, in spite of the fact that these cells play an important role in the induction of the antibody and cytotoxic CD8+ T cell responses. In this study, we set out to understand the role of antigen presentation in shaping the antigen-specific human Th cell repertoire, and its contribution to the development of immunological memory following vaccination. By combining antigenic stimulation of naïve and memory T cell libraries, T cell cloning, peptide mapping and TCR sequencing, we provide a comprehensive description of the clonal composition of human Th cell repertoire against HA. Furthermore, using mass spectrometry-based MHC-II immunopeptidomics we define the HA-derived peptides naturally presented by monocytes-derived dendritic cells and HA-specific B cell clones. This study can shed new light on the mechanisms underlying T cell repertoire selection, immunodominance, and formation of immunological memory, and could have important implications for vaccine design and prediction of immunization outcome.

S2

Chronic immune activation as a cause of poor T helper cell migration in successfully ART treated HIV-1 infected individuals

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Background: CD4+ T-cell depletion, and in particular Th17 cell loss in the mucosal compartment, is a hallmark of HIV-1 infection in humans. Alterations in the integrity of the mucosal barrier have been indicated as cause for chronic immune activation and disease progression that occurs despite successful anti-retroviral therapy (ART). In this study, we have investigated the effect of chronic immune activation or selective TLR triggering on the homing capacity of lymphocytes, with the aim of understanding the mechanisms at the basis of a poor repopulation of T-helper cells in the gut during successful treatment with ART.

Methods: Blood samples were collected from a total of 58 HIV-1-infected individuals, either ART-naïve (n=15) or on long-term ART (n=43), and from healthy donors (HD). T-helper cell dynamics, migration capacity, and levels of soluble CD14 (sCD14) were assessed. A mouse model, mimicking the alterations observed in HIV-1 infection was also used to assess the impact of chronic immune activation on T-helper cell migration. In vitro triggering of selective TLRs, was performed on HD samples to dissect the involvement of the different TLRs in modulating cell migration capacity.

Results: CCR6+ and CXCR3+ T-helper cells accumulate in the blood of ART-treated HIV 1-infected patients, and their frequency correlates with the levels of sCD14. In HIV-1-infected individuals, migration of T-helper cells in response to chemotactic stimuli is impaired, regardless therapy. Chronic immune activation induced by TLR signaling is sufficient to dampen T-helper cell migration, which can be restored by pharmacological modulation of cytoskeleton activity.

Conclusions: In patients under long-term ART, chronic immune activation results in an altered T-helper cell response to chemotactic cues, and clarifies the poor gut repopulation observed. More in general, persistent triggering of different TLRs leads to changes in the T cell cytoskeleton machinery and to an impairment of cell migration.

This study calls for novel pharmacological approaches in those pathological conditions characterized by persistent immune activation and loss of trafficking of T-cell subsets to niches that sustain their maturation and activities.

POSTER VIEWING TOUR 1 (THURSDAY, SEPTEMBER 5, 2019, 13.15–14.15 H)

P1

Investigating the role of keratinocytes in the pathogenesis of equine insect bite hypersensitivity

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Equine insect bite hypersensitivity (IBH) is a type I IgE-mediated skin allergy of horses caused by bites of Culicoides midges. Keratinocytes play a role in development of type I skin allergies such as atopic dermatitis by producing cytokines like thymic stromal lymphopoietin (TSLP). Although the involvement of epithelial cells in development of type I hypersensitivities has been described, the response of keratinocytes from atopic and healthy individuals to causative allergens remains to be elucidated.

We aimed to investigate TSLP expression in lesional skin of IBH-affected horses and control horses. Additionally, we investigated whether Toll like receptor ligands (TLR-L) 1-9 are exogenous triggers of TSLP in equine keratinocytes. Moreover, we examined whether response of keratinocytes to TLR-L changes in cytokine milieu that mimics allergic environment of IBH. Finally, we aimed to investigate the transcriptome of equine keratinocytes derived form IBH-affected and control horses stimulated with causative allergens of IBH.

Keratinocytes from six non-allergic horses were stimulated with TLR-L 1-9, or left unstimulated for 4h and 24h, in the presence or absence of IL-4 and TNF-α. Gene expression of TSLP was investigated by qRT-PCR. For transcriptome research, skin samples were collected from 9 IBH-affected and 9 control horses, from which keratinocytes were derived and stimulated with causative allergens of IBH. RNA was extracted and sent for RNA sequencing. RNA-Seq data is presently being analyzed.

Expression of TSLP was significantly higher in lesional skin of IBH-affected horses compared to healthy controls. In equine keratinocytes, TLR-L 1-8 induced significant upregulation of TSLP. The strongest upregulation was induced by TLR-1/2L and TLR-3L. Additionally, combination of allergic inflammation cytokine milieu and TLR-1/2L or TLR-3L further upregulated TSLP expression.

Our data suggests that upregulation of TSLP in lesional skin of IBH-affected horses might play a role in IBH development. Moreover, TSLP expression is induced by TLR-L, in particular by TLR-1/2L and TLR-3L and is further increased by atopic cytokine milieu. Considering that secondary bacterial infections additionally exacerbate clinical signs of IBH, these findings suggest that secondary bacterial infections amplify TH2 inflammation via the induction of TSLP expression.

P2

Dupilumab treatment for severe atopic dermatitis: South Switzerland experience

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Introduction: Atopic dermatitis (AD) is a frequent dermatosis characterized by pruritus, dry skin, and eczema. Her pathophysiology is multifactorial and complex, involving epidermal dysfunction, alterations in cell mediated immune responses, IgE mediated hypersensitivity and environmental factors. Dupilumab is a new approved monoclonal antibody for treatment of moderate-to-severe AD. The molecule acts as an IL-4-Rα blocker and inhibits the T cells cascade responsible for clinical manifestations of AD. We report our experience using this therapy to treat moderate-to-severe AD.

Cases Report: We describe 6 cases of south Switzerland adults' patients (4 male and 2 female) all with severe AD (SCORAD >50 points) at different treatment time with Dupilumab. We initially started by performing a DLQI score, SCORAD and QuantiFERON. If the patients had a history of tropical travel, we performed a complete screening for intestinal parasites. 1 patient was affected with chronic hepatitis B and 2 with hypercholesterolemia. We administered firstly 600 mg of Dupilumab subcutaneous and continued with 300 mg every two weeks according to guidelines. After the first month of treatment, patients started self-administration of Dupilumab and we performed a clinical control (DLQI and SCORAD) every three months. We objectified an improvement in 5 of 6 threated patients, especially a reduction of itchy and sleepless. In 5 of 6 patient we observed a reduction of SCORAD and DLQI >75%. The major reduction of the SCORAD was obtained during first 14 days from the beginning of the therapy. Instead, for the DLQI they got best results after 28 days. For one female patient we have interrupted the treatment because she developed an erythroderma 3 months after the first doses. Investigations to determine if Dupilumab was the cause of the erythroderma are on course. One patient experienced mild conjunctivitis as side effect during first month; he was treated with artificial tears with resolution of the symptomatology.

Discussion: The experience with Dupilumab in patients with moderate-to-severe AD results very promising, confirming what other larger studies have already showed. In our case series we found the major reduction of the SCORAD during the first 14 days and for the DLQI after 28 days from treatment start. In conclusion, Dupilumab is a new and effective drug in the therapeutic arsenal of dermatologists that revolutionize the systemic treatment and management of moderate-to-severe AD.

P3

A Phase II Randomized Double-blind Placebo-controlled Single Center Study of Canakinumab Treatment of Adult Patients with Moderate to Severe Chronic Idiopathic Urticaria. (URTICANA-Study)

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Introduction: Chronic idiopathic urticaria (CIU) is a common disease and a significant proportion of patients does not respond to standard therapy with antihistamines and optionally corticosteroids/immunosuppressants. The IL-1β antagonist Canakinumab is effective in cryopyrin associated periodic syndromes (CAPS) associated with urticarial symptoms, so it was suspected that it could be effective also in patients with CIU.

Methods: The effect of canakinumab was investigated in 20 patients with moderate to severe CIU in in a randomized, double-blind placebo-controlled single-dose crossover trial. 10 patients were initially randomized to the canakinumab group, 10 to placebo. Canakinumab was given once in the dose approved for CAPS. Patients who had received placebo were able to receive canakinumab at week 4 if they did not improve. Primary endpoint was clinical improvement at week 4 calculated from the change in UAS7 at week 4 compared to baseline.

Secondary endpoints were the clinical improvement at week 8 calculated by UAS7 and clinical improvement as measured by physician score and DLQI at week 1, 2, 4, and 8.

Result: At week 4 two patients with canakinumab and three with placebo showed a clinical improvement. The difference was not statistically significant (p=1.000). Even if the patients who switched to canakinumab after four weeks were included, the result did not change. Likewise, there was no significant difference between the verum and placebo groups for all secondary endpoints. The therapy was well tolerated, only mild AEs occurred, which did not differ in frequency and type between verum and placebo.

Discussion: Although the study with a total number of 20 patients was rather small, in view of the clear effect in all the parameters investigated, it must be assumed that canakinumab has no effect on lesions of CIU. In conclusion, this suggests that IL1b may not play a pathogenetically crucial role in the majority of patients with CIU, unlike e.g. in hereditary fevers, where influencing IL1 is a key effect in therapy. However, the good tolerability of canakinumab could be confirmed again.

P4

Dupilumab: Clinical findings of a targeted therapy in patients with severe and refractory atopic dermatitis (Follow-up)

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Background. Dupilumab blocks IL-4 and IL-13 signaling and is the first efficacious monoclonal antibody for the systemic treatment of atopic dermatitis (AD). For this indication it is approved in Switzerland since April 2019.

Objective. To depict the real-world experience on the efficacy and adverse events of dupilumab treatment of severe AD at the Department of Dermatology Zurich, Switzerland.
Methods. We assessed the efficacy and adverse events of dupilumab treatment in patients with severe AD Dupilumab wasordered within a compassionate use program Patientsreceived dupilumab 300 mg subcutaneously every 2 weeks, continued emollients and topical steroids on demand.
Results. Since September 2017, 27 patients started dupilumab treatment (20 males, 74%; median age 43.9 years). Median SCORAD before treatment was 65.7, decreasing to median 50.3 (p=.002) after 2 weeks of treatment (n=27 patients), to median 22.3 (p<.001) after 12 weeks (n=22), to median 18 (p<.001) after 70 weeks (n=13) (Fig.1). Regarding side effects, 12 patients (44.4%) reported ocular symptoms starting in median 10 weeks after onset of treatment (Fig.2). 7 of these patients (58.3%) had a mild conjunctivitis, resolving with moisturizing eye drops and avoidance of mechanical irritation (e.g. glasses instead of contact lenses); 4 patients (33.3%) had a moderate conjunctivitis that resolved with topical steroids; 1 patient (8.3%) stopped dupilumab treatment due to severe conjunctivitis. Interestingly we observed that patients with this side effect had elevated eosinophils counts before treatment compared to patients without this side effect (Fig.3). Other side effects were, more frequent (>4 within 12 months) viral infections of the upper respiratory tract in 4 patients (33%), a mucosal wound healing disturbance in 1 patient (3.7%) and in 1 patient (3.7%) a reactivation of his mollusca contagiosa lesions, which resolved spontaneously during next weeks of treatment.
Conclusion. Dupilumab is an efficient treatment for severe AD in reallife, starting to be efficacious already after the first injection and also remains to be efficacious after 70 weeks. Most common side effect is a conjunctivitis, which we observed more frequently in our cohort than in recent clinical studies. The role of the eosinophils in this condition needs further investigation.

P5

Novel insights in allergy by using low-affinity IgE antibodies against Feld 1, the major allergen in cats

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Allergic diseases have become a severe problem worldwide and affect more than 25% of the population in Western countries. The only disease-modifying treatment for allergy available is allergen-specific immunotherapy (SIT), in which the patients are exposed to increasing doses of allergen to induce immunological changes promoting unresponsiveness to the allergen. Even though the mechanisms of SIT are still not well defined, one remarkable change is production of allergen-specific IgG antibodies. Allergen-specific IgG antibodies are believed to neutralize the allergen or engage the inhibitory receptor FcgRIIB. We recently demonstrated that low-affinity IgG antibodies failed to neutralize allergen, but engaged FcgRIIB to block mast cell degranulation. Since allergy is directly mediated by IgE antibodies, we aimed at revealing the role of low-affinity IgE antibodies in the process. We have identified three different antibodies, named A044, F127 and G078 recognizing independent epitopes on Feld 1, the major allergen in cats. To obtain low-affinity IgE antibodies against Feld 1, we back-mutated the variable regions of mature antibodies to germ-line configuration and assembled them in expression vectors, which were transfected to mammalian cells. Then ELISA assays showed germ-line IgE antibodies bound with much lower affinity to Feld 1 than mature counterparts. However, the difference between germ-line and mature IgE antibodies was not as large as expected because avidity contributed to the binding of IgE antibodies to allergens. Importantly, the same pattern emerged if binding of germ-line and mature IgE antibodies were assessed on surface of mast cells. And the binding was readily translated to activation of mast cells. This work demonstrates for the first time the significance of antibody affinity and avidity in driving and blocking allergies and may explain unexpected cross-reactive allergies between allergens that do not seem to be related to a high degree.

P6

Scaling of effector CD8+ T cell responses by non-cognate DCs

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Naive CD8+ T cells integrate signals from peptide antigen-MHC complex (pMHC; signal 1), costimulatory molecules (signal 2), and cytokines (signal 3) during their activation. Despite the vast knowledge on molecular regulators of effector CD8+ T cell responses, it remains elusive whether CD8+ T cells integrate all three signals through interactions with cognate pMHC-presenting dendritic cells (DCs); it is also plausible that signals from the priming microenvironment play a role in achieving full-fledged clonal expansion of CD8+ T cells. To address this question, we combined conventional flow cytometry with intravital two-photon microscopy (2PM) and light sheet fluorescence microscopy of lymph nodes (LNs) in a DC vaccination model. Subcutaneous injection of a quorum of 20,000 peptide-pulsed DCs was necessary to induce exponential expansion of a starting population of 15–25 antigen-specific CD8+ cells in a draining LN. These numbers corresponded to a DC : T cell ratio of 8 in the draining LN. 2PM imaging showed that responding CD8+ T cells established stable contacts selectively with pMHC-presenting DCs without staying proximal to other activated DCs that are not presenting cognate pMHC (non-cognate DCs). Unexpectedly, however, non-cognate DCs restored clonal expansion and effector cell differentiation of reactive CD8+ T cells when we immunised mice with below-quorum pMHC-presenting DCs. In the presence of non-cognate DCs, injection of as few as 1,250 pMHC-presenting DCs sufficed for exponential expansion and effector differentiation of CD8+ cells. In contrast, Il12a–/– non-cognate DCs did not exhibit such effect. Interestingly, Il12rb2–/– CD8+ T cells also benefited from the presence of non-cognate DCs for their clonal expansion and effector cell differentiation. Thus, non-cognate DCs support CD8+ T cell response by conditioning the microenvironment through local production of inflammatory cytokines, and not by directly stimulating CD8+ T cells. As a result, non-cognate DCs significantly lower the pMHC requirement for clonal expansion and effector cell differentiation of CD8+ T cells. Our data suggest that CD8+ T cell expansion follows a “coincidence detection” model in which cognate interactions with single DCs do not suffice for full-fledged clonal expansion without additional signal 3 from the surrounding immune microenvironment.

P7

Transcriptional and post-transcriptional regulation of miR-150 expression in primary human T helper lymphocytes modulates cell proliferation and homeostasis

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MicroRNAs (miRNAs) are small regulatory molecules that negatively modulate gene expression in all cell types, including T lymphocytes of the immune system, by binding to target mRNAs and inducing their translational repression and destabilization. Investigating the molecular network underlying miRNA functions and the regulation of miRNA themselves is crucial to gain a comprehensive understanding of T cell responses. By profiling the miRNome of resting human naïve and memory T helper (Th) cells, we found that miR-150 was the most highly expressed miRNA in these cells, representing about half of the total miRNA content in each subset, and suggesting a potential homeostatic role for this miRNA in maintaining the resting T cell state. Importantly, miR-150 levels dropped rapidly upon T cell activation, even in the absence of proliferation, pointing towards the existence of one or more active mechanisms to down-regulate miR-150 expression. To determine the mRNAs that are targeted by miR-150 specifically in primary human T lymphocytes, we pulled down miR-150 and sequenced its associated targets. The most regulated target of miR-150 was c-MYB, a transcription factor (TF) involved in regulating the proliferation and differentiation of B cells and cytotoxic CD8 T lymphocytes. By modulating both miR-150 and MYB levels, we found that miR-150 restrained Th cell proliferation through direct regulation of MYB expression. Because miR-150 acted as a key negative regulator of T cell proliferation, we further investigated mechanisms of regulation of miR-150 expression. We found that miR-150 levels were regulated by

IRE1α, an Endoplasmic Reticulum (ER) transluminal endonuclease involved in the cleavage of miRNAs, which contributed to miR-150 down-modulation during T cell activation, most likely through its direct degradation. IRE1α also modulated miR-150 transcriptionally by inducing the splicing of the TF XBP1. Finally, we observed that while miR-150 intracellular levels decreased after T cell activation, its release via extracellular vesicles increased. Taken together, our data suggest a homeostatic role for miR-150, whose expression is regulated transcriptionally and post-transcriptionally by a series of mechanisms tailored to rapidly diminish its expression levels in response to T cell activation.

P8

Regulator of G-Protein Signalling 1 (Rgs1) mediates the efficient functional differentiation and maintenance of intestinal tissue resident memory CD8 T cells

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Tissue resident memory T cells (TRM) were recently identified as a distinct T cell subset, primarily located in barrier tissues, including the intestinal mucosa. They are primarily distinguished from the other memory T cell subsets (TEM, TCM cells) by their non-recirculating properties.
When the gene expression signatures of TRM cells from different tissues were compared to those of circulating T cells, one of the consistently most up-regulated gene in TRM cells in both human and mouse tissues was the gene encoding regulator of G-protein signaling1 (Rgs1). Rgs1 inhibits signal transduction by increasing the GTPase activity of the G-protein alphai subunit. This terminates G-protein-coupled-receptor (GPCR)-mediated signaling, known to modulate chemokine receptor-mediated immune cell trafficking. Accordingly we hypothesized that Rgs1 regulates the non-recirculating properties of TRM CD8 T cells and controls the generation, maintenance and function of TRM CD8 T cells, notably, the TRM cell-mediated protection from pathogen dissemination after local infection.
Following intestinal infection with recombinant, Ova expressing Listeria monocytogenes (Lm-ova), Rgs1-/- CD8+ T cells are underrepresented in the CD69+ CD103+ CD8+ TRM compartment of the small intestine at 30 days post-infection. Intriguingly, in contrast to Rgs1+/+ CD8+ TRM cells these Rgs1-/- CD8+ TRM cells cannot efficiently control the dissemination of this pathogen to extraintestinal organs during reinfection.
In vitro Rgs1 attenuates the chemotactic activity of CD8 T cells, and in vivo antigen-specific Rgs1-/- CD8+ T cells accumulate less efficiently in the small intestinal mucosa following primary infection. Accordingly, the local Rgs1-/- CD8+ memory precursor effector T cells (MPEC), which will further differentiate in situ into CD8+ TRM cells, are also markedly diminished upon primary infection. Collectively, the enhanced Rgs1 expression in activated and recruited CD8+ T cells promotes the local accumulation of MPEC's; their differentiation to local TRM cells, and their local retention at the site of initial antigen contact/infection. Altogether, Rgs1 expression in CD8 TRM cells contributes to the local protection of a primed host from local reinfection by potential pathogens.

P9

Poster cancelled

P10

Similar CD8 T cell functional avidity in vivo induced by homologous vaccinations with altered prime/boost intervals or varied antigen density

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It has long been known that B cell responses employ affinity maturation to produce higher affinity antibodies over time. Consequently, most vaccine regimens are designed according to B cell responses with optimal antibody affinity, by using vaccines with well-chosen antigen doses/densities, and by vaccination at prime/boost (P/B) intervals of at least four weeks. The situation for improving the quality of T cells (functional avidity; FA) is much less clear; although it is established that the strength of TCR binding to peptide-MHC (pMHC) has a major influence of FA. Most studies investigating changes in the FA of the peptide-specific CD8 T cell response have focused on using altered peptide ligands (APLs) to artificially modulate the TCR pool recruited, by modifying the affinity of the pMHC. However, changing epitopes from prime to boost and/or effector phase preclude precise conclusions on avidity maturation for a defined epitope. Evidence from early studies showed that using low antigen doses produced T cells with higher avidity, compared to high doses. However, more recent evidence found that CD8 T cell FA was not altered by changes in vaccine dose.
Therefore, the practical question remains open whether short-term homologous P/B vaccinations can be optimized to achieve high FA T cell responses, through strategies comparable to vaccination for high affinity antibody responses. To address this question, we compared two-week and four-week intervals between priming and boosting with highly potent sub-unit vaccines in C57BL/6 mice. Interestingly, we found no difference in the FA. Equally, similar FA was observed after vaccination with virus-like particles (VLPs) decorated with low vs. high density peptide. In vitro, using murine monoclonal T cells, we found higher FA after stimulation with a lower antigen dose. The finding of variable FA in vitro but stable FA in vivo suggests efficient in vivo regulation despite different vaccination schedules or antigen densities. The mechanisms of efficient modulation for equal FA in vivo remain to be elucidated. Nevertheless, our findings suggest that low antigen density vaccines or a long (four-week) prime/boost interval are not crucial for the T cell's FA, in contrast to immunizations for affinity maturation in antibody responses.

P11

How Immunoproteasome inhibition dampens IL-23 production by human blood leukocytes

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The immunoproteasome is a variant of the standard proteasome expressing the interferon-inducible subunits LMP2 (β1i), MECL-1 (β2i), and LMP7 (β5i). Targeting the immunoproteasome in autoimmune diseases and colorectal carcinoma proved to be therapeutically effective in preclinical mouse models. In endotoxin-stimulated human peripheral blood mononuclear cells (PBMCs), immunoproteasome inhibition reduced the secretion of several pro-inflammatory cytokines with suppression of IL-23 being the most prominent. In this study, we have investigated why the production of IL-23, a key mediator of inflammation in autoimmunity, is most effectively dampened when the immunoproteasome is inhibited in LPS-stimulated human PBMCs. CD14+ monocytes could be identified as the main producers of IL-23 in PBMCs. Immunoproteasome inhibition with the irreversible LMP7/LMP2 inhibitor ONX 0914 induced apoptosis in CD14+ monocytes whereas CD4+, CD3+, CD19+, and CD56+ cells survived. High expression of immunoproteasome subunits rendered monocytes susceptible to immunoproteasome inhibition, leading to accumulation of poly-ubiquitylated proteins and induction of the unfolded protein response. Similar to immunoproteasome inhibition, other pharmacological inducers of the unfolded protein response also selectively killed CD14+ monocytes in human PBMCs. Blockage of translation in monocytes protected them from ONX 0914-induced cell death, indicating that the immunoproteasome is required to maintain a high protein turnover in monocytes.

P12

A new in vitro model of T-cell-independent B-cell activation based on short peptides presented on MPLA-containing liposomes

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Background: B-cell effector and memory functions generally depend on CD4 T cells to provide co-stimulatory help, e.g. CD40-CD40L interactions. Large polysaccharides and lipopolysaccharides with repetitive structures can activate B cells independent of T cells. Recently, we demonstrated that nanosized liposomes, containing a high density of short

peptides as well as monophosphoryl lipid A (MPLA) on the surface, stimulated T-independent (TI) B-cell activation with IgG class switch and memory response.
Objective: The aim of this project is to study TI B-cell activation further using liposomes, and especially to study the mechanisms and signalling pathways of TI B-cell effector and memory functions in vitro and in vivo. Finally, we will investigate the liposomes in disease models where TI-B-cell activation may be beneficial, e.g. immunosuppression.
Method: B cells were sorted from spleens of naïve wild type and immune-signalling-deficient mice. The cells were stimulated in vitro with liposomes containing MPLA and 15mer peptides derived from either ovalbumin or amyloid beta (Aβ). The production of antibodies and cytokines was assessed by means of ELISA and flow cytometry.
Results: Cytokines such as IL-10, TNFα, IL-6, and IL-1β were detected 24 hours after stimulation of the B cells. Secretion of IgM was observed from day 3 on and it was dependent on TLR4, MyD88, and, for B cells stimulated with Aβ-containing liposomes, TRIF. The IgM secretion was also caspase-dependent, as shown in B cells from caspase 1/11-deficient mice and in cultures treated with the pan-caspase inhibitor zVAD.
Conclusion: Liposomes with MPLA and short peptides could be applied for TI activation of B cells in vitro for production of antibodies. This model can be used to scrutinize immune signalling during TI B-cell responses. Next to basic knowledge of B-cell signalling, the data may guide design of liposome-based vaccines in situations where T-cell responses are absent or not wanted, e.g. for use in immunosuppressed patients.

P13

MHC II-dependent activation of regulatory T cells in the bone marrow of leukemia mice leads to immune evasion and disease progression

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Leukemia stem cells (LSCs) in the bone marrow (BM) are the origin of leukemia and resistant against conventional therapies and immune control. This resistance is partially mediated by protective mechanisms of the hematopoietic stem cell niche in the BM. In leukemia, the BM microenvironment changes dramatically with regulatory T cells (Tregs) accumulating. However, little is known how Tregs interact with LSCs.
We induced chronic myeloid leukemia (CML) in a murine model with BL/6 BCR-ABL-1 transduced LSKs (lineage-/Sca-1+/c-kit+) in FoxP3 DTR GFP mice. We investigated the frequency, origin, activation and proliferation capacity of BM Tregs in CML compared to naïve mice and analyzed the Treg-accumulation and activation mechanisms during disease progression.
BM Tregs in CML mice were mostly thymic-derived, activated and showed higher proliferation capacity compared to controls. Treg-depletion resulted in long-term survival in the majority of the mice. Importantly, Treg-depleted CML mice showed phenotypically decreased LSC numbers compared to controls (FACS-analysis) and also functionally by colony forming assays and secondary transplantation experiments. To investigate the possibility of an indirect preservation of LSCs via Tregs by inhibiting CD8 T cell mediated eradication, we depleted both Tregs and CD8 T cells in leukemia-bearing mice. Double-depletion restored LSC numbers, suggesting that Tregs protect LSCs from CD8-mediated elimination. The oncogene BCR-ABL-1 seems to be immunogenic since specific CD8 T cells derived from BM of CML mice could efficiently decrease the colony forming capacity of LSCs in comparison to CD8 T cells derived from BM of healthy mice. To investigate the mechanism how Tregs get activated in the BM of leukemia mice, we induced leukemia derived from MHC-II-deficient LSCs since we observed high MHC II expression on leukemic stem and progenitor cells. MHC II KO CML developed significantly slower than control CML and showed the same phenotype as the Treg-depleted CML mice. This effect could be reversed by depleting CD8 T cells during disease progression in MHC II-deficient CML and those mice succumbed to the disease, supporting the previous findings. Our data indicate that thymic-derived, MHC-II-activated Tregs protect LSCs from elimination by specific CD8 T cells and promote leukemia development.

P14

Gut commensal bacteria modulate functions of tumor-associated neutrophils in human colorectal cancer

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Tumor infiltration by immune cells critically impacts on clinical outcome of human colorectal cancer (CRC). While high densities of CD8+ T cells and T-helper type 1 cells are associated with prolonged patient survival, the role of tumor-associated neutrophils (TANs) is debated.
CRC arise in an environment heavily populated by microrganisms. Upon CRC oncogenesis, gut commensal bacteria translocate into the submucosa where they directly interact with residing immune cells. Neutrophils represent a front-line arm of the immune system in the response to bacteria. However, little is known about their interaction with commensal bacteria within CRC tissues. We investigated the interplay between neutrophils and commensal bacterial species present within the CRC microenvironment.
Using an orthotopic CRC xenograft model, we found that commensal bacteria stimulate CRC cells to produce neutrophil recruiting chemokines. We then compared in vitro chemokine induction capacity of Fusobacterium nucleatum (Fn) and Bacteroides fragilis (Bf), two most abundant bacterial species of CRC microenvironment. Fn induced significantly higher levels of IL-8 by CRC cells than Bf, thus more effectively promoting neutrophil recruitment. Accordingly, in human CRC samples, abundance of Fn, but not Bf, significantly correlated with high infiltration by CD66b+ cells. Functional studies indicate that neutrophils cultured in the presence of Fn, but not Bf, lose their ability to enhance proliferation and cytokine release by CD8+ T cells undergoing antigenic stimulation. Moreover, neutrophils exposed to Fn, but not Bf, stimulate release of IL-6 by tumor-associated stromal cells, leading to enhanced tumor cell proliferation.
Our data cumulatively suggest that distinct bacterial components of the human gut flora might differentially modulate functions of CRC infiltrating neutrophils, thus ultimately influencing their prognostic significance.

P15

Survival and fitness of the TFH memory cell compartment

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T follicular helper (TFH) cells regulate antibody production. Following infection or vaccination, effector TFH cells give rise to long-lived memory TFH cells. The survival requirements, lineage flexibility and impact of TFH memory cells on subsequent immune responses have not been fully elucidated. In this study we determined that TFH memory cells are uniquely sensitive to NAD-induced cell death during isolation. Inhibition of NICD prior to memory cell isolation specifically rescues the TFH memory cell compartment and highlights the survival of this population to at least 400 days after infection. Single cell RNA sequencing reveals that TFH memory cells express many genes associated with stemness and self-renewal; reconstruction of a developmental trajectory suggests that TFH memory cells occur earlier in pseudotime, giving rise to other Th cell subsets in a linear fashion. Surprisingly, TFH memory cells concurrently express a distinct metabolic signature normally associated with trained innate immunity, including elevated expression of mTOR, HIF-1a and cAMP regulated genes. Integration of glycolytic and self-renewal programming is mediated through ICOS. Late inhibition of ICOS signaling impairs TFH memory cell survival and correlates with decreased LCMV-specific antibody titers. These results highlight a previously unrecognized role for metabolism in linking memory T cell heterogeneity and long-term humoral immunity.

P16

Non-apoptotic TRAIL function modulates NK cell activity during viral infection

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The role of death receptor signaling for pathogen control and infection-associated pathogenesis is multifaceted and controversial. Here, we show that during viral infection, tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) modulates NK cell activity independently of its pro-apoptotic function. In mice infected with lymphocytic choriomeningitis virus (LCMV), Trail-deficiency led to improved specific CD8+ T cell responses, resulting in faster pathogen clearance and reduced liver pathology. Depletion experiments indicated that this effect was mediated by NK cells. Mechanistically, TRAIL expressed by immune cells positively and dose-dependently modulates IL-15 signaling-induced granzyme B production in NK cells, leading to enhanced NK cell-mediated T cell killing. TRAIL also regulates the signaling downstream of IL-15 receptor in human NK cells. In addition, TRAIL restricts NK1.1-triggered IFNg production by NK cells. Our study reveals a hitherto unappreciated immunoregulatory role of TRAIL signaling on NK cells for the granzyme B-dependent elimination of antiviral T cells.

P17

RNA and Toll-like receptor 7 are essential for the generation of secondary plasma cells in a B cell intrinsic fashion

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Due to their highly repetitive nature and bacterial RNA content, virus-like particles (VLPs) derived from the bacteriophage Qβ elicit strong and long lasting humoral immune responses. After immunization memory B cells (MBC) and plasma cells (PCs) are generated in germinal center (GC) responses. It has been reported that MBC differentiate to secondary PCs after reactivation. Secondary PCs produce higher amounts of antibodies early during the recall response than their primary counterparts (1). To determine the influence of bacterial RNA, a ligand for Toll-like receptor (TLR) 7/8 inside the VLPs, on MBC and secondary PC generation, adoptive transfers of MBCs generated in the presence or absence of TLR7/8 stimulation followed by challenge with Qβ VLPs were performed. The antibody response of secondary PCs, derived from Qβ RNA induced MBCs, is higher and starts earlier, than the primary response. In contrast, the response of secondary PCs, derived from MBCs induced by Qβ without RNA is more similar to the primary response. Moreover, the avidity of antibodies produced by secondary PCs is higher, when they were induced with VLPs containing RNA. Similar results were obtained when MBC were generated in TLR7 knockout mice. Moreover, TLR7 signalling was needed B cell intrinsically for the generation of MBC capable of producing secondary PCs. This was proven using bone marrow chimeric mice that lack TLR7 only in B cells (2). Thus, TLR7/8 signalling in B cells seems to drive the differentiation of MBCs capable of generating secondary plasma cells. These cells are responsible for rapid high avidity IgG responses during secondary antibody responses.

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P18

Tissue-specific conditioning of ILC3s is critical for antigen presentation and T-cell responses

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Splenic and intestinal group 3 innate lymphoid cells (ILC3s) have been reported to differ in their MHC class II (MHC II)-dependent interaction with T cells such that CD4+ T-cell responses were induced in the spleen and prevented in the small intestine. How ILC3-T cell interactions are regulated has not been determined. Here we found that splenic natural cytotoxicity receptor (NCR)- ILC3s surpassed intestinal NCR- ILC3s in the expression of transcripts associated with MHC II antigen presentation. In the spleen interferon (IFN)-γ supported CD4+ T-cell stimulation by induction of MHC II expression in ILC3s. In contrast, microbiota-induced interleukin (IL)-23 reduced the frequency of MHC II+ ILC3s and their capacity to induce antigen-dependent T-cell proliferation in the intestine. Moreover, mTORC1 (mechanistic target of rapamycin complex 1) and STAT3 (signal transducer and activator of transcription 3) signaling are essential for IL-23-mediated suppression of MHC II. Our findings identify biological signals, which promote or prevent ILC3-dependent T-cell responses in a tissue-dependent manner.

P19

Discovery of RhoH as a negative regulator in neutrophil extracellular trap formation

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Neutrophil extracellular trap (NET) formation is a novel function of neutrophils that facilitates the immobilization and killing of invading microorganisms. In spite of its protective role in microbial infection, if dysregulated, NETs contribute to the pathogenesis of inflammatory diseases. Thus, exploring the regulation mechanisms of NET formation could not only add knowledge to neutrophil biology but may also provide new effective therapeutic targets for the related diseases.
RhoH, an atypical small GTPase, is exclusively expressed in the hematopoietic cells and mutated in a number of lymphomas and leukemia. Though, it's known to be involved in diverse cellular processes, including T cell activation and migration, mast cell signaling transduction and eosinophil differentiation, it remains to be investigated whether RhoH would regulate NET formation.
We found that freshly isolated neutrophils from blood contained only little or no detectable RhoH protein, but its expression was induced by GM-CSF under in vitro or in vivo inflammatory conditions, such as cystic fibrosis (CF). Interestingly, the aberrant expression of RhoH in CF neutrophils led to less NET formation upon activation compared with control neutrophils. Consistent with this, overexpression of RhoH in neutrophils differentiated from Hoxb8-immortalized myeloid progenitor cells also resulted in deficient NET formation, indicating that RhoH plays a negative role in NET formation. In addition, neutrophils derived from bone marrow of Rhoh-knockout mice regained the ability in NET formation upon activation compared with neutrophils from WT mice, which further supports the notion that RhoH is a negative regulator in NET formation. Further investigation showed that RhoH had no effects on ROS production and tubulin polymerization during NET formation but inhibited actin rearrangement possibly by suppressing cofilin activity. Though the interaction partners of RhoH and the underlying mechanism are still under investigation, we have considerable evidence to show that RhoH is a negative regulator in NET formation and plays an important role in innate immune response.

P20

Microbiota-mediated shaping of gut secretory IgA in systemic metabolism

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The human gastrointestinal (GI) tract is a complex ecosystem, in which all the three domains of life (Archaea, Bacteria and Eukarya) and Viruses co-exist in close association with the host. This microbial community, re-

ferred to as the gut microbiota, has co-evolved with the host in a mutualistic relationship that influences many physiological functions such as energy harvesting, development and function of the immune system. The equilibrium between the gut microbiota and the host is a key element in human health. In fact, alterations in the composition of the microbial community, termed dysbiosis, have been associated to an increasing number of medical conditions. Central in the homeostatic relationship between immune system and gut microbiota is the local production of secretory immunoglobulin A (SIgA).
Adenosine triphosphate (ATP) is a ubiquitous extracellular messenger, which activates purinergic receptors in the plasma membrane termed P2 receptors. The P2X7 receptor subtype is a widely expressed ATP-gated nonselective cationic channel; in the Peyer's patches of the small intestine, it regulates T follicular helper (Tfh) cell abundance and thereby T dependent SIgA. Depletion of bacteria-derived eATP in the small intestine results in enhanced SIgA response, altered enterocyte transcriptional regulation and dysregulated systemic metabolism. We hypothesize eATP constitutes an inter-kingdom signalling molecule with an important function in shaping a beneficial gut ecosystem for host metabolism via modulation of the SIgA response.

P21

Myelin-specific Th17 cell traffic and proliferate in the large intestine enhancing CNS autoimmunity

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Background: Multiple sclerosis (MS) and its animal model, the experimental autoimmune encephalomyelitis (EAE), are demyelinating diseases of the central nervous system (CNS). They are mediated by auto reactive T cells that are activated in the periphery and then penetrate through the blood brain barrier (BBB) into the brain parenchyma. The gut-brain axis, including gut microbiota changes, has been proposed to promote neuroinflammation. However, the underlying mechanisms, in particular how immune responses are modulated in the gut during EAE, remain unclear.
Aim: To investigate the fate of gut immune cell responses during EAE and to assess the contribution of the gut microbiota in promoting neuroinflammation.
Methods: Th17 cell adoptive transfer model of murine EAE was used. Large spectrum antibiotic treatments were administered before and during EAE to assess the role of gut microbiota in Th17 adoptive EAE model. Histological evaluations were performed to assess intestinal morphology and to quantify inflammatory infiltrates. Immune cells isolated from the intestinal lamina propria were analyzed by flow cytometry, and specific myelin reactive CD4+ T cells from the intestinal compartment were further evaluated ex-vivo.
Results: We observed infiltration of encephalitogenic myelin-specific Th17 cells (TCR MOG Th17 cells) within the lamina propria of the large intestine before clinical signs onset in adoptive experimental autoimmune encephalomyelitis (EAE). Using 3D image reconstruction, we found that more than 60% of the myelin-specific Th17 cells were contacting the colon blood capillaries and showed an elongated morphology, suggesting a migratory phenotype. Specifically targeting myelin-specific Th17 intestinal homing by blocking integrin α4β7-MAdCAM-1 pathway not only impaired T cell migration to the large intestine but further dampened EAE severity. Mechanistically, two third of myelin-specific Th17 cells were found to proliferate near the colonic lumen at preclinical stage of the disease. Antibiotic treatment did not reduce myelin-specific Th17 cell infiltration but shaped their cytokine productions.
Conclusion: Those results show that myelin-specific Th17 cell traffic to the large intestine and proliferate within the lamina propria before appearance of the first neurological symptoms. Furthermore, antibiotic treatment during EAE dampens the pro-inflammatory encephalitogenic properties of Th17 cell and reduces EAE severity.

P22

Intestinal fibroblasts – phenotype and function for IgA plasma cells

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One of the most active sites of immune defense in our body is the intestinal mucosa where we harbor billions of commensals and several potential pathogens. Secretory immunoglobulin A (sIgA) serves as a first-line defense that limits the access of microbes to the lamina propria (LP) and plays critical roles in the regulation of host-commensal interactions. The amount of secreted IgA exceeds all other isotypes (>70%) with most IgA being produced by plasma cells (PCs) residing in the LP of the small and large intestine. The sustained IgA production is maintained by the continuous development of new IgA+ PCs, their migration into the intestinal lamina propria (LP), and the maintenance of IgA+ PCs in this site allowing IgA secretion and transport into the gut lumen. Regarding their survival, PCs are known to be particularly prone to die when extracted from their tissue and their survival is thought to be regulated by extracellular stimuli existing in limiting amounts and provided by a specialized microenvironment, called survival niche. Currently, the cell types and factors defining and regulating this niche for IgA+ PCs are poorly defined. Therefore, the aim of this study was to explore the nature of this particularly important PC niche. Here, we show that intestinal collagen1α1+ podoplanin+ fibroblasts (iFB) form a dense and organized network throughout the LP making extensive physical contacts with all immune cells including IgA+ PCs and constituting the main source of known PC survival factors including baff and cxcl12, besides being one of the april sources. We have established a new in vitro culture system mimicking the complex LP microenvironment where IgA+ PCs reside in order to define the cells and factors that are critical for PC homeostasis. We observed that purified iFB as well as macrophages (Mϕ) are the LP cell types most efficient at promoting IgA+ PC survival and IgA production. Interestingly, they achieve PC survival in a synergistic and cell contact dependent fashion. When screening for membrane-bound factors using inhibitors in our coculture system, we identified CD44 as positive regulator of PC survival and function. In line with this, investigation of the CD44-deficient mouse phenotype revealed that IgA+ PCs and IgA secretion into the gut lumen are selectively reduced.

P23

Characterisation of a novel tissue resident memory CD4 T cell population

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During the last ten years, a population of clonally expanded T cells has been identified that take up permanent residence in the lung and other mucosal tissues. The non-circulating status of these T resident memory (TRM) cells allows them to rapidly respond at the site of antigen exposure, making them an attractive therapeutic target for vaccination. Very little is currently known about the cellular regulation or transcriptional programs leading to CD4 TRM generation, survival and function. Here we used single cell RNA sequencing to investigate heterogeneity within the polyclonal lung CD4 TRM cell compartment after influenza infection. Unbiased clustering analysis revealed several distinct subsets of non-circulating cells whose transcriptional profiles resemble follicular helper T cells (TFH) or Th1 cells. Maintenance of TFH-like TRM cells depend on B cells and Bcl6 expression, consistent with their co-localization with B cells in lymphoid clusters called inducible bronchus associated tissue (iBALT). CD4 T cell intrinsic deletion of Bcl6 at late time points results in disruption of iBALT, a reduction in resident memory B cells, and increased lung pathology. Within the TFH-like TRM compartment, we additionally identified a distinct subset of CD4 TRM cells that expresses amphiregulin, an epidermal growth factor that mediates tissue remodelling and repair. We are currently investigating the hypothesis that HIF-1α dependent amphiregulin production promotes the formation of iBALT, which in turn provides a structured niche for continued TRM cell survival and renewal.

P24

Poster cancelled

P25

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

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Background: Cancer is a public health matter and one of the leading causes of death worldwide. Cancer vaccines aim to stimulate anti-tumor immune response, especially cytotoxic T lymphocytes (CTLs). CTLs recognize tumor antigens presented on antigen presenting cells (APCs) in complex with major histocompatibility complex (MHC) class I molecules. One major problem of cancer vaccines is the inefficacious delivery of antigens to the MHC class I pathway of antigen presentation. Here, photochemical internalization (PCI) may bypass this problem based on the co-delivery of antigens and photosensitizer. Upon uptake into APCs, the photosensitizer localizes in the endosomal membranes. Subsequent light treatment causes activation of the photosensitizer and disruption of the membrane with release of the endosomal content into the cytosol for association to MHC class I.
Objectives: The primary objective is to develop PCI-based vaccination as a method for the stimulation of CTLs. As a secondary objective, the current project studies treatment-associated innate immune reactions in the skin.
Methods: Mice received intradermal injections of photosensitizer and antigen. Eighteen hours later, light was administered, and at various time points thereafter, organs were harvested for analysis of immune responses. Antigen-specific CD8 T-cell responses were measured in blood by flow cytometry, while the treated skin was characterized for innate immune responses by histology and fluorescence microscopy.
Results: PCI improved proliferation of antigen-specific CD8 T cells and their cytokine production as compared with PCI-free vaccination. Proliferation was independent on CD4 T-helper cells as observed using MHC class II-deficient mice. Histological and microscopic analysis of the skin revealed light- and photosensitizer-dose-dependent innate inflammatory responses in the skin. The symptoms of inflammation observed in the treated skin included acanthosis, edema and infiltration of immune cells.
Conclusions: The results demonstrate that PCI can facilitate stimulation of CTLs and that this reaction is strictly independent on CD4 T cells and MHC class II. The results further suggest that early innate immune responses may be an important part of the mechanism of action of PCI-based vaccines. Further studies will focus on how these innate immune responses translate into effective anti-tumor CTL responses.

P26

Combining oral vaccination and niche competition to fight antimicrobial resistance gene-reservoirs

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Antibiotic resistance poses an existential threat to global health. Despite the big effort of European countries to minimize antibiotic use, antibiotic resistance is still on the rise. Many antibiotic multi-resistant (AMR) bacteria are found in the intestinal tracts of humans and livestock. This large AMR-gene reservoir and the low fitness cost for bacteria of carrying AMR genes makes solitary reduction of antibiotic usage insufficient to tackle the current crisis.
To approach this problem we follow a two-pronged strategy that combines inactivated oral vaccines with engineered bacterial competitors. When targeting bacteria, anti-glycan vaccines have been found to be most effective. These are often tedious and expensive to manufacture. A novel system enabling site-specific glycosylation of virus-like particles (VLPs) in E. coli, enables us to produce glycoconjugate vaccines at a fraction of the price, and with increased immunogenicity.
We could show that oral vaccination with VLPs induces a strong antibody response at mucosal surfaces as well as in serum of mice. Moreover, we have validated our approach of combining oral vaccinations with niche competition in a murine model of non-typhoidal Salmonellosis. The strong specific IgA response together with our engineered competitor clearly depletes the targeted strain in vaccinated mice.
Currently, our oral vaccination techniques are being tested in pigs, and a preliminary study has demonstrated no adverse reactions to the vaccine preparations.
Taken together, we have proven the effectiveness of our approach in a mouse model of pathogen infection. While challenges still remain in translation to farmed pigs, this technique holds considerable promise in the fight against AMR.

P27

Deciphering macrophage role in Multiple sclerosis: invading routes and regulation by insulin-like growth factor 1

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Mononuclear phagocytes guide disease development in neuroinflammatory conditions including Multiple Sclerosis (MS) and in the animal model Experimental Autoimmune Encephalomyelitis (EAE). These cells act as damaging forces (pro-inflammatory) and as contributors to recovery (anti-inflammatory), with a high heterogeneity which makes them elusive pharmacological targets.
Before the beginning of clinical disease, pro-inflammatory phagocytes invade the central nervous system (CNS) through unclear gateways and, guided by unknown cues, can evolve their phenotype into anti-inflammatory cells. Once in the parenchyma, activated macrophages can locally secrete toxic substances among which reactive oxygen/nitrogen species, altogether contributing to the damage observed in MS and EAE.
Our overall aim is to ameliorate CNS inflammation by (1) describing the invading routes of phagocytes to the CNS; (2) defining pathways regulating the balance between pro- and anti-inflammatory phagocytes.
To test pathways of invasion, we use a combination of in vivo 2-photon imaging in reporter mouse models (visualizing spinal cord meninges) and in vitro primary models of brain barriers (endothelial blood-brain barrier and choroid plexus).
To control phagocyte plasticity, we will test a promising immunomodulatory candidate, i.e. Insulin-like Growth factor 1 (IGF-1). IGF-1 strongly affects the inflammatory properties of phagocytes; furthermore, the factor has been therapeutically tested for several CNS pathologies, but data are contrasting and need cell-specific elucidation. We will study the role of IGF-1 in new animal models carrying genetic deletion of the IGF-1 receptor (IGF1R) specifically in pro-inflammatory phagocytes or in resident phagocytes. Different immunological and microscopy techniques allow studying the differential role of resident and invading phagocytes during EAE at a single-cell level.
Altogether, through a high specificity in genetic targeting of defined cell states and locations, we will shed light on macrophage contribution to disability, finally clarifying the disease-ameliorating role of IGF-1 selectively in CNS phagocytes, thus potentially re-assessing such therapeutic option for MS.

P28

Mechanisms of myeloid cell invasion and polarization in autoimmune CNS inflammation

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Multiple sclerosis (MS) is a chronic disease of the central nervous system (CNS) characterized by multifocal invasion of inflammatory cells. Microglia/macrophages are the main cell type found in CNS lesions in patients and in mice suffering from experimental autoimmune encephalomyelitis (EAE), an animal model for MS. These cells guide disease development as damaging forces (pro-inflammatory cells)
but are also contributors to recovery (anti-inflammatory cells). Unfortunately, given their complex heterogeneity in origin, location and phenotype, these cells remain elusive pharmacological targets. To shed light on macrophage dynamics during EAE, we recently showed that peripherally-originated phagocytes invade the CNS and dynamically adapt their phenotype to anti-inflammatory cells over time. We hypothesize that selectively blocking pro-inflammatory myeloid cell invasion and -in parallel- accelerating their anti-inflammatory phenotype evolution will be of great benefit to MS patients.

Main objectives: To decrease inflammation in MS and EAE, we thus need to: (1) discover the specific CNS entry points of different macrophages; (2) define the signalling pathways leading to phenotype change, to accelerate this evolution; (3) understand the regulatory role of CNS-resident myeloid cells, and how they influence the phenotype of invading myeloid cells.
Methods: We use a reporter mouse model, iNOS-Tomato-cre x Arginase-EYFP (TomY), allowing real-time imaging of individual macrophage polarizations. Transmigration of macrophages from TomY mice is tested using state-of-the-art in vitro models of different mouse CNS barriers. Myeloid cell function is studied in new transgenic mice carrying genetic deletion in key signalling pathways, specifically in proinflammatory cells or in CNS-resident macrophages. Cutting-edge immunological and in vivo microscopy techniques will be used to define myeloid phenotype dynamics and parallel neuronal pathology during EAE at a single-cell level.
Impact: This approach aims at clarifying the pathogenesis of MS progression and how to ameliorate it. Microglia/macrophages are at the center of every chronic inflammatory process, but are difficult to study in light of their dynamic multilayered complexity. Our in vivo and in vitro approach can set the stage to accurately foresee targeted interventions altering the balance of destructive and protective myeloid cells in the inflamed CNS.

P29

LCMV-specific antibody sequencing and characterization upon chronic infection

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Some viruses (notably HIV, HCV in human and LCMV in mice) are able to cause chronic infection in the respective host, meaning that their clearance by the host immune system is significantly delayed, or even entirely impaired. Once inside the host, chronically infecting RNA viruses mutate at a rapid pace due to selective pressure exerted by the host immune system, allowing them to continuously escape the recognition through the adaptive immune defense. B cells often lag behind with the specificity adaptation of their receptors (surface bound immunoglobulin, B cell receptor (BCR)) and the recruitment of novel T clones is delayed, whose T cell receptors (TCR) would be able to recognize the virus.
Plasma cells (PC) are a differentiated B cell subtype, releasing large amounts of antibodies (the secretory form of the B cell receptor), able to bind and eventually neutralize free viral particles. The purpose of this study is to identify pathogen (LCMV)-specific single plasma cells and their genomic sequence upon chronic infection with LCMV, after neutralizing antibodies have developed and the virus is cleared. The so-obtained information will be used to trace back pathogen-specific antibody ancestors (clones with very similar or even identical sequence) in bulk isolated blood-derived PC from longitudinal blood samples (every 10 days) applying Ig-sequencing through NGS. The final purpose of this project is to better understand the evolution of specific antibodies against LCMV upon chronic infection in mice, as well as analyse the viral escape mutants which evolved in the same time period.

P30

Optimized pMHC class II staining procedure for the detection of tumor, viral and bacterial antigen-specific CD4+T cells

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In contrast to the well-known role of CD8 T cells and their detection with pMHC classI multimers, CD4 T have only recently gained increasing importance especially in tumor immunity. However, due to the lack of tools to efficiently detect specific CD4 T cells as well as their low affinity for MHC classII in the case of tumor epitopes, has made their characterization very limited. Our goal is to develop a procedure which will allow a better detection of specific CD4 T cells. To that aim we have been using new peptide MHC class II multimer (pMHCII) constructs with the following MHC restrictions: DR7, DP4 and DR4. These multimer structures have been loaded with tumor, viral or bacterial associated peptides averaging 15aa in length. By pre-treating CD4 T cells with a combination of 1) molecules inhibiting the TCR internalization, 2) molecules cleaving cell surface sugars and 3) molecules capable of having TCR co-localisation with co-receptor molecules. 4) In addition, after the pMHCII surface staining, purified anti-PE or APC antibody were added. Their function being of cross-links pMHCII molecules at the surface of the cell stabilizing the multimer complexes. By using these optimization methods we were able to boost the detection of tumor, viral and bacterial specific cells compared to the non-optimized staining. By increasing the variety of fluorochromes used to label pMHCII multimers we were also able to increase the number of specificities investigated within one samples. This new staining procedure is a step towards a better detection and analysis of antigen specific CD4 T cells.

P31

Development of high-throughput pMHC tetramer screening method for CD8 T cell specificity readouts

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Tumor specific somatic mutations lead to the formation of new protein sequences, giving rise to so called neoantigens. By their presentation on major histocompatibility complex (MHC) molecules on tumor cells or antigen presenting cells (APCs), some neoantigens can trigger T cell activation via the engagement of the T cell receptor (TCR). The rapid identification of neoantigen-reactive TCRs has been challenging, given the diversity of potential neoantigen candidates detected in tumors and the relatively laborious antigen screening approaches. While the identification of neoantigens has dramatically improved by recent developments in innovative sequencing technologies and advances in bioinformatics, methodologies allowing the rapid validation of their immunogenicity are still limited. Thus, we chose to develop a high-throughput approach to screen for specific T cells. As a first step, to optimize the assay, we use viral antigen followed then by predicted neoantigens. We took advantage of the MHC class I tetramer technology that can be used to identify antigen-specific CD8 T cells without further in vitro manipulations. It is a simple approach for the monitoring of specific T cells, but it is time-consuming and the different pMHC tetramer production steps are technically challenging. To circumvent these difficulties, we decide to develop the tetramerization in a miniaturized assay in a 96-well plate format, with a micro-scale approach occurring in as little as 150 uL containing the biotinylated heavy chain, the B-2-microglobulin and the predicted neoantigen. We screen and assess the refolding efficiency by ELISA and do the direct tetramerization of hundreds of molecules in the wells by adding streptavidin-phycoerythrin (PE) conjugates, without purification. Next, patients' cells are stained with an antibody specific for CD8 and all the tetramers validated and selected in the micro-scale assay. This novel methodology provides a simple, fast and efficient approach of high-throughput pMHC tetramer screening for the identification of several predicted neoantigens specific CD8 T cells across a wide range of MHC class I alleles. As recently reported, the newly identified immunogenic neoantigens can be injected as a vaccine to induce potent immune responses against the mutated sequence, or neoantigen specific TCRs can be sequenced and cloned for T cell engineering purposes.

P32

Circulating ILCPs regulate endothelial cell activation through NF-kB

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Innate lymphoid cells (ILCs) represent the most recently identified subset of lymphocytes, which lack rearranged antigen-specific receptors. Despite their established involvement in inflammatory immune responses, the role of ILCs in cancer remains poorly defined.
Our aim is to assess whether ILCs might exert an active role in controlling or promoting tumor growth through the interaction with the endothelium. Therefore, short-term in vitro-expanded ILC subsets, isolated from the peripheral blood of healthy donors, were used in 3h co-culture experiments with an endothelial cell line (HUVEC, human umbilical vascular endothelial cell line) at 1:1 ratio. The activation state of endothelial cells (ECs) was assessed by flow cytometry, by evaluating the expression level of the adhesion molecules E-Selectin, ICAM-1 and VCAM-1.
Among all ILC subsets, ILCPs elicited the strongest upregulation of adhesion molecules in ECs,through NF-kB activation. Indeed, by specifically blocking the NF-kB pathway in ECs, the expression level of adhesion molecules was reverted to basal levels. In particular, EC activation

occurred in a contact-dependent manner. Interestingly, the pre-incubation of ILCPs with bladder cancer cell lines inhibited the ILCP capacity of upregulating the adhesion molecules on ECs.
The in vivo relevance of these in vitro findings will be tested with the use of tumor-bearing mice, to unravel if this capacity of ILCPs could represent a way for facilitating the immune cell infiltration in the tumor and, therefore, impact tumor progression and/or growth.

P33

Human "TH9" cells are a subpopulation of pparγ+ th2 cells

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T helper (TH) cells are crucial mediators of adaptive immune responses and react to the various infectious and non-infectious challenges. While TH1, TH2, and TH17 cells are well-defined T helper cell lineages in humans, the existence of an IL-9-producing "TH9" lineage remains debated. Given the functional importance of "TH9" cells in murine models of allergic inflammation and tumor immunity, we here set out to better understand the identity of human IL-9-producing TH cells by first characterizing their subset-defining properties. We found that IL-9-producing TH cells are better described as a subpopulation of TH2 cells that express IL-9 transiently post activation, rather than as a bona fide TH cell lineage. This is based on our findings that IL-9-producing TH cells show key TH2-lineage-defining properties: IL-9-producing TH cells express TH2-lineage-defining cytokines (IL-5, IL-13), chemokine receptors (CCR4+/CCR8+), and transcription factors (GATA3) when analyzed irrespective of activation status.
Next, we investigated the transcriptional program that differentiates IL-9+ TH2 cells from "conventional" TH2 cells that lack IL-9 expression. To this end, we performed transcriptional profiling before and after activation of different human TH cell subsets. We found that IL-9+ TH2 cells specifically express high levels of the transcription factor PPARγ. Accordingly, PPARγ was strongly induced in naive TH cells by priming with IL-4 and TGF-β ("TH9" priming), just as IL-9 itself. Functional importance of PPARγ for IL-9 expression was confirmed by pharmacological antagonism with GW9662 or gene silencing of PPARγ by siRNA. PPARγ inhibition reduced IL-9 production in TH2 cells while leaving production of other cytokines in TH2, TH1, and TH17 cells largely unaffected.
In human skin disease, we found high numbers of IL-9+ TH2 cells in acute but not chronic allergic skin inflammation and these numbers correlated with the presence of PPARγ+ cells. Correspondingly, antagonism of PPARγ in T cells isolated from acute allergic contact dermatitis resulted in specific downregulation of IL-9. Taken together, these findings suggest PPARγ as novel regulator of IL-9 in TH2 cells and identify TGF-β as an important factor inducing PPARγ in human TH2 cells. Our findings in humans are in line with recent findings in murine models of allergy and parasite infection where PPARγ emerged as a driver of pathogenic TH2 inflammation.

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The pathobiont Helicobacter Typhlonius promotes inflammation by skewing intestinal macrophage activation in a standardized gnotobiotic mouse model of colitis

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The intestine harbors trillions of microbes that live in a mutualistic relationship with its host. These symbionts have profound effects on the host by shaping the immune system and by providing essential metabolic compounds. Alterations in this sophisticated host-immune-microbiota crosstalk have been associated with the development of acute and chronic intestinal inflammatory disorders such as Crohn's disease and ulcerative colitis. Accordingly, differences in the microbial composition including the presence of disease promoting commensals (pathobionts) have been shown to critically impact on the immune system in many disease models, thus providing a strong rationale for the standardization of the microbiota. Here we describe a novel gnotobiotic mouse model to investigate the host microbial relationship during colitis in which germ free (GF) mice are colonized with the recently described sDMDMm2 flora supplemented with or without the mouse pathobiont Helicobacter Typhlonius (HT). In the adoptive CD4 T cell transfer model of colitis, germ free Rag1-/- mice colonized with sDMDMm2 + HT develop colitis with a more severe pathogenesis and faster kinetic than mice without HT. Flow cytometry analysis of lamina propria infiltrating immune cells shows that CD4 T cell accumulate to a similar extent in the presence or absence of HT. Interestingly, however, there is a strong accumulation of colonic macrophage subsets in the presence of HT which exhibit a colitis promoting pro-inflammatory phenotype. Our data further indicate that the T cell response is directed against the commensals in general rather than the pathobiont itself. In summary, we suggest that HT is able to alter intestinal macrophage activation which in the presence of activated T cells can drive the devastating immunopathologies in our gnotobiotic model of colitis. The precise mechanisms, however, how HT boosts the inflammatory response in our gnotobiotic mouse model is subject of current investigations. Nonetheless, the described model offers as yet unavailable possibilities to study in parallel the host immune response and its relationship with microbiota on a species level. In addition, the model is universally applicable and is independent on the microbial status of the local animal facilities and thus antagonizes contradictory results in disease models.

P35

Colonic bacterial peptide mimics activate heart-specific CD4+ T cells and drive lethal inflammatory cardiomyopathy

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Myocarditis is a frequent inflammatory heart disease that develops into lethal dilated cardiomyopathy (DCM) in 20–30% of the patients. Immune activation after infectious myocarditis is associated with the establishment of autoimmune responses against myosin heavy chain 6 (MYH6) with chronic stimulation of MYH6-specific Th1 and Th17 cells that mediate the inflammatory cardiomyopathy. However, the mechanisms that govern the cardiotoxicity programming of heart-specific T cells have remained elusive. Likewise, therapeutic approaches that mitigate the activity of such pathogenic T cells and that prevent the severe consequences of inflammatory cardiomyopathy are still limited. Using a T cell receptor transgenic mouse model of spontaneous autoimmune myocarditis, we show here that progression of myocarditis to lethal heart disease is dependent on MYH6-specific Th17 cells that are imprinted in the colonic lamina propria by a commensal Bacteroides species peptide mimic. Antibiotic therapy against the colonic commensals reduced intestinal activation of heart-specific T cells and thereby rescued genetically predisposed mice from lethal cardiac disease. Moreover, activation of MYH6-specific T cells in mice triggered the generation of anti-Bacteroides IgG antibodies. Importantly, significantly elevated anti-Bacteroides CD4+ T cell and B cell responses in myocarditis patients were observed, suggesting that cardiac inflammation in humans is also linked to the recognition of bacterial antigens by heart-specific CD4+ T cells. In sum, this study reveals that commensal bacteria such as Bacteroides can serve as a source of mimic peptides that promote the progression of myocarditis to lethal cardiomyopathy in genetically susceptible individuals. Thus, manipulation of the microbiome restrains the commitment of cardiotoxic, IL-17-producing T cells and thereby transforms inflammatory cardiomyopathy into a targetable disease.

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Interim analysis of a postauthorization safety study on long-term safety of hyaluronidase-facilitated subcutaneous immunoglobulin 10% in primary immunodeficiency disease in Europe

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Introduction: HyQvia (fSCIG) is a recombinant human hyaluronidase (rHuPH20)-facilitated subcutaneous immunoglobulin 10% replacement therapy approved in Europe for treatment of patients with primary immunodeficiency disease (PID). The aim of this study is to acquire additional data on the long-term safety of fSCIG and to assess the prescribed treatment regimens and administration in routine clinical practice.
Methods: This ongoing prospective, non-interventional, open-label, uncontrolled, multicenter study, initiated in July 2014 in Europe, includes patients aged ≥ 18 years with PID who are currently receiving or prescribed fSCIG (EUPAS5812). The treatment regimens are prescribed at the discretion of the attending physician in accordance with standard clinical practice. Assessments of anti-rHuPH20 antibodies are performed on a voluntary basis.
Results and Conclusions: As of March 15, 2018, out of 111 enrolled patients, 101 patients had received ≥ 1 dose of fSCIG and were included in the the safety analysis population; the mean (SD) fSCIG exposure duration was 1.72 (0.92) years. The incidence rate of nonserious adverse events (treatment related and unrelated, excluding infections) in the safety population (n = 101) was 2.45 events per person-year; 426 events were observed in 70 patients. Two patients with immunogenicity data presented with positive binding antibodies (defined as titer ≥160) to rHuPH20. There were no neutralizing antibodies to rHuPH20. The average annualized per-patient rates for both hospitalizations and emergency room visits were <0.2. Most treatments were administered at home during the first (90.5%), second (93.7%), and third year (90.9%) in the study. This interim analysis of prospectively collected data of fSCIG use in routine clinical practice suggests that fSCIG is well tolerated.

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Preliminary report on the Swiss Autoimmune Hepatitis Cohort Study

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Background and aims: Autoimmune hepatitis (AIH) is a rare disease affecting children and adults. The Swiss AIH cohort study was established in 2017 with the aim of collecting high quality, standardized data and biosamples on AIH in Switzerland.
Methods: Inclusion criteria: diagnosis of AIH, residency in Switzerland.
Results: 134 adult and 11 pediatric patients were enrolled by March 2019 within 17 centres. Adult population: 98 female; median age at diagnosis 56 years (IQR 42-65); 127 white, 3 Asian, 2 black, 1 native American, 1 other; 9 with AIH/primary sclerosing cholangitis overlap; 23 with AIH/primary biliary cholangitis (PBC) overlap; 2 with de novo AIH following liver transplantation (LT). Three patients had required LT before enrollment, none died after enrollment. Median retrospective observation was 52 months (IQR 11-110), median prospective follow-up 11 months (IQR 9-15); 72% were positive for anti-nuclear antibody (ANA), 49% for anti-smooth muscle antibody (SMA), 40% being double positive for ANA and SMA, 7% for anti-soluble liver antigen (SLA), 1% for anti-liver kidney microsomal antibody (LKM1). Initial treatment (available for 90 patients): 57 received prednis(ol)ne (dose range 2.5 -70 mg/day, median 40), of whom 33 received also azathioprine (dose range 25-200 mg/day, median 50); 10 patients were treated with budesonide, 9 mg/day. Other initial treatments included mycophenolate mofetil in 2 patients, combined with tacrolimus in one with de novo AIH. Rescue treatments included mycophenolate mofetil in 15 patients, 6-mercaptopurine in 6, cyclosporine in 3, one patient with concomitant inflammatory bowel disease and one without were treated with infliximab. Pediatric population: 5 female; median age at diagnosis 10 years (IQR 5-12); 7 white, 2 black, 2 Asian; median retrospective observation 31 months (IQR 3-51), median prospective follow up 12 months (IQR 10-14); 6 had a magnetic resonance cholangiopancreatography at diagnosis, 1 at follow-up: 2 had abnormal bile ducts; three out of five tested at diagnosis for anti-SLA were positive; 3 were anti-LKM1 positive. All were treated with predniso(lo)ne and azathioprine. None underwent LT or died.
Conclusion: The Swiss AIH cohort study provides novel information on AIH in Switzerland, raising awareness on the uneven management, and establishes a platform for collaborative national and international research projects.

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The Fcε-Receptor I pathway is crucial for basophil activation in patients with autoimmune forms of chronic spontaneous urticaria

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Background: Donor basophils incubated with serum of patients with chronic spontaneous urticaria (CSU), are used as in vitro read-out system for the detection of autoreactive serum components (CU-BAT). Ibrutinib, an irreversible, specific and highly potent Bruton tyrosine kinase (BTK) inhibitor, has been shown to completely inhibit the IgE/FcεRI-BTK dependant degranulation of human basophils. This allows to further discriminate between different autoreactive serum components in CSU patients.
Methods: We examined the effects of Ibrutinib on basophils. Basophils of well characterized donors were pretreated with Ibrutinib (100nM) for 15min, primed with optimal IL-3 concentration and stimulated with controls (none, anti-IgE, C5a, fMLP) or serum, obtained from patients with CSU (n=13).
Results: Ibrutinib led to a complete inhibition of IgE/FcεRI-BTK degranulation of human basophils without affecting the signaling via G-protein coupled receptors (C5a, fMLP). When stimulated with serum of CSU patients, Ibrutinib was able to inhibit CU-BAT in all study participants. Interestingly, the inhibition was only partial in some patients.
Conclusion: This study confirms the previous observation that ibrutinib is a potent inhibitor of the IgE/FcεRI-BTK pathway in basophils. The IgE/FcεRI-BTK pathway is required for degranulation of basophils treated with serum of CSU patients. Possibly a second signal in terms of a primer together with anti-IgE/anti-FcεRI autoantibodies might be needed for the clinical manifestation of some forms of CSU.

POSTER VIEWING TOUR 2 (FRIDAY, SEPTEMBER 6, 2019, 13.15–14.15 H)

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Prophylactic allergen vaccination of horses against insect bite hypersensitivity

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Insect bite hypersensitivity (IBH) is an allergic dermatitis of horses caused by IgE-mediated reactions to Culicoides midges and characterized by an imbalance between T-cell subsets. IBH does not affect horses in Iceland as the causative midges are not found there. However, the eczema is highly prevalent in horses born in Iceland and exported to Culicoides infested areas. IBH in Icelandic horses is therefore an excellent model for development and study of prophylactic immunotherapy as the horses are only sensitized after export. Numerous Culicoides allergens have been identified and produced as recombinant proteins.
In our previous study intralymphatic (i.l.) vaccination with purified allergens in Alum/monophosphoryl lipid A (MPL), but not in Alum alone, resulted in a Th1/Treg focused immune response. The IgG antibodies induced efficiently blocked the binding of IgE from IBH horses to the allergens.
The i.l. injection route is presently being compared to the more practical subcutaneous injection (s.c.). Twelve healthy Icelandic horses were injected three times with 4 week interval; either i.l. or s.c. with Cul n 4, Cul o 2p and Cul o 3 in alum/MPL. Allergen-specific IgG subclasses were measured in serum at week 0, 2, 6, 10, 18 and 20 by ELISA. Cytokine profile was evaluated in cell supernatants by bead-based multiplex assay following in vitro re-stimulation of PBMC with the allergens.
Our preliminary results show that both injection routes result in strong allergen-specific IgG antibody responses, mostly IgG1 and IgG4/7. The IgG antibodies induced are able to inhibit the binding of the IgE from IBH-affected horse to the allergens. The horses vaccinated i.l. produced slightly more IFN-γ upon in vitro re-stimulation of PMBC and slightly less IL-4 as compared to the horses vaccinated s.c., although the difference does not reach significance.
Based on our current vaccination experiment a challenge will be conducted where horses in Iceland will be vaccinated with the most important allergens. The horses will subsequently be exported to Culicoides infested area in Switzerland and followed for three years in order to investigate whether the vaccination protects them against IBH.

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CD23 provides a non-inflammatory pathway for IgE-allergen complex elimination

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Type I allergic inflammation is mediated by IgE that triggers inflammatory reactions when it has bound the high-affinity IgE receptor FcεRI present on mast cells or basophils. The second receptor engaged by IgE is the low-affinity IgE receptor CD23, most prominently expressed in B cells, which has been shown to negatively regulate IgE synthesis in B cells in vitro and in vivo. However, the mechanisms that regulate IgE targeting to FcεRI versus CD23 and thereby control the two pathways are still poorly understood. Here, we could show that free IgE preferentially binds to FcεRI whereas IgE immune complexes (IgE-ICs) preferentially bind to CD23. These different binding properties directly translate to distinct biological functions: monomeric IgE initiates allergic inflammation via FcεRI on allergic effector cells while binding of IgE-ICs to CD23 is non inflammatory. It can on one hand facilitate the antigen presentation by delivering antigen to dendritic cells and on the other hand prevents effector cell activation because of reduced IgE binding to FcεRI and enhanced IgE serum clearance. In vivo data using CD23 wild type and knockout mice showed that CD23 is responsible for clearing IgE-ICs and that IgE clearance was mediated through the presence of IgG anti-IgE autoantibodies. Furthermore, IgE-allergen immunization protected mice from passive as well as active IgE sensitization and subsequent allergen challenge. Hence, we propose a novel mechanism of IgE regulation via IgE-allergen complexes which might open the possibility for novel treatment approaches to control IgE-dependent hypersensitivity diseases.

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Bugs are everywhere – Anaphylaxis after Orange Juice

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Case report: A 58-year old saleswoman presented to our outpatient allergy unit after one episode of urticaria, asthma and hypotension following 15 minutes after ingestion of an orange juice cocktail, raising suspicion of an immediate type hypersensitivity to orange juice.
Diagnostic work up:
Skin prick test was negative for inhalant seasonal/ perennial allergens, for orange juice and citrus fruit pulp. Total IgE was slightly elevated to 103 kU/l (UNL: 100 kU/l), specific IgE to fx5 and orange were not detectable (<0.35 kU/l). An ISAC® Microarray screening test showed a strong but isolated sensitization to tropomyosins of various origins such as shrimps, house dust mites, cockroaches and anisakis respectively (nPen m1, rDer p10, aBlg g7, rAni s3).
Correlation of tropomyosin sensitization or cross- reactivity and anaphylactic reaction after orange juice consumption was not evident, as tropomyosin is contained only in avertebrate animal sources. However, we learned that citrus fruit are coated in a protection wax after harvesting in order to improve shininess of the shell and as a protection to prevent water loss. Wax coatings can contain compounds such as shellac from insect origin.
This time also testing for orange peel, we repeated skin prick tests which resulted in a positive test to the peel but not the juice. The patient also reported sucking on an orange slice which was decorating the drink – possibly leading to the culprit allergen exposure. Our final diagnosis therefore was a severe anaphylactic reaction due to orange peel treated with insect containing wax, based on IgE-mediated tropomyosin sensitization.
Conclusion: Allergic reactions to tropomyosin-containing food such as seafood or parasite-infested fish in Sushi is well-known. However, food allergy to insect hitherto was rarely considered and possibly underestimated due to the often-hidden character of insects in food sources. Shellac, a resin secreted by the female lac bug or carmine, a red food coloring originating from cochineal lice as well as other insect products have long been commercially used.
Since Swiss food safety authorities have licensed 3 insect species to be used in processed food (e.g. insect burgers) in May 2017, such allergies might become more common and have to be expected in seafood allergic patients due to high cross-reactivity between tropomyosins. Patients should be alerted to this possibility; an upcoming study together with the BAG will address this issue.

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Hypersensitivity reaction following dupilumab injection due to excipient polysorbate-80

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Background: Dupilumab is a fully human monoclonal antibody targeting the alpha-subunit of the interleukin (IL)-4 receptor, thus interfering with the IL-4 and IL-13 pathway. It has shown efficacy in various clinical trials in atopic dermatitis (AD) and asthma. Syringes for subcutaneous injection were recently admitted for the treatment of moderate to severe atopic AD. Beside cutaneous reactions at the injection site, hypersensitivity to the drug appears to be rare.
Methods: We report the case of a 22-year-old men with severe AD and baker's asthma experiencing an immediate-type hypersensitivity reaction after subcutaneous dupilumab (Dupixent) and the results of skin-tests to the drug formulation including the solubilizing agent polysorbate-80 (polyoxyethylene-sorbitan-20-monooleate, aka Tween- 80).
Results: The patient was treated with dupilumab every two weeks for 6 months, with significant improvement of AD and asthma. However, one minute after the 13th injection, the patient developed facial angioedema, nausea, chest tightness and wheezing. He received immediately in-

tramuscular adrenaline, inhaled albuterol, oral antihistamine and intravenous corticosteroids with rapid relief. Serum tryptase measured 2 hours after symptom start was not increased. Skin prick with the undiluted drug formulation (Dupixent pre-filled syringe containing dupilumab 150 mg/ml) were negative. We then performed intradermal testing (IDT) with the diluted drug formulation and the excipient polysorbate-80 (100%). Intradermal were positive at 15' with both solutions at a 1/100 dilution. IDT with the same dilutions were negative in a control subject. Skin prick and IDT were negative for chlorhexidine. Twenty minutes after the positive IDT, the patient complained about dyspnoea, with a 200 L/min decrease in the peak expiratory flow rate. Pulmonary auscultation and saturation remained normal. The patient received inhaled albuterol with rapid relief of symptoms. We considered Polysorbate-80 as the culprit for these hypersensitivity reactions. Treatment with Dupixent was suspended.
Conclusions: Immediate-type hypersensitivity to subcutaneous dupilumab appears to be exceedingly rare, possibly due to reduced immunogenicity of a fully human antibody. However, patients may get sensitized to excipients in the drug formulation, as in our case. Allergy to polysorbate-80 is uncommon, but of particular concern in sensitized individuals, given its presence in a vast array of medical products.

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Baseline and peak serum tryptase compared in acute mast cell activation

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Background: The diagnosis of anaphylaxis relies on suggestive clinical history after exposure to a potential triggering factor. Serum tryptase concentrations increase in case of degranulation of mast cells and measurement of is used to diagnose anaphylaxis. There is no standardized method for assessing serum total mast cell tryptase (MCT) in anaphylaxis. The consensus equation (peak MCT should be >1.2x baseline tryptase + 2 µg/L) has been proposed to interpret acute mast cell activation syndrome (aMCAS) since the Working Conference in 2010.
Objective: Our objective was to study the current literature since the consensus equation and other equations evaluating baseline and peak serum tryptase during aMCAS since the Working Conference in 2010.
Methods: Computerized bibliographic searches of PUBMED and EMBASE were supplemented with a manual search of reference lists. English-language studies were included.
Results: Ten studies met our inclusion criteria with 4450 participants. However, only three studies with 552 participants referred to the consensus equation. Seven studies were using other peak and baseline serum tryptase comparison.
Conclusion: Serum tryptase makes a valuable contribution to the diagnosis of anaphylaxis but is unable to detect all anaphylactic reactions. Based on the current literature the consensus equation is underused. Other alternative biomarkers should be explored in parallel to peak and baseline serum tryptase in aMCAS.

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Severe asthma diagnosis and management by Swiss general practitioners

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Introduction: In these days, the majority of asthma patients can be effectively treated with currently available medications. However, 5-10% of them develop a severe asthma. As no national data on the diagnosis and treatment of severe asthma patients at general practitioners is available, we developed a survey to investigate how this is currently handled by general practitioners in Switzerland.
Methods: We are conducting a retrospective survey among Swiss general practitioners regarding the diagnosis, evaluation and treatment of their severe asthmatic patients. The survey consisted of 16 questions and was distributed by mail to 3'500 randomly selected Swiss general practitioners from July 2017 to April 2019. Completion of the questionnaire was made on a voluntary basis, without any counterpart or fee.
Results: The project is ongoing and was started in March 2019. The survey was completed by 154 Swiss general practitioners, 60,4% of whom were German-speaking, 34,4% French-speaking and 5,2% Italian-speaking. Results of the survey with cutoff date 30 June 2019 will be presented here.
Conclusions: This survey is expected to provide a description of the diagnosis and management of severe asthma by Swiss general practitioners. The analysis will provide us insight into the daily challenges faced by general practitioners in real life in terms of management and polymorbidity of their severe asthma patients. In addition, we expect to gain insights where improvements in education and in interdisciplinary care with asthma specialists could be achieved with the aim of good asthma control.

P45

Allergen-specific profiling of sera from patients with peanut allergy

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Background: Peanut allergy is one of the most common IgE-mediated food allergies and can cause potentially deadly hypersensitivity reactions such as anaphylaxis and asthma. Allergic patients potentially also have peanut-specific IgG antibodies that may prevent IgE binding to allergens, and the IgE-IgG balance, the peanut-protein specificity of IgE and IgG, as well as the IgG subclasses may determine whether the patient develops symptoms or not.
Among 16 identified peanut allergens, Ara h 1, Ara h 2, Ara h 3, and Ara h 6 bind IgE in the majority of peanut-allergic patients. Sensitization to Ara h 2 and Ara h 6 have been shown to cause more severe allergic reactions than Ara h 1 and Ara h 3, indicating that certain allergens are more prone to elicit an anaphylactic shock.
The objective of this study was to describe the serological profile of a Swiss cohort of peanut allergic patients.
Methods: Sera from 95 male and female patients with peanut allergy was obtained from a Swiss cohort. Both adults and children were included. The sera were tested for reactivity against different peanut allergens by an in-house sandwich IgG ELISA and by Immuno-CAP to detect IgE.
Results: The majority of patients were sensitized to multiple peanut allergens. Among the 95 sera, 84 (88%) and 79 (83%) were positive to anti-Ara h 2 IgE and anti-Ara h 6 IgE, respectively. Fifty patients (53%) were sensitized to Ara h 1, 37 (39%) to Ara h 3, 52 (55%) to Ara h 8 and only 20 sera (21%) were reactive against Ara h 9. When comparing peanut-specific IgE concentrations, we observed that Ara h 2 and Ara h 6 were the most frequently and most strongly IgE-binding peanut allergens. Peanut-specific IgG were also detected in all patient sera, but the protein specificities varied. All patients were positive for anti-Ara h3 IgG. Against Ara h 1, 92 sera (97%) had detectable IgG. Ninety sera (95%) were positive for Ara h 2 IgG, 88 (93%) for Ara h 6 IgG, and 78 (83%) for Ara h 8 IgG.
Conclusion: The study confirmed that sensitization to Ara h 2 and Ara h 6 may be predictive of peanut allergy, since the majority of the patients in the analyzed cohort were strongly sensitized to these proteins. Ara h 2 and Ara h 6 are moderately homologous proteins and they showed indeed comparable IgE-binding potential and similar prevalence.

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Production of Tregitope–allergen vaccine for immunotherapy in cockroach allergy

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Allergen-specific immunotherapy (AIT) facilitates long-term resolution of allergic morbidity resulting in reduced drug use and increased refractoriness to new sensitization. AIT effectiveness has been demonstrated in seasonal and perennial allergies, and insect stings. However, data and studies in AIT relative to cockroach (CR) allergy are relatively scarce. In this study, mice allergic to American CR (Periplaneta americana) were treated with a liposome (L)-entrapped vaccine made of mouse Tregitope289-Per a 9 of the CR, Tregitope167-Per a 9, or Per a 9 alone – or placebo. Allergic mice that received an individual vaccine intranasally had reduced Th2 response, reduced lung inflammation, and reduced respiratory tissue remodeling. However, only L-Tregitope289-Per a 9 and L-Tregitope167-Per a 9 induced expression of immunosuppressive cytokine genes (IL-10, TGF-β, and IL-35 for L-Tregitope289-Per a 9, and IL-10 and TGF-β for L-Tregitope167-Per a 9) and increment of idoleamine-2,3-dioxygenase 1 (IDO1), indicating that these vaccines caused allergic disease suppression and reversal of respiratory tissue remodeling via generation of regulatory lymphocytes. Liposome entrapped-recombinant Per a 9 (L-Per a 9) did not cause upregulation of immunosuppressive cytokine genes and IDO1 increment; rather, L-Per a 9 induced high expression of IFN-γ in lungs of treated mice, which resulted in mitigation of allergic manifestations. This study provides compelling evidence that both liposome-entrapped vaccines made of single refined major allergen alone and single refined major allergen linked with Tregitopes are effective for reducing allergen-mediated respiratory tissue inflammation and remodeling, but through different mechanisms.

P47

Prevalence of Bronchial Asthma in Indian Adults: A Systematic Review & Meta-analysis of Observational Studies

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Objective: There is limited data available on epidemiology of asthma among Indian adults. The aim of this study was to conduct a comprehensive systematic review and meta-analysis of observational studies done on prevalence of bronchial asthma.
Methods: The investigator systematically searched MEDLINE for population based prevalence studies that had reported the prevalence of bronchial asthma in the last 20 years. The keywords used were “Bronchial Asthma”, “Prevalence”, and “India”. The inclusion criteria were studies done among non-occupational, adult population consisting of both genders. The articles chosen were in English language. We conducted the meta-analysis using Microsoft Excel software. Fixed and Random effects model were used to know the effect summary. I2 & Cochrane Q Statistics were applied to know the heterogeneity of the studies.
Results: In the first phase, 534 articles were screened and in the second phase 508 articles were excluded after reading the title and abstract. Then 16 more studies were excluded after reading the full texts, leaving 10 studies for the analysis. In this study, the asthma was self-diagnosed and not physician confirmed. The total population that had been studied for prevalence of asthma was 6,20,226. The overall estimation of prevalence of bronchial asthma was 0.19% (95% C.I: 0.18-0.20). Also, the I-2 value of 99.8% & Cochrane Q Statistics suggested heterogeneity among the studies. Forest plot of the prevalence of bronchial asthma also supports the findings.
Conclusion: The analysis of population based studies emphasize upon the high number of Indian adults suffering from self-reported asthma.

P48

Local allergic reaction to yellow fever vaccine as first manifestation of a bird-egg syndrome

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Bird-egg syndrome is characterized by primary sensitization to bird allergens followed by secondary sensitization to serum albumins present in egg and chicken meat. Allergic reactions to yellow fever vaccine have been described in patients with primary sensitization to egg. Yellow fever vaccines are produced by culture of live attenuated yellow fever virus on chicken embryos. We here present a rare case with a local allergic reaction to yellow fever vaccination as first manifestation of a bird-egg syndrome.
A 20-year-old bird keeper developed erythema, pruritus and swelling of the whole arm following yellow fever vaccination in the upper arm. Additionally, she had been suffering from nasal obstruction for years, but previous examinations had failed to reveal corresponding sensitizations. Skin prick tests and specific IgE were positive to chicken egg yolk and white, chicken meat and to the feathers of her canaries. On the other hand, specific IgE to ovalbumin, ovomucoid and α-livetin were negative. The patient also reported that she tolerates eggs in her diet quite well, but avoids eating chicken meat for personal reasons.
In the absence of a primary egg allergy, the patient’s current history and clinical and laboratory findings point to the diagnosis of a bird-egg syndrome. Sensitization in bird-egg syndrome usually occurs through α-livetin, a serum albumin present on bird feathers and in egg yolk. Although the eliciting allergen in our patient could not be defined conclusively, dietary egg tolerance suggests a heat-labile allergen as causative allergen. According to current guidelines, egg allergy does not represent a general contraindication to egg-containing vaccines. In any case, however, skin testing and tolerance induction are recommended prior to vaccination.

P49

Autophagy in lung epithelial Club cells confers protection against house dust mite (HDM)-induced allergic airway disease (AAD)

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Bronchiolar non-ciliated Club cells are a population of the lung epithelium with self-renewing and progenitor properties in bronchioles. Although Club cells were reported to be important in a model of allergic airway disease (AAD), clarification of their specific functions in allergic immune responses emerges. Autophagy is a fundamental cell fate pathway also involved in AAD. In fact, bronchial epithelial cells isolated from patients with moderately severe asthma had greater numbers of autophagosomes. The current study was focused on the autophagy-associated gene Atg5, as two of its single nucleotide polymorphisms were reported to be linked with AAD pathogenesis. Deletion of Atg5 in Club cells and the subsequent autophagy impairment exacerbated house dust mite (HDM)-induced AAD. The HDM model that includes one allergen sensitization, followed by multiple challenges, is the closest model to the human disease. Lack of autophagy in Club cells resulted in exacerbated lung inflammation with enhanced mucus production, elevated numbers of eosinophils, neutrophils, lymphocytes and total cells in bronchoalveolar lavage (BAL). Higher IL-17 production in BAL was consistent with increased neutrophil and lymphocyte accumulation. Interestingly, in mice with autophagy-deficient Club cells, IL-33, CCL22 and CCL17 chemokine secretion and consequently IL-4 levels were significantly enhanced in the lung. Lack of autophagy in Club cells also facilitated the enrichment of T1ST2+ (IL-33R+) T cells in mediastinal lymph nodes (MLNs), in agreement with elevated IL-33 lung and BAL secretion. Moreover, deficiency in autophagy of Club cells resulted in HDM-specific recruitment of TH cell populations into MLNs. Supportively, MLN cell allergen-specific responses from the autophagy-deficient group exhibited enhanced IL-17, concomitantly with reduced IL-10 production. TH17 responses have been reported to be inhibited by Club cells in allergy and they have also been associated with steroid resistant AAD. Thus, our data suggest a possible link between Club cell autophagy and suppression of lung inflammation in AAD.

P50

Lymph node Migratory Dendritic Cells Modulate HIV-1 Transcription through PD-1 Engagement

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Background. PD-1+/T follicular helper (Tfh) cells, serve as a major cell reservoir for HIV-1 and are responsible for active and persistent HIV-1 transcription after prolonged ART. However, the precise mechanisms regulating HIV-1 transcription in lymph nodes (LNs) remain unclear. The present study aimed to determine the potential role of immune checkpoint (IC)/IC-Ligand (IC-L) interactions on HIV transcription in lymph node (LN).
Methods. To address this issue, we assessed the expression of ICs and IC-Ls on LN cell populations and the impact of IC/IC-L interactions on T-cell proliferation, reactivation of HIV transcription and production in LN

memory CD4 T-cell populations from viremic and ART-treated HIV-infected subjects (n=57).
Results. We showed that PD-1 and TIGIT are the two major ICs expressed on Tfh cells directly ex vivo. We therefore explored the phenotype, frequency and tissue distribution of IC-L expressing cells and showed that PD-L1, PD-L2 and/or CD155 (TIGIT-ligand) were predominantly co-expressed on LN migratory dendritic cells (DCs). The frequencies of migratory DCs in viremic HIV-infected subjects directly correlated with HIV viral load (r=0.875 P=0.0017) and significantly dropped after prolonged ART (P<0.05). Interestingly, PD-L1 expressing cells were detected in both extra-follicular and germinal center (GC) areas of viremic HIV-infected subjects and ART initiation was associated with a significant reduction of the proportion of PD-L1 positive areas (P<0.05), which was even more pronounced in GCs. These data indicate that ART initiation had a profound impact on IC-L expressing cell frequency and tissue distribution and that PD-1/PD-L1 interactions might be selectively reduced in GCs of ART-treated subjects. We subsequently showed that TCR-mediated HIV production was suppressed in vitro when LN migratory DCs were co-cultured with PD-1+/Tfh cells (P<0.05), demonstrating that PD-1 and TIGIT signaling pathways modulate TCR-mediated HIV transcription and production. Finally, the frequencies of LN migratory DCs inversely correlated with HIV transcription (r=-0,828; P<0.05) from LN memory CD4 T cells, indicating that LN migratory DCs may contribute to control HIV transcription in vivo.
Conclusions. These results indicate that LN migratory DCs expressing IC-Ls may more efficiently restrict HIV-1 transcription in the extra-follicular areas versus GCs and explain the persistent HIV transcription in PD-1+/Tfh cells after prolonged ART.

P51

Single-cell analysis of tumor-infiltrating CD8 T-cell transcriptomic states in endogenous anti-tumor responses

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CD8 T cells are the main effectors of the adaptive immune response against intracellular pathogens and cancer. However, antigen persistence in chronic infections and cancer profoundly alters CD8 T-cell differentiation and function. In the past few years, single-cell RNA-sequencing (scRNA-seq) has revealed a heterogeneous landscape of cellular states among tumor-infiltrating CD8 T lymphocytes (TILs). In this work we built an unbiased map of transcriptomic states of CD8 T cells infiltrating murine B16 melanoma tumors based on a new scRNA-seq dataset of >3500 CD8 TILs from individual mice. Unsupervised clustering and systematic gene signature analysis revealed four main transcriptomic states: naïve, exhausted, memory-like and an effector memory-like state that preferentially express Gzmk, Cxcr3 and Ccl5 (Gzmk_Cxcr3). Moreover, common expanded T-cell clones were identified among the three (non-naïve) differentiated states by TCR sequencing, suggesting that the these states are clonally related and tumor-specific. Integrated knowledge on CD8 TIL states was used to develop and validate a novel single-cell transcriptomic classifier of CD8 TIL states (TILPRED) for murine and human TILs. We illustrate the utility of TILPRED by mapping CD8 T-cell states in published data on different murine cancer types and human melanoma patients. These results suggest a compositional shift towards the Gzmk_Cxcr3 population upon checkpoint blockade.

P52

Chronic immune activation as a cause of poor T helper cell migration in successfully ART treated HIV-1 infected individuals

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Background: CD4+ T-cell depletion, and in particular Th17 cell loss in the mucosal compartment, is a hallmark of HIV-1 infection in humans. Alterations in the integrity of the mucosal barrier have been indicated as cause for chronic immune activation and disease progression that occurs despite successful anti-retroviral therapy (ART). In this study, we have investigated the effect of chronic immune activation or selective TLR triggering on the homing capacity of lymphocytes, with the aim of understanding the mechanisms at the basis of a poor repopulation of T-helper cells in the gut during successful treatment with ART.
Methods: Blood samples were collected from a total of 58 HIV-1-infected individuals, either ART-naïve (n=15) or on long-term ART (n=43), and from healthy donors (HD). T-helper cell dynamics, migration capacity, and levels of soluble CD14 (sCD14) were assessed. A mouse model, mimicking the alterations observed in HIV-1 infection was also used to assess the impact of chronic immune activation on T-helper cell migration. In vitro triggering of selective TLRs, was performed on HD samples to dissect the involvement of the different TLRs in modulating cell migration capacity.
Results: CCR6+ and CXCR3+ T-helper cells accumulate in the blood of ART-treated HIV 1-infected patients, and their frequency correlates with the levels of sCD14. In HIV-1-infected individuals, migration of T-helper cells in response to chemotactic stimuli is impaired, regardless therapy. Chronic immune activation induced by TLR signaling is sufficient to dampen T-helper cell migration, which can be restored by pharmacological modulation of cytoskeleton activity.
Conclusions: In patients under long-term ART, chronic immune activation results in an altered T-helper cell response to chemotactic cues, and clarifies the poor gut repopulation observed. More in general, persistent triggering of different TLRs leads to changes in the T cell cytoskeleton machinery and to an impairment of cell migration.
This study calls for novel pharmacological approaches in those pathological conditions characterized by persistent immune activation and loss of trafficking of T-cell subsets to niches that sustain their maturation and activities.

P53

Characterization of melanoma cell responses to T cell-derived cytokines

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We characterized the immunological response of 21 melanoma cell lines in response to a variety of T cell-derived cytokines present in the tumor microenvironment. We found that only IFNγ and TNFα led to major changes in vitro, such as upregulation of MHC, chemokines and/or IDO. Interestingly, the combination of both cytokines was often strongly additive, and was even required in some melanoma cells. Although all cell lines seem to have a shared pattern of response based on the reporters investigated, we still see that the amplitude of the response is diverse and sometimes abnormal. We are now going deeper by investigating what could be the mechanisms explaining the observed differences and whether these changes affect antitumoral immune responses in absence and presence of immunotherapy. In addition to the changes in the level of expression of immune related genes, we also investigated the differentiation states of our melanoma cell lines in presence of the cytokines, as well as the ability of our treatment at inducing cell proliferation arrest and cell death. This characterization already allowed us to correlate some immunological behaviours of melanoma cells with the survival of the patients of which the cells were derived.
Currently, we are analyzing whole exome sequencing data of these cells with the aim to identify mutations related to the behaviours of the melanoma cells. We have a high number of hits that remain to be investigated. We also found that higher mutational load correlates with shorter patient survival, which is however the opposite to what the literature describes for immunotherapy treated patients. We are also characterizing by western blot the IFNγ and TNFα signaling pathways of the melanoma cells with the aim to find molecular alterations that may explain the differences of the cytokine response.
Our data confirm that melanoma cells are not immunologically inactive and should therefore be considered as part of the factors shaping the immunological state of the tumor microenvironment. It is of interest to characterize their behaviour to find molecular alterations that may potentially influence disease and immunotherapy outcomes.

P54

How obesity changes the dynamics, diversity and function of macrophages in adipose tissue: search for culprits

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Immune cell infiltration in visceral adipose tissue (VAT) during obesity is associated with local chronic inflammation and the development of metabolic syndrome, a plague of aging societies. Adipose tissue macrophages (ATMs), one of the major leukocyte fractions in the VAT, are not only the key cells for maintenance of VAT homeostasis, but they could also play a major role in a metabolic dysregulation during aging and obesity. Similarly what we have observed in a tumour microenvironment, at least three phenotypically distinct subpopulations of ATMs are present in the VAT showing different frequencies during the progression of obesity suggesting that the tissue environment drives ATM heterogeneity and vice versa that ATMs contribute to VAT homeostasis and inflammation. I will present data on the turn-over kinetics and potential roles of ATMs during development of obesity probing beyond the superficial M1/M2 dichotomy.

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P55

Mutually exclusive lymphatic vessel formation or perineural infiltration in human skin squamous-cell carcinoma

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Tumor-associated lymphangiogenesis is correlated with increased metastasis formation and poor prognosis in many cancers. In melanoma, lymphatic vessels promote an immunotolerant microenvironment. Recently, we have shown that lymphatic vessels support T-cell recruitment into the tumor, thus potentiating immunotherapy. The role of lymphatic vessels in skin squamous-cell carcinoma (sSCC) remains mostly unknown. Although limited to localized disease in the majority of cases, sSCC can occasionally metastasize into lymph nodes, to distant organs and/or spread through nerves. Existing chemo- and targeted therapies are ineffective against metastatic sSCC, whereas anti-PD1 therapy may be beneficial, with however only low response rates. A greater understanding of disease mechanisms is needed to develop better therapies.
We performed a comprehensive analysis of the abundance and localization of lymphatic vessels in 37 cases of primary sSCC patients classified into 4 groups according to metastases and/or perineural infiltration (PNI). We studied sSCC patients without metastasis (n=15), with PNI only (7), with loco-regional metastases only (6) and with PNI and loco-regional metastases (9). We investigated possible associations of lymphatic vessel density (LVD) with the density of stroma and tumor infiltrating CD8 T-cells.
Using multiplex fluorescent immunohistochemistry followed by computed quantitative image analysis, we found that overall lymphatic vessels, identified by Prox-1+ and Podoplanin+ labeling, are located almost exclusively in the stroma surrounding the tumor. As expected, CD8 T-cell infiltration was significantly denser in the stroma compared to the tumor.
Similar to our data in melanoma, sSCC with high LVD showed increased CD8 T-cell density in stroma and tumor areas. An entirely new observation is that perineural infiltrated sSCC, especially in absence of metastases, were characterized by low LVD compared to tumors without PNI, suggesting that lymphangiogenesis differs according to the PNI status of sSCC.
Our data suggest that the mechanisms underlying PNI may be linked with the biology of lymphatic vessels and thus stroma. Possibly, both PNI and lymphatic vessels have common drivers that trigger branching into one or the other. Since both are known to affect tumor progression and patients' prognosis, it may be particularly important to identify the drivers, opening future options for therapeutic targeting.

P56

Combination of anti-CD40 and anti-PD1 revert M2 polarization to limit tumor growth in a genetically engineered blander cancer mouse model

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Bladder cancer (BCa) represents the fourth most common cancer in men with a poor patient prognosis for advanced disease. The recent advances in immunotherapy allowed to improve the treatment of these patients, but only a fraction of them respond to immune checkpoint blockade (anti-PD-1/anti-PDL1). To understand better the immune contexture of the disease and its relevance for the patient's outcome we used a genetic based mouse model for BCa that recapitulates the various stages of the disease. Specific deletion of tp53 and pten in the bladder led to the development of non-muscle invasive BCa followed by transition toward muscle invasive BCa and the development of metastases. As tumor evolves, we observed a transition from an inflammatory microenvironment to a pro-tumoral/immunossupressive one, going along with a M1 to M2 transition of the macrophages, an increased expression of PD-1 by T cells and accumulation of regulatory T cells. Interestingly, this model is resistant to anti-PD1 treatment. After testing various combinations on mice with MIBC, we found that αCD40+αPD1 led to an increase of CD8 TILs infiltration and IFNγ production, a re-polarization of macrophages toward a M1-like phenotype and to a significant increase of the survival of the mice. We showed that CD8 T cells, Macrophages and IFNγ were necessary for the control of the tumor, and that IFNγ production by CD8 T cells was directly related to the repolarization of the macrophages.

P57

Immune-checkpoints in the regulation of leukemia and cancer stem cells

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Chronic myeloid leukemia (CML) shows many characteristics also present in its healthy counterpart, normal hematopoiesis: a rare (cancer) stem cell on top of the hierarchy, which divides only infrequently, shows self-renewal and can give rise to the different lineages present in the healthy tissue or, in case of CML, in the cancer. As chemotherapy mainly targets bulk cancer cells but spares the more resistant cancer stem cells (CSCs), CSC-targeting therapies are needed. Immune-Checkpoints like PD1, LAG3 or TIM3 are receptors regulating T-cell activation, but it has been shown that they can also be expressed on subsets of cancer cells in different types of cancer. For example, TIM3 is upregulated on acute myeloid leukemia CSCs where it drives their self-renewal via an autocrine loop with its ligand. To screen for immune checkpoint receptors and ligands of importance for CML and CML CSCs, we will set up a pooled small-scale in vivo CRISPR-Knock Out (KO) screening. We are generating a small pooled library of single-guide RNAs (sgRNAs) targeting 25 different genes of interest, and clone it into a lentiviral vector to transduce isolated Cas9-expressing CML stem cells. The mixed cell population will then be used to generate a secondary CML in non-irradiated recipients. Positive and negative effects of KO can be assessed by measurement of the abundance of the respective sgRNAs by next generation sequencing. Effects of KO can also be analysed in vitro by colony formation. Once this system is established it will be a versatile tool to study different cancer models and genes.

P58

Extracellular ATP limits T cell effector response in the tumor microenvironment via ionotropic P2X7 receptor

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Extracellular ATP (eATP) contributes to the determination of cell fate, cell proliferation, differentiation or apoptosis, thereby possibly taking part in promoting or preventing carcinogenesis. ATP released during tissue damage acts as a danger-associated molecular pattern (DAMP) for cells of the innate immune system through stimulation of P2 receptors. P2rx7, which encodes the ATP-gated P2X7 receptor, is a signature gene of

effector T cell subsets; however, its function in these cell subsets has not been addressed so far. P2X7 activation by extracellular ATP causes the efflux of Na+ and Ca2+ and the influx of K+. The tumor microenvironment is rich in eATP that affects TEM cells survival and function. We hypothesize P2X7 constitutes a checkpoint regulator for potentially immunopathogenic CD4 and CD8 effector/memory cells that restrains the tumoricidal activity of tumor infiltrating lymphocytes (TILs). We show that P2X7 stimulation leads to generation of mitochondrial reactive oxygen species (ROS) and p38 MAPK dependent upregulation of cyclin-dependent kinase inhibitor 1A (Cdkn1a, encoding for p21Waf1/Cip1) that result in cell cycle arrest and cellular senescence of T effector memory cells. Our study suggests targeting P2X7 in TILs might improve their tumoricidal activity.

P59

Role of ER stress and lipid metabolism in tumor-associated macrophages

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The presence of tumor-associated macrophages (TAMs) in several solid tumors is associated with poor prognosis and decreased overall survival. TAMs are mainly trapped in the hypoxic area where they exert immunosuppressive and/or proangiogenic functions. These protumoral TAMs show a M2-like phenotype. Re-education of TAMs towards an anti-tumoral M1-like phenotype has been proposed as potential immunotherapy, alone or in combination with checkpoint blockade inhibitors. However, in order to design specific therapy aiming at TAMs reprogramming, it is necessary to unravel the driving cause of the M2-like switch of macrophages in the tumor microenvironment. Here, we show that TAMs have higher lipid content and upregulated ER stress response compared to splenic macrophages. The uptake of lipids released by cancer cells plays an important role in skewing macrophages towards an immunosuppressive M2-like phenotype. Uptake of cancer cells-derived lipids by macrophages induces an IRE1/XBP1-mediated ER stress response that induces STAT3 activation and together with it affects the regulation of important immunosuppressive genes. In vitro, pharmacological or genetic inhibition of XBP1 and STAT3 could rescue the M2-like switch caused by cancer cell-derived lipids. These data open new therapeutic possibilities by either targeting lipid metabolism in cancer cells or ER stress in TAMs.

P60

Constructing accessory lymph nodes in situ for local control of tumor growth

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Lymph nodes (LNs) are strategically situated throughout the body at junctures of the blood vascular and lymphatic systems to drain antigens from peripheral tissues. LNs that are located in the vicinity of a tumor, known as tumor-draining LNs, play an important role in the host's immune responses against the tumor. Here, we have addressed the hypotheses that generation of accessory LNs can be exploited to foster the initiation and maintenance of anti-tumor immunity. Following the implementation of a robust protocol for the induction of accessory LNs in mice, we have elaborated the cellular composition and functionality of such novel immunological "base camps". We found that, despite moderate structural deviations, immune cell content and activation parameters were comparable to constitutive LNs. In particular, accessory LNs contributed efficiently to mounting of anti-viral and anti-tumor immune responses. Currently, single-cell RNA-seq and high-resolution microscopy analyses are performed to decipher the cellular and molecular composition of the stromal cell infrastructure of accessory LNs. In sum, our findings provide novel avenues towards improvement of immune reactivity and options to interfere with immune-regulatory circuits during malignant diseases.

P61

On the role of Rfx7 in the development of tumors

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Metabolism is important for the normal function and development of immune cells. During development and activation, lymphocytes proliferate and increase their energy consumption in a tightly controlled process. Dysregulation of metabolism can contribute to the development of malignancies and is a sign of cancer. The transcription factor Rfx7 was shown to attenuate metabolism in lymphocytes. In fact, using RNA-sequencing, we discovered that Rfx7 controlled several negative regulators of metabolic pathways. Additionally, indications for a link between Rfx7 and blood cancers exist. Mutations of Rfx7 are present in lymphoma and associations of Rfx7 to leukemia were found in genome-wide association studies. We therefore speculated that lack of Rfx7 in lymphocytes could contribute to the development of lymphoid malignancies. To test the contribution of Rfx7 to tumour development, we generated mice with Rfx7 and p53 deletion in the hematopoietic system. p53 knock-out mice develop T cell lymphoma at a mean age of 20 weeks. Mice lacking p53 and Rfx7 in the hematopoietic cells started to show symptoms of lymphoproliferation starting at age 9 weeks, mainly due to B cell expansion. This suggests that Rfx7 contributes to the suppression of tumour formation. To uncover the underlying mechanisms, we plan to study altered signalling pathways using RNA-sequencing, molecular, and biochemical techniques. The discovery and characterization of Rfx7 as a tumour suppressor could give insights into new mechanisms of cancer development and help understanding tumour formation in patients.

P62

PGC-1α engineered CD8 T cell for enhanced anti-tumor immunity

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Introduction: Memory CD8 T cells can provide long-term protection against tumors, which depends on their enhanced proliferative capacity, self-renewal and unique metabolic rewiring to sustain cellular fitness. Specifically, memory CD8 T cells engage oxidative phosphorylation (OXPHOS) and fatty acid oxidation (FAO) to fulfill their metabolic demands. In contrast, tumor infiltrating lymphocytes (TILs) display severe metabolic defects, which may underlie their functional decline.
Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) is a master regulator of mitochondrial biogenesis and controls pathways known to be crucial in memory differentiation. We thus hypothesized that overexpression of PGC-1α in CD8 T cells might improve cell fitness and memory lineage differentiation in tumor and infection settings.
Methods: To explore the precise role of PGC-1α in CD8 T cell memory formation and persistence, we transduced OT-1 T cells (transgenic CD8 T cell bearing TCRs specific for ovalbumin) with a retroviral-based system overexpressing PGC-1α or mock control vector (SCR).
Results: Here, we show that overexpression of PGC-1α indeed favors CD8 T cell central memory formation rather than resident memory generation. PGC-1α-overexpressing CD8 T cells exhibit increased persistence and mediate more robust recall responses to bacterial infection or peptide vaccination. Importantly, CD8 T cells with enhanced PGC-1α expression provide stronger anti-tumor immunity in a mouse melanoma model. Moreover, TILs overexpressing PGC-1α maintain higher mitochondrial activity and improved expansion when re-challenged in a tumor-free host.
Discussion: Altogether, our findings indicate that enforcing mitochondrial biogenesis promotes CD8 T cell memory formation, metabolic fitness

and anti-tumor response *in vivo*. Therefore, development of novel strategies improving metabolic fitness of adoptively transferred T cells could enhance the therapeutic efficacy of anti-tumor immunity.

P63

Metabolic Reprogramming of Antitumor CD8+ T Cell Immunity

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Introduction: Adoptive cell transfer (ACT) therapies are successfully used in the clinic, however a large fraction of patients remains unresponsive, and the therapeutic effectiveness of the responding fraction can be improved. The limited efficacy of this therapy is mainly due to the terminally differentiated state of transferred T cells, which limits their proliferation and long-lasting antitumor response. Memory $CD8^+$ T cells display specific phenotypic and functional characteristics endowing them with the ability to provide a more potent and long-lasting antitumor immune response than their terminally differentiated counterparts. The development and fitness of memory T cells was recently shown to be associated with specific metabolic pathways. We aimed to metabolically reprogram $CD8^+$ T cells in order to generate fitter memory-like T cells prior to ACT. To do so, we performed a pharmacological inhibition of a metabolic enzyme involved in the tricarboxylic acid cycle during the priming of $CD8^+$ T cells.
Methods: OT-1 cells were activated with SIINFEKL peptide and cultured in vitro for 3 days in the presence of recombinant human IL-2 and either DMSO or a small molecule metabolic inhibitor. At day 3 the inhibitor was washed out and the cells were cultured for an additional 4 days in the presence of recombinant human IL-2 and IL-7. These cells were then adoptively transferred, followed by vaccination with CpG and SIINFEKL peptide into ovalbumine-expressing B16 melanoma-bearing mice. Memory markers were analysed by flow cytometry and histone modifications by western blot analyses.
Results: We have found that metabolic inhibition during T cell activation led to an increased memory formation and to an enhanced tumor growth inhibition upon ACT into melanoma tumor-bearing mice. Interestingly, the metabolic inhibition was associated with increased histone methylation and acetylation. These histone modifications were required to induce the observed memory phenotype, since the concurrent treatment of metabolically reprogrammed cells with a histone acetyltransferase inhibitor abrogated the phenotypic changes and the enhanced antitumoral function induced by single agent metabolic inhibition. We hypothesize the metabolic intervention resulted in altered levels of metabolic intermediates used in epigenetic modifications, such as α-ketoglutarate and acetyl-CoA.
Discussion: These results suggest a novel strategy to promote stable memory T cell differentiation by epigenetic processes induced by metabolic reprogramming during T cell priming. These findings might be exploited to optimize ACT immunotherapy against cancer.

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Understanding the susceptibility of CART cells to Fas- and DR5-mediated cell death

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Introduction: Adoptive transfer of T cells engineered to express a CD19-specific Chimeric Antigen Receptor (CART) has demonstrated impressive clinical success against haematological cancers. In contrast, CART cells have proven inefficient so far against solid tumors. We recently discovered that antigen specific CART cells are highly prone to Programmed Cell Death (PCD) independently of either TCR or CAR ligation. We hypothesized that the susceptibility of CART cells to apoptosis may result from 1) a tonic signaling resulting from the supra-physiological number of CAR signaling domains; 2) an unfolded protein response (UPR) due to the high load of CAR molecules to be folded and transferred to the cell surface.
Methods: We tested if lowering CAR expression could rescue CART cells from apoptosis and improve their persistence *in-vivo*. We first tried to destabilize the CAR at the protein level, by introducing various destabilizing domains (DD) into HER2-CAR molecules. Then, we tested destabilizing the CAR at the mRNA level, by developing HER2-CAR constructs expressing the miR-responsive element (MRE) of miR-155 in the 3'UTR of the CAR transcript. We also looked at different UPR markers in CART cells.
Results: Destabilization of the CAR at the protein level indeed led to reduced CAR expression and to functional CART cells *in-vitro*. Yet, it failed to prevent massive OT-I CART cell death upon *Listeria*-OVA infection. Further, destabilization of the CAR at the mRNA level was tested. Despite a decrease in the CAR expression levels correlating with increased miR-155 levels *in-vitro*, rescue of CART OT-1 T cells from apoptosis upon either *Listeria*-OVA infection or in B16-OVA tumor settings was not possible. In view of these negative results, we investigated the ER-stress and UPR occurrence and found that some markers were up-regulated in *ex-vivo* sorted CART OT-1 cells, such as total XBP1 and the spliced variant XBP1s. Moreover, the addition of the chemical chaperone 4PBA *in-vitro* greatly ameliorated the surface expression of the CAR. However, XBP1s over-expression in CART cells did not prevent CART cell death upon *Listeria*-OVA infection.
Discussion: Overall, these results suggest that several molecular mechanisms are likely involved in the CART cell pronounced susceptibility to death. We are now developing different translational strategies to more specifically protect engineered T cell from apoptosis.

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Impaired mitochondrial fitness orchestrates t cell dysfunction in the tumor microenvironment

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Cancer immunotherapy, including checkpoint blockade and adoptive transfer of tumor-reactive T cells, represents a paradigm shift in the treatment of malignancies in recent years, and yields remarkable responses by reawakening anti-tumor immunity in established tumors. Nevertheless, a significant portion of patients are refractory to cancer immunotherapies, which may be in part due to the persistent impairment of anti-tumor effector functions in T cells, a phenomenon referred to as T cell exhaustion. Emerging evidence reveal that alterations in global chromatin accessibility and de novo DNA methylation patterns are keys events to drive development of T cell exhaustion under chronic antigenic stresses. However, it remains elusive how T cells engage epigenetic reprogramming to orchestrate exhausted state. Here, we found that tumor-infiltrating tumor-reactive T cells with accumulation of damaged mitochondria, characterized by increased mitochondrial mass but reduced mitochondrial membrane potential and cristae, display more severe exhausted phenotypes, including decreased proliferation capacity, reduced cytokine production and up-regulation of co-inhibitory receptors. The accumulation of damaged mitochondria is in part due to the deficiency of mitophagy machinery. Importantly, we found that the accumulation of dysfunctional mitochondria is corelated to the specificity and affinity of antigen, and also supported by the PD-1 expression. Ultimately, the combination of glucose deprivation, hypoxia and TCR signaling in vitro can drastically weaken T cell immunity with the accumulation of dysfunctional mitochondria as seen in TILs previously. Taken together, our study suggests that mitochondrial fitness is pivotal for T cell-mediated immunity and the accumulation of dysfunctional mitochondria could result in exhaustion phenotypes in T cells. This further provides pillars for better harnessing T cell immune responses with metabolic regulations for immunotherapy.

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Arginase-2 serves as an immunometabolic checkpoint regulator of anti-tumor CD8+ T cell function and persistence

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As arginine is a crucial amino acid for T cell responses, tumors often exert competition for arginine as a strategy for escaping immune control. Although arginine depletion within the tumor microenvironment by Arginase 1 (Arg1) is a well-established immunosuppressive mechanism, roles of Arginase 2 (Arg2) in anti-tumor responses remain unexplored. We show here that Arg2 is a cell-intrinsic immunometabolic regulator of anti-tumor CD8+ T cell activity. Both germ-line Arg2 deletion and adoptive transfer of Arg2-/- CD8+ T cells significantly reduced tumor growth in mouse tumor models by enhancing CD8+ T cell function and persistence. Transcriptomics, proteomics and high-dimensional flow cytometry experiments revealed a cell-intrinsic role for Arg2-mediated arginine metabolism in CD8+ T cells, regulating their activation and anti-tumor functions. Furthermore, CD8+ T cell-specifc Arg2 deletion exhibited strong synergy with PD-1 blockade in controlling tumor growth and animal survival, hence unveiling Arg2 as a promising target for T cell based cancer therapies

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Type I interferon signalling in fibroblastic reticular cells secures protective antiviral immunity

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Fibroblastic reticular cells (FRC) shape the organization of secondary lymphoid organs and actively promote the induction of immune responses by coordinating the interaction of innate and adaptive immune cells. However, the mechanisms underlying FRC functions during viral infections have remained largely unexplored. Here, we genetically ablated the type I interferon receptor (IFNAR) in Ccl19-Cre+ cells and found that lymph node FRC sense type I IFN to prevent viral dissemination and to promote T cell-mediated immunity. Under homeostatic conditions, single cell transcriptomic analysis revealed that IFNAR signaling is required to maintain an antiviral and immune-stimulating state of Ccl19-Cre+ lymph node FRC. During localized infection with the lymphocytic choriomeningitis virus, IFNAR signaling precipitated rapid reprogramming of Ccl19-Cre+ FRC that prevented systemic dissemination of the virus. Moreover, the profound IFNAR-dependent shift of all FRC subsets towards an immune-stimulatory state was required to sustain effective antiviral CD8+ T cell responses. In sum, these results unveil the critical role of lymph node FRC at the nexus of innate and adaptive immune responses during viral infection.

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Virus-like particles (VLPs) for vaccination against cancer

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Active immunotherapy of cancer aims to treat the disease by inducing effective cellular and humoral immune responses. VLP-based vaccines have evolved dramatically over the last few decades, however therapeutic VLP-based vaccines remain to demonstrate clinical benefit. Here we demonstrate the ability of VLP-based vaccine in inducing potent anti-tumour immune response in an aggressive transplanted melanoma murine model.
In the first study, we proposed a platform for the development of a personalized VLP-based vaccine. Germline or mutated CTL epitopes of the B16F10 melanoma murine cell line were identified by immunopeptidomics or predicted by WES, respectively. Qb-VLP were loaded with TLR-9 ligands and the CTL epitopes were coupled to Qb-VLPs using the bioorthogonal Cu-free click chemistry method enabling bedside production of a personalized cancer vaccine, ready for clinical translation. Three sets of multi-target vaccine (MTV) were developed, the 1st set was based on germline epitopes (germline-MTV), the 2nd set was based on mutated epitopes (mutated-MTV) and the 3rd set combined both epitopes (Mix-MTV). Our results showed that both germline and mutated MTV induced protection but the best therapeutic effect was achieved when combining both. The Mix-MTV could enhanced the infiltration of CD8+ T-cells into the tumour and changed its myeloid composition.
In the second study, we harnessed the physiological properties of the lymphatic system to optimize the induction of a protective T cell response. We used our previously developed cucumber-mosaic virus-derived nanoparticles termed (CuMVTT-VLPs) incorporating a universal Tetanus toxoid epitope TT830–843 and displaying gp33 epitope as a model antigen using Cu-free click chemistry. The CuMVTT-p33 nano-sized vaccine has been next formulated with MCT adjuvant. Our results showed that formulating the nanoparticles with the micron-sized MCT adjuvant of ~ 5 µM resulted in a local depot for the nanoparticles and a longer exposure time for the immune system. The preclinical nano-vaccine CuMVTT-p33 formulated with the MCT has enhanced the specific T cell response in the stringent B16F10p33 murine melanoma model. Furthermore, MCT adjuvant was as potent as B type CpGs and clearly superior to the commonly used Alum adjuvant when total CD8+, specific p33 T cell response or tumour protection were assessed.

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Vaccination with nanoparticles combined with micro-adjuvants protects against cancer

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Active immunotherapy of cancer aims to treat the disease by inducing an effective cellular immune response, in particular cytotoxic T cells. VLP-based vaccines have evolved dramatically over the last few decades, however therapeutic VLP-based vaccines remain to demonstrate clinical benefit. Here we demonstrate that it is possible to harness the physiological properties of the lymphatic system to optimize the induction of a protective T cell response. Indeed, the lymphatic system sharply distinguishes between nanoscale and microscale particles. The former reaches the fenestrated lymphatic system via diffusion, while the latter either need to be transported by dendritic cells or form a local depot. In this study, we used our previously developed cucumber-mosaic virus-derived nanoparticles termed (CuMVTT-VLPs) incorporating a universal Tetanus toxoid epitope TT830–843 and displaying gp33 epitope as a model antigen using Cu-free click chemistry. The CuMVTT-p33 nano-sized vaccine has been next formulated with the micron-sized microcrystalline tyrosine (MCT) adjuvant and the formed depot effect was studied using confocal microscopy and trafficking experiments. The immunogenicity of the nanoparticles combined with the micron-sized adjuvant was next assessed in an aggressive transplanted murine melanoma model. The obtained results were compared to other commonly used adjuvants such as B type CpGs and Alum. Our results showed that CuMVTT-VLPs can efficiently and rapidly drain into the lymphatic system due to their nano-size of ~30 nm. However, formulating the nanoparticles with the micron-sized MCT adjuvant of ~5µM resulted in a local depot for the nanoparticles and a longer exposure time for the immune system. The preclinical nano-vaccine CuMVTT-p33 formulated with the micron-sized MCT adjuvant has enhanced the specific T cell response in the stringent B16F10p33 murine melanoma model. Furthermore, the micron-sized MCT adjuvant was as potent as B type CpGs and clearly superior to the commonly used Alum adjuvant when total CD8+, specific p33 T cell response or tumour protection were assessed.

P70

IL-21 secreted by CD4 T cells reduces leukemia stem cell function in human and murine acute myeloid leukemia

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Leukemia stem cells (LSCs) are resistant to standard treatment and to elimination by the immune system. Consequently, they represent the main reason for treatment failure and disease relapse. IL-21 receptor (IL-21R) forms a heterodimeric complex with the common γ chain and is widely expressed by hematopoietic cell populations. Upon binding of its ligand interleukin 21 (IL-21), it modulates function of lymphoid and myeloid cells. The role of IL-21/IL-21R signalling in leukemia and LSCs is unknown.

In this study, we show that IL-21R is heterogeneously expressed on leukemic stem/progenitor cells (LSPCs) from newly diagnosed acute myeloid leukemia (AML) patients and that IL-21 is exclusively expressed by CD4 T cells in AML. IL-21 was significantly increased in serum of AML patients compared to healthy controls (median: 45.9 vs 1.3 pg/mL) and was identified as an independent positive prognostic marker for overall survival. Functionally, IL-21 significantly reduced colony-forming and replating capacity of primary LSPCs ex vivo. Importantly, hematopoietic stem/progenitor cells from healthy bone marrow (BM) donors did not express IL-21R and were unaffected by IL-21 treatment.

Based on these findings, we hypothesized that IL-21/IL-21R signalling restricts the functionality of AML LSCs. We used a well-established syngeneic murine AML model to study how IL-21/IL-21R signalling regulates LSCs function in vivo. IL-21R -proficient and -deficient lineage- Sca-1+ c-kit+ cells were transduced with three different AML oncogenes (MLL-AF9, MLL-ENL and BCR-ABL1/NUP98-HOXA9) followed by transplantation into immuno-competent IL-21R-proficient recipient mice. Similar to our findings in human AML, murine AML LSCs expressed IL-21R and IL-21 was exclusively secreted by conventional CD4 T cells in the BM but not in the blood of AML mice. IL-21R deficiency on LSCs resulted in a faster disease progression and reduced survival in all three AML models. Genetic blockade of IL-21/IL-21R signalling in AML increased LSCs number in the bone marrow of AML mice after leukemia transplantation, as analysed by colony assay and in secondary transplantation experiments.

Similarly, treatment of human AML LSCs with recombinant IL-21 reduced the frequency of human AML LSCs in patient-derived xenografts. In summary, IL-21 secreted by CD4 T cells regulates LSCs function in vivo and contributes to leukemia control. Further investigations will focus on the underlying cellular and molecular mechanisms.

P71

The human palatine tonsil microbiome in HPV-associated oropharyngeal squamous cell carcinoma

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Oropharyngeal squamous cell carcinoma (OPSCC) can be caused by viral transformation of epithelial cells in the oropharynx such as high risk-human papilloma virus (HR-HPV). It has been suggested that the containment of HR-HPV can be influenced by the microbiome present at mucosal surfaces and by microbial agents that persist within immune cells and thereby alter immune responses against the pathogen. In a pilot study, we characterized the microbiome in crypts of human palatine tonsils in patients suffering from OPSCC in comparison to an age-matched control cohort of patients undergoing tonsillectomy due to sleep apnea. We found that patients suffering from OPSCC display a characteristic tonsillar microbiome composition that significantly differs from the microbiome of healthy tonsils. In sum, our study provides valuable information on the composition of the tonsillar microbiome in adults and will help to clarify the role of the microbiome in HPV-driven OPSCC.

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Progressive encephalomyelitis with rigidity and myoclonus with antibodies to glycine receptor presenting with temporo-mandibular joint dislocation

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Introduction. Progressive encephalomyelitis with rigidity and myoclonus (PERM) is a rare immune-mediated neurological disorder. It manifests with limb and axial rigidity, myoclonus, hyperekplexia, brainstem (oculomotor and bulbar), sensory and autonomic disturbances, as well as pyramidal signs. Antibodies (Ab) to the glycine receptor (GlyR) are detected in up to 50% of reported cases. PERM with anti-GlyR Ab is generally associated with a better prognosis than PERM with Ab to glutamic acid decarboxylase.

Methods. We report a case of severe PERM with anti-GlyR Ab, which was successfully treated with high-dose corticosteroids (CS), plasma exchanges (PE) and rituximab (RTX).

Results. A 68-year-old male presented with sudden-onset nocturnal right ear pain, worsened by any mouth movement. He was unable to eat, but drank sufficiently over the next few days. He came to the emergency room (ER) on day 5 of symptom onset. The ENT specialists diagnosed a right temporo-mandibular joint (TMJ) dislocation. After unsuccessful attempts to relocate the jaw, tizanidine was prescribed. The patient also complained of constipation and difficult micturition. Within a week he developed vertigo and binocular vertical diplopia. Cerebral imaging showed a microvascular leukoencephalopathy. He was started on Aspirin, but returned on day 9 to the ER with an acute confusional state. Upon hospitalization, his condition worsened, with development of hyperthermia, generalized stiffness and jerky movements, requiring intubation, sedation and admission to the intensive care unit (ICU). He received vasopressor support for severe autonomic dysfunction. Routine lab work and EEG were unremarkable. Cerebrospinal fluid (CSF) showed lymphocytic pleocytosis. Anti-GlyR Ab were detected in both serum and CSF. A whole-body PET-CT was normal. The patient was treated with five PE and five methylprednisolone 1 g pulses, followed by slow CS tapering and two RTX 1 g infusions. Myoclonus and autonomic dysfunction subsided over 1-month period. The patient underwent tracheostomy and was transferred to the neurological ward after a 38-day ICU stay. Further course was characterized by slow but steady reduction of rigidity.

Conclusions. PERM in this case started with unilateral TMJ dislocation, followed by diplopia. Within ten days, the patient developed the full-blown neurological picture. The identification of anti-GlyR Ab was crucial for the diagnosis. Immunosuppression led to slow but progressive improvement.

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Protection from Clostridium difficile infection by apyrase-bearing oral vaccine

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Clostridium difficile has emerged as a major public health threat as its infection was recognised as the most common cause of antibiotic-associated diarrhoea and colitis. The stimulation of a potent immune response by oral vaccination is a desirable strategy to protect from the infection of enteric pathogens, such as C. difficile. Notably, the production of high-affinity secretory IgA (SIgA) fosters the enchainment and aggregation of enteropathogens, thus promoting their clearance from the gut lumen. A transient depletion of intestinal ATP, via administration of ATP-diphosphohydrolase (apyrase), dramatically improves the induction of a specific SIgA response against enteropathogenic species. In the present work, the immunization of mice with a non-pathogenic E. coli co-expressing Shigella flexneri periplasmic apyrase and a C. difficile antigen was found to significantly enhance the production of protective SIgA and promote the recovery in a murine model of C. difficile infection. Overall, these data provide a promising proof of concept for our ultimate aim, which is to develop an apyrase-bearing oral vaccine to provide high-level protection from C. difficile infection

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A new drug-modifying therapy for Type 2 Diabetes Mellitus

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Amyloid aggregates composed of extracellular fibrils of islet amyloid polypeptide (IAPP, also called amylin) – a peptide synthetized in the pancreatic β-cells and co-secreted with insulin- are found in most type 2 diabetes mellitus (T2DM) patients and has been associated with the progression of the disease. As aggregates are considered to be a key factor in β cell death, we aim at developing a vaccine targeting these pathogenic aggregates to prevent and/or reverse accumulation and enhancing β cell survival. To study this, a transgenic mouse model expressing human IAPP (hIAPP) was used.

The vaccines were designed using different IAPP peptide sequences chemically cross-linked to virus like particles (VLPs). The induced antibody response against each peptide, was analyzed by ELISA with serum obtained from immunized C57BL/6JRccHsd mice. The peptide coupled to the VLPs inducing the best IgG response against IAPP was then tested in a transgenic mouse model developing spontaneously T2DM. Interestingly, mice immunized with the candidate vaccine showed significantly delayed increased fasting blood glucose level compared to the control ones, which received the uncoupled VLPs. Furthermore, pancreatic islets of vaccinated mice show significantly less amyloidogenic aggregates, higher insulin content and significantly less IL-1β positive cells in comparison to the control mice. Moreover, we could prove that the generated polyclonal antibodies recognize aggregates but not monomeric amylin, leaving a physiological function intact.

In addition, based on recent data suggesting that hIAPP can promote βcells death through the synthesis of IL-1β via activation of the inflammasome NLRP3 we aimed at assessing whether a monoclonal IgG antibody directed against hIAPP can decrease inflammasome activation. For this purpose bone marrow derived dendritic cells (BMDC) were obtained after stimulation with the granulocyte-macrophage colony-stimulating factor (GM-CSF) from C57BL/6JRccHsd mice; primed first with LPS from E.coli to induce the transcription of pro-IL-1β, and later activated either with hIAPP or rat IAPP (rIAPP) for the secretion of mature IL-1β. As expected, only hIAPP could induce the activation of the inflammasome after priming; and, as hypothesized, the anti-hIAPP IgG prevented the release of IL-1β in a dose-dependent manner. A mechanistic approach is now under investigation to discover if the Fc-Receptor plays an important role in the functional role of IgG.

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Preclinical development of VLP-based vaccines targeting all four Dengue virus serotypes

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The Dengue virus (DENV) is a member of Flavivirus genus and is among the most prevalent and fastest spreading mosquito-transmitted viruses capable of causing human diseases. However, the urgent need to develop an efficient vaccination strategy against DENV is challenged by complex immune responses against its four antigenically distinct serotypes (DENV1-4). The major problem is that pre-existing immunity to one of the DENV serotypes and subsequent infection with a heterotypic DENV serotype increases the risk of developing severe clinical manifestations such as dengue haemorrhagic fever (DHF)/dengue shock syndrome (DSS). An effective vaccine against DENV must therefore be capable to neutralize all four serotypes in order to avoid disease enhancement. The potential targets of neutralizing antibody generation are three structural proteins: a capsid protein (C), a membrane-associated protein (prM) and an envelope protein (E). The E protein of DENV consists of three distinct domains (EI-EIII) and is structurally conserved among Flaviviruses. Although EIII-specific antibodies are not predominant in DENV-infected patients, solid evidence suggests that these antibodies are strongly neutralizing and do not cross-react with other dengue serotypes.

Here, we show that coupling of EIII-domains of all 4 serotypes to bacteriophage derived virus-like particles (VLPs) is feasible and results in high titers of EDIII-specific, neutralizing antibodies against all four serotypes. Furthermore, combining the four vaccine candidates results in simultaneous induction of neutralizing antibodies against all four serotypes in the same mouse. Thus, it may be possible to generate a combination vaccine against all four Dengue serotypes based on EIII-domain coupled to VLPs. As next, we will address the possibility to genetically fuse the EIII-domains to next generation VLPs, such as immunologically optimized Cucumber Mosaic Virus (CuMV). This will result in easily produced, highly immunogenic forms of the EIII-domains displayed on the surface of VLPs.

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