European Journal of Immunology

Abstracts

II Joint Meeting of the German Society for Immunology (DGfI) and the Italian Society of Immunology, Clinical Immunology and Allergology (SIICA)

10-13 September, 2019

Munich, Germany

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Abstract title. Authors. Conference: Abstracts. Location Munich, Germany. Date September 10-13, 2019. Eur. J. Immunol. 2019.49, S1, page number(s). Meeting Abstract number [the Abstract number can be found above the title]

DOI: 10.1002/eji.201970300

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ORAL PRESENTATIONS

POSTER PRESENTATIONS

DOI: 10.1002/eji.201970300

Satellite Symposium of the Research Focus Group "Natural Killer Cells" (SAT1-SAT9)

SAT1

The role of the chemokine receptor CCR8 in migration and effector function of innate lymphoid cells type 2

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Introduction: Group 2 innate lymphoid cells (ILC2s) are a newly described branch of type 2 immunity implicated in protective anti-helminth immune responses and the pathogenesis of airway inflammatory diseases. While their immunoregulatory capacity is largely mediated by providing a major source of type 2 signature cytokines at mucosal barrier surfaces, the factors and mechanisms involved in migration and tissue tropism of ILC2s are poorly understood. In this context, chemokine receptors are thought to be involved in organ-specific homing of ILC2s.

Objectives: Since we and others revealed, that the type 2 immune response-associated chemokine receptor CCR8 is within the ILC subsets specifically expressed in both human and mouse ILC2s, we aimed to investigate the role of CCR8 in ILC2 migration or effector function.

Methods: We sort-purified ILC2s and performed migration assays towards chemokine ligands *in vitro*. To investigate the migration capacity *in vivo*, we adoptively transferred ILC2s and visualized cells seeded to the lungs by lightsheet microscopy. Further, we infected WT and Ccr8-/- mice with the helminth *Nippostrongylus brasiliensis (N.b.)*. To create ILC2-specific CCR8-deficient mice, we generated mixed bone marrow chimeras. Finally, we adoptively transferred ILC2s in Rag2-/-Il2rg-/- mice and performed *N.b.* infection. In line with murine experiments, we also analyzed sort-purified human ILC2s.

Results: Here, we revealed substantially diminished lung ILC2 counts in Ccr8-/- mice upon *N.b.* infection, accompanied by decreased amounts of type 2 effector cytokines, especially IL-9. Consequently, these mice exhibited significantly less eosinophilia and were overall impaired in worm clearance. Infection of ILC2-specific CCR8-deficient mice and adoptive transfer of WT or Ccr8-/- ILC2s proved that CCR8 specifically expressed by ILC2s is critical for parasite clearing. However, migration assays with WT and Ccr8-/- ILC2s clearly indicated no migration towards CCR8 ligands *in vitro* and *in vivo*. By contrast, *in vitro* stimulation experiments with the CCR8 ligand CCL1 and specific CCL1 inhibitors identified autocrine CCL1/CCR8-signalling as critical for regulating the growth and effector cytokine production of mouse and human ILC2s.

Conclusion: These data add to our understanding of ILC2 biology and may support the further development of clinical approaches, as CCR8 is a promising target for monoclonal antibody therapy of asthma.

SAT2

Cytokine release, degranulation and migratory capacity of NK cells depends on ADAP during *Listeria monocytogenes* infection of mice

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Introduction: ADAP serves as a scaffold protein while known to be involved in the formation of immune signaling complexes. To date only limited and conflicting data exist regarding the role of ADAP in NK cells. While on the one hand it was shown that ADAP is dispensable for NK cell development and function, another study revealed that ADAP is essential for inflammatory cytokine production in NK cells but is not required for their cytotoxicity.

Objectives: We thought to extend existing data by studying ADAP-dependency of NK cells in the context of *in vivo* infection with the intracellular pathogen *Listeria monocytogenes (Lm)* were NK cell activation occurs within their natural environment.

Materials & methods: Systemic *Lm*-infections were carried out in wildtype and ADAPko mice as well as in NKp46$^{cre/het}$ × ADAP$^{fl/fl}$ mice lacking ADAP exclusively in NK cells followed by isolation and characterization of NK cells

DOI: 10.1002/eji.201970300

via FACS, real-time qPCR, proteomic profiling and transwell migration assays with or without prior re-activation *in vitro*, respectively.

Results: n line with previous observations *ex vivo* analysis of NK cells from infected wildtype and ADAPko mice revealed impaired expression of IFN-γ, CCL3, CCL4 and CCL5 in ADAPko NK cells. However, in direct contrast to previous reports focusing on naïve NK cells, *in vivo* activated ADAPko NK cells show impaired degranulation capacity as indicated by reduced CD107 surface expression following *in vitro* re-stimulation with anti-NK1.1 +/- cytokines or YAC-1 target cells on day 1 and 3 *post Lm* infection. In line with reduced cytotoxic capacity, impaired perforin production was found in ADAPko NK cells on day 3 *post Lm* infection. In addition, CXCL12-stimulated *in vitro* migration assays revealed significantly reduced migration capacity of ADAPko NK cells from *Lm* infected mice. Strikingly, this defect was also observed *in vivo* during *Lm* infection of conventional and conditional ADAPko mice. Here, we found significantly reduced numbers of NK cells in spleen and livers, the major sites of *Lm* replication. Ongoing experiments focus on potential differences in vesicular transport and actin dynamics in order to explain the observed impact of ADAP on NK cell degranulation and migration during *in vivo* infection.

Conclusion: We found that during *in vivo Listeria monocytogenes* infection in mice cytokine and chemokine production, cytotoxic and migratory capacity in NK cells depends on ADAP.

SAT3

Diminished allergen-induced intestinal and airway inflammation in humanized mice by depletion of natural killer cells prior to PBMC engraftment

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Recently, we have developed a humanized mouse model of allergen-induced IgE-dependent gut and lung inflammation in PBMC-engrafted immunodeficient mice. As natural killer (NK) cells have been shown to promote allergen sensitization, type-2 immune responses and airway hyperreactivity, the aim of the present study was to investigate the impact of NK cells in this model. Therefore, NOD-*scid*-γc$^{-/-}$ mice were injected intraperitoneally with human PBMC or NK cell-depleted PBMC from highly sensitized birch or grass pollen allergic donors together with the respective allergen or with NaCl as control. After an additional allergen boost one week later, mice were challenged with the allergen rectally on day 21 and gut inflammation was monitored by video mini-endoscopy evaluating translucency, granularity, fibrin production, vascularity, and stool. Then, mice were further challenged intranasally on two subsequent days and airway inflammation was measured by invasive body plethysmography and by histology. Allergen-specific human IgE in mouse sera, if detectable after co-injection of the respective allergen, was reduced in mice being injected with NK cell-depleted PBMC compared to mice which received non-depleted PBMC. Additionally, allergen-induced IgE-dependent colitis, airway hyperreactivity and mucus-producing goblet cells were significantly inhibited in these mice. Importantly, infiltration of the colon and lung with human CD45+ cells was similar in all groups. These results demonstrate that allergen-specific gut and lung inflammation in PBMC-engrafted humanized mice can be diminished by depletion of NK cells prior to PBMC transfer, which may be of great interest for therapeutic intervention of allergic diseases.

SAT4

NK cells express target-cell specific receptors associated with degranulation

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Introduction: Natural killer (NK) cells play an important role in the recognition and subsequent elimination of virus-infected and transformed cells. NK cells differentially express a large array of surface receptors mediating NK-cell effector functions and contributing to the diversity of an individual's NK-cell compartment. Given the variety of potential

DOI: 10.1002/eji.201970300

target cells and their phenotype NK cells may require distinct receptor profiles for effective recognition of different target cells.

Objectives: In this study we sought to identify NK-cell receptor expression patterns associated with NK-cell activation after exposure to different target cells using a large-scale flow cytometry-based screening approach.

Materials and methods: Primary peripheral blood NK cells from healthy human individuals were co-incubated with either cell lines K562 (n=18), 721.221 (n=12), Raji (n=14, with anti-hCD20-hIgG1) or autologous HIV-1 (NL4-3) infected CD4 T cells (n=15) for 5h. NK-cell activation levels were measured using CD107a as a surrogate marker for degranulation. Subsequently, expression of 346 individual surface proteins was measured using flow cytometry and compared between CD107a+ and CD107a- NK-cell subsets.

Results: Expression of CD107a on NK cells was upregulated following co-incubation with any of the target cells compared to no-target controls. Approximately 60% of the individually tested 346 markers were expressed on NK cells. A subset of those markers were differentially expressed (median difference >10% points cut-off) between activated (CD107a+) and non-activated (CD107a-) NK cells: K562: n=26, 721.221: n=42, Raji: n=78, HIV-1 infected CD4 T cells: n=32. 15 receptors with differential expression between CD107a+ and CD107a- NK cells were identified regardless of the target-cell type used for co-incubation, including known markers for activation (e.g. CD69). Interestingly, we identified receptors that were differentially expressed between CD107a+ and CD107a- NK-cell subsets only in the presence of a specific target-cell type (K562: none, 721.221: n=6, Raji: n=40, HIV-1 infected CD4 T cells: n=3).

Conclusion: These findings indicate that NK cells may utilize common but also target-cell specific receptors for the efficient recognition of different target cells. Target-cell specific NK-cell receptor profiles may be utilized in the development of novel approaches in cancer or anti-viral therapies.

SAT6
The role of checkpoint molecules in human intrahepatic NK cells

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Introduction: NK cells represent a large fraction of all lymphocytes in the human liver, where they play a central role in controlling infections and cancer. While NK cells have been shown to be involved in early control of hepatitis virus infections and protection against HCC, they have also been implicated in causing liver damage. The mechanisms by which the liver instructs a tolerogenic environment and controls NK cell function during steady state conditions is only incompletely understood.

Objectives: The aim of this study was to investigate the role of checkpoint molecules in controlling the function of NK cells in the human liver.

Patients & methods: A total of 25 individuals were enrolled in this study. Peripheral blood and matched liver samples were collected from individuals undergoing liver transplantation due to end stage liver diseases (n=12) and liver resection due to liver metastases or adenoma (n=8). Additionally, PBMC samples from healthy control donors (n=5) were used. We performed detailed analysis of peripheral blood-derived and intrahepatic NK cells in those samples using flow cytometry as well as single cell qPCR on flow-sorted NK cells.

Results: In line with previous results, the intrahepatic (ih) lymphocyte compartment was enriched for NK cells with a shift towards higher proportions of CD56bright NK cells, expressing markers of liver residency (CXCR6 and CD69). In addition, ihNK cells expressed a distinct pattern of checkpoint molecules, characterized by higher levels of TIGIT,

DOI: 10.1002/eji.201970300

TGFBR1, TGFBR2 (all p<0.0001), PD-1 (p=0.0004) and lower levels of DNAM-1, ICOS, IL7R (all p<0.0001) as well as CD96 (p=0.007) compared to peripheral blood-derived NK cells. Furthermore, liver-resident NK cells expressed higher levels of TIGIT, CD96 (all p=0.005), TGFBR2 (p=0.02), IL7R (p=0.01) and lower levels of DNAM-1 (p=0.005). These results were independent of the underlying liver disease and also observed in tumor-free liver resection samples.

Conclusion: Taken together, our data showed that peripheral, intrahepatic and liver-resident NK cells expressed distinct patterns of checkpoint molecules, suggesting these molecules might contribute to a tissue-specific control of NK cell function. A better understanding of the mechanisms involved in the control of NK cell function might allow for future NK cell-based therapeutic intervention strategies.

SAT7

Identification of PLZF^{hi}CD56^{bright} NK cells in human peripheral blood sharing phenotypical and functional characteristics with liver-resident NK cells.

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Introduction: Human liver-resident NK cells (lrNK cells) have been characterized by their expression of specific surface molecules (e.g. CXCR6, CD69, CD56^{bright}) and certain transcriptional profiles (e.g. Eomes^{hi}T-bet^{lo}). The promyelocytic leukemia zinc finger protein (PLZF) is a transcription factor shown to be involved in the development of lrNK cells and innate lymphoid cells but not of conventional NK cells in mice. However, the potential role of PLZF in facilitating liver-residency in human NK cells remains unknown.

Objectives: The aim of this study was to investigate expression of PLZF in matched human peripheral blood-derived NK cells (pbNK cells) and intrahepatic NK cells (ihNK cells) and its association with expression of tissue-residency markers.

Materials & methods: Isolated liver-derived lymphocytes and matched PBMCs retrieved from patients undergoing resection of metastases or liver transplantation were phenotypically characterized using multiparameter flow cytometry. To determine functional characteristics of ihNK and pbNK cells, expression of granzymes and perforin was determined in unstimulated NK cells and IFN-γ- and TNF-α-production as well as degranulation (CD107a-expression) were assessed after stimulation with K562 cells.

Results: CD56^{bright} ihNK cells exhibited high expression of PLZF, while the majority of CD56^{bright} pbNK cells expressed low levels of PLZF (Median FI 480 vs.168: p=0.008). Highest expression levels of PLZF were detected among CXCR6^{+}CD69^{+}CD56^{bright} lrNK cells, compared to other CD56^{bright} ihNK cells (Median FI: 516 vs. 375; p=0.001). Surprisingly, peripheral blood harbored a small subset of CXCR6^{+}CD69^{+}CD56^{bright} NK cells that similarly expressed high levels of PLZF. Furthermore, these CXCR6^{+}CD69^{+}CD56^{bright} pbNK cells shared functional characteristics with lrNK cells by exhibiting low expression of granzyme B and perforin and high expression of granzyme K, together with poor IFN-γ- and TNF-α-response to K562 stimulation. However, CXCR6^{+}CD69^{+}CD56^{bright} pbNK cells did not display an Eomes^{hi}T-bet^{lo} profile, normally observed in lrNK cells.

Conclusion: These data implicate an involvement of PLZF in the regulation of liver-residency in human NK cells. PLZF^{hi}CD56^{bright} pbNK cells expressing liver-homing receptors and sharing functional characteristics with lrNK cells might represent a transitional state of NK cells that can contribute to the replenishment of lrNK cells.

DOI: 10.1002/eji.201970300

SAT8

Circulating passenger donor T and NK cells in lung transplantation recipients are derived from the donor lung parenchyma and represent tissue-resident memory cells

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Question: After lung transplantation (Tx), donor T and NK cells were detected in recipient blood immediately after Tx, persisting at least 3 weeks and characterized by the tissue retention marker CD69. In order to determine their origin, we hypothesized that donor T and NK cells have a peculiar tissue-resident memory phenotype that represents a unique subset of resident cells in the lung.

Methods: Donor lymphocytes in recipient blood were determined in 27 lung transplant patients at T0, T24, 3 weeks post-Tx by staining of donor HLA class I molecules in combination with lineage- and tissue-specific markers using flow cytometry. The phenotype of T and NK cells in perfusates (n=30), donor trachea (n=9), lymph nodes (n=15) and recipient explanted parenchyma (n=19) was compared to circulating cells using the same markers.

Results: In blood of all lung transplant recipients, donor derived T and NK cells were detected at T0, T24 and 3 weeks post-Tx, showed higher CD69 expression compared to recipient cells (p=0.001 to 0.03) and were mostly CD25-. This phenotype was similar to T and NK cells in perfusates, with significantly increased CD69 without CD25 expression compared to PBMCs (all p<0.005). NK cells from donor perfusate and tracheas were CD56dim CD16+, in contrast to lung-draining lymph nodes with highly enriched CD56++CD16- NK cells. T cells from trachea and lymph nodes showed also high CD69 expression and significant enrichment of effector memory (CCR7- CD45RO+) T cells (all p<0.03). In donor trachea and recipient parenchyma, CD69+ T cells showed co-expression of other tissue residency markers such as CD103, CD49a and PD-1. These were not found in perfusates, highlighting differences between these compartments.

Conclusion: Our results suggest that donor T and NK cells found in the periphery of lung transplant recipients are derived from lung parenchyma and represent a subset tissue-resident memory cells. This transient chimerism in recipient blood may have clinically relevant implications for the induction of tolerance after transplantation.

SAT9

Functional characterization of NK cells in the human and murine kidney

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Purpose: Natural killer (NK) cells represent specialized effector lymphocytes of the innate immune system that besides their ability to kill tumor and virus-infected cells are crucially involved in immune cell homeostasis and regulation of immune responses in tissues. However, our understanding of NK cell function in the kidney in homeostasis and inflammation is still very limited.

Notably, the NK cell population can be subdivided into conventional (circulating) and tissue-resident NK cells defined by differences in surface marker expression and transcription factor profile, but how these two subsets differ functionally, especially in the context of autoimmune diseases, remains incompletely understood. Here, we investigate the phenotype and function of the different NK cell populations in the healthy and inflamed kidney.

Methods*:* Human NK cell populations were isolated from paired kidney and blood samples of patients and phenotyped by flow cytometric analyses and subsequent tSNE-based clustering. For characterization in mice, we

DOI: 10.1002/eji.201970300

applied a combination of flow cytometric analyses and bulk RNA sequencing approaches. In order to assess NK cell function, we used an antibody directed against the NK cell receptor NK1.1 for total NK cell depletion or anti-asGM1 antibodies for "selective" depletion of conventional NK cells. The effects of different depletion strategies on disease outcome were analysed in a mouse model for autoimmune renal disease (crescentic glomerulonephritis, cGN).

Results*:* Here, we demonstrate that in addition to CD49b$^+$T-bet$^+$Eomes$^+$CD127$^-$ conventional NK (cNK) cells, a substantial fraction of kidney lymphocytes are CD49a$^+$T-bet$^+$Eomes$^-$CD127$^+$ tissue-resident NK (trNK) cells /ILC1s in the murine kidney. Our RNA sequencing data underline the substantial transcriptional differences of the two distinct NK subpopulations and identified potential mediators of trNK cell function in the kidney (e.g Hobit, CXCR6). Depletion of total NK cells caused an increase of histopathological kidney damage in mice with cGN, suggesting a regulatory function of NK cells in immune-mediated renal disease. Importantly, tissue-resident NK cells with a CD69$^+$CD49a$^+$ and/or CD103$^+$ phenotype could also be identified in the healthy human kidney.

Conclusion*:* Our preliminary data indicate a distinct tissue-resident subset of NK cells in the murine and human kidney and suggest a regulatory role of NK cells in a mouse model of autoimmune renal disease.

President's Symposium (Award 1-Award 6)

Award 1 – Otto-Westphal Award
Instructive role of the BCR in B1 cell development

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B1 and B2 lymphocytes exert distinct functions in humoral immunity. B2 lymphocytes engage in classical antibody responses, typically involving T cell-dependent affinity maturation as part of the adaptive immune response. B1 cells, in contrast, use the germline-encoded Ig repertoire and are considered carriers of natural immunity, biased towards the recognition of common pathogens and self-antigens. To explore the role of B cell antigen receptor (BCR) specificity in driving B1 cell differentiation, we developed a transgenic system that allowed us to change the BCR specificity in mature B2 cells to a B1-typical BCR specificity. Using this system, we show that mature B2 cells differentiate into bona fide B1 cells upon acquisition of a B1 cell-typical, self-reactive BCR. Thus, B2 cells retain plasticity towards B1 differentiation, and B1 differentiation can be instructed by BCR-mediated self-reactivity, in the absence of B1 lineage pre-commitment. These findings support the notion that the B1 cell identity is defined by BCR specificity. Science, Vol. 363, Issue 6428, pp. 748-753. DOI: 10.1126/science.aau8475

Award 2 – Hans-Hench Award
T cell activation versus tolerance induction in islet autoimmunity

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Type 1 diabetes (T1D) afflicts millions of people worldwide with rising incidence. Islet autoimmunity, the presymptomatic phase of T1D, is characterized by aberrations in immune activation vs. immune tolerance and a heterogeneous disease progression. CD25$^+$Foxp3$^+$ regulatory T cells (Tregs) are the main mediators of peripheral T cell tolerance and their *de novo* induction is a long envisioned goal for the tolerance restoration in autoimmunity. However, requirements for efficient Treg induction, especially in the human immune system, remain ill defined. Here, we used of humanized HLA-DQ8 transgenic NSG mice and demonstrate, that strong agonistic insulin variants can induce human Tregs *in vivo* and that induced Tregs are stable and functional. We furthermore used these variants to device tetramer reagents and show that during recent onset of islet autoimmunity, insulin-specific Treg frequencies are reduced, while T follicular helper (TFH) precursor frequencies are increased in the blood. This is however reversed in children with longterm autoimmunity, with autoantibodies for more than 10 years without the symptomatic disease, directly supporting the rationale of inducing these cells for delaying or preventing T1D progression. In line, we demonstrate that Treg induction is impaired specifically during recent onset of islet autoimmunity and that this impairment is linked to increased CD4$^+$T cell activation and proliferation. During recent onset of islet autoimmunity,

DOI: 10.1002/eji.201970300

increased miRNA92a and miRNA181a expression in CD4$^+$T cells contribute to aberrant immune activation and impaired Treg induction. Mechanistically, miRNA92a increases TFH cell differentiation, while miRNA181a enhances sensitivity of CD4$^+$T cells to antigenic stimulation involving increased NFAT5 signaling. Both miRNAs inhibit negative regulators of T cell activation and impair Treg induction *in vitro*. Accordingly, blocking either miRNA or their signaling intermediates improves Treg induction *in vitro* and increases Treg frequencies while reducing insulitis scores in NOD mice *in vivo*. The data presented here contribute to the understanding of requirements for efficient Treg induction as well as to the mechanistic basis of aberrant immune activation and defective tolerance in islet autoimmunity. Additionally, the introduced tetramer reagents and humanized mice are new tools that will help to improve clinical trial readouts, as well as the evaluation of translatability of new reagents to the human disease.

Award 3 – Fritz-and Ursula-Melchers Award
Antibody diversification through non-VDJ insertions

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Antibody diversity is created by the somatic recombination and imprecise joining of variable (V),diversity (D) and joining (J) segments. Recently, we discovered a new mechanism whereby a non-VDJ-sequence is somatically inserted into the antibody heavy chain gene and becomes the crucial antigen-binding element. Specifically, more than 5% of malaria exposed individuals produce antibodies that integrated the extracellular domain of the inhibitory receptor LAIR1 thereby gaining broad reactivity to Plasmodium falciparum infected erythrocytes. Although we found somatic integrations of extra exons from all over the genome into the antibody switch region of European individuals, it remains elusive if such genomic exon shuffling may give rise to productive antibody transcripts. Repertoire studies have so far systematically excluded the detection of hybrid antibodies. Thus, we developed a methodology based on suppression PCR to selectively amplify and study rare, extra-long antibody mRNA templates. Interestingly, we found several protein-encoding exons inserted in-frame into antibody mRNAs of healthy individuals. Although rare, their detection in antigen experienced B cell populations indicate that this novel mechanism can contribute to antibody diversity.

As the integration of pathogen-binding receptors bears the potential for the generation of antibodies to key-targets of pathogen virulence, the suppression PCR method provides a general approach for the detection of novel antibodies in the human repertoire.

Award 4 – Werner-Müller Award
Maturation and potency of cross reactive memory B cell response to *Plasmodium falciparum* circumsporozoite protein in humans

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Plasmodium falciparum circumsporozoite protein (PfCSP) is the major surface protein on Pf sporozoites, the malaria parasite stage that is transmitted to humans by the mosquito vector. PfCSP is composed of an N-terminus, a central NANP repeat region, and a C-terminal domain. Antibodies against the repeat and the junctional peptide that links the N-terminus and the repeat domain can block the infection in experimental animal models. However, natural parasite exposure fails to induce sterilizing anti-PfCSP titers and RTS,S, the most advanced PfCSP-based malaria vaccine, which lack the junctional epitopes and N-terminus mediates only partial protection with no clear immune correlate. By single B cell antibody sequencing and cloning we followed the clonal evolution and quality of the anti-PfCSP memory B cell antibody response after three immunizations with high numbers of live Pf sporozoites under chemoprophylaxis (PfSPZ-CVac). We performed an in-depth analysis of a panel of 200 human monoclonal anti-PfCSP antibodies cloned from memory B cells and plasmablasts after repeated parasite exposure. ELISA and SPR measurements show that the majority anti-PfCSP antibodies bind to more than one epitope. The data show that cross-reactivity with the N-terminal junction was associated with high antibody affinity and provide insights in the role of clonal selection and affinity maturation for the development of cross-reactive antibodies with parasite inhibition activity. Our findings have

DOI: 10.1002/eji.201970300

wide implications for the design of malaria vaccination strategies aiming to induce PfCSP B cell memory responses that mediate sterile protection and prevent disease.

Award 6 – German Immunology Award
Treg-Up or Treg-Down to control immune responses
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Regulatory T (Treg) cells, which are expressing the transcription factor Foxp3 in the nucleus, CD25 and CTLA-4 on the cell surface, are actively engaged in the maintenance of immunological self-tolerance and homeostasis. Depletion or functional impairment of Treg cells is able to enhance cancer and microbial immunity, while their numerical expansion or functional augmentation is instrumental in treating autoimmune disease and establishing graft tolerance. How to achieve these aims by targeting Treg cells with biologicals (such as monoclonal antibodies) or chemicals has been an issue of intense investigation. We have recently made attempts to pharmacologically control Treg-specific transcriptional and epigenetic changes and thereby control Treg cell development and function. We found that certain tyrosine kinase inhibitors that blocked T-cell receptor-proximal signaling in T cells were able to specifically deplete mature Treg cells, thereby enhancing tumor immunity in humans. On the other hand, inhibitors of a serine threonine kinase involved in a T-cell signaling pathway evoked Foxp3 expression in conventional T cells including effector/memory T cells and converted them to functionally competent Treg-like cells, which effectively suppressed autoimmune disease and allergy in animal models. It will be discussed how the development and function of Treg cells can be controlled by transcriptional and epigenetic interventions and how Treg cells be pharmacologically targeted to control a variety of physiological and pathological immune responses.

Young Immunologists (YI1-YI6)

YI1
Circulating CD8+ *helper*-type T cell memory subsets represent CD4+ *helper*-alike cells with a skin resident memory T cell signature
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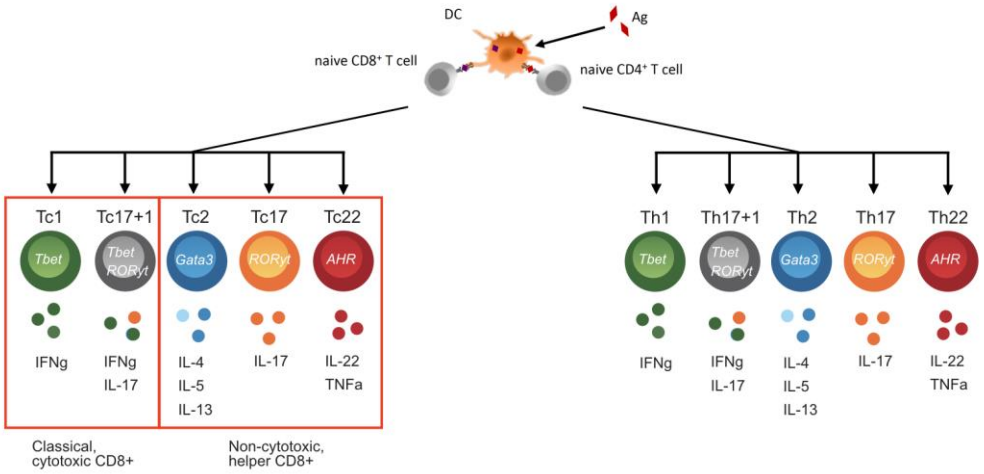
The prevailing `division of labor´ concept in cellular immunity is that CD8+ T cells primarily utilize cytotoxic functions to kill target cells, while CD4+ T cells exert helper/inducer functions. CD4+ memory T cells are organized into diverse T *helper* subsets such as Th1, Th2, Th17, Th17+1 and Th22 type cells characterized by distinct functions with highly specialized cytokine secretion and chemokine receptor expression patterns. We here demonstrate that identical chemokine receptor patterns utilized for T *helper* subset delineation are sufficient to systematically separate corresponding specialized CD8+ memory T cell subsets Tc1, Tc2, Tc17, Tc17+1 and Tc22 in blood. While Tc1 and Tc17+1 display classical CD8+ T cell related cytotoxic signature, Tc2, Tc17 and Tc22 cells possess a non-cytotoxic phenotype and express the prototype *helper* molecule CD40L after activation (see Figure1). *Helper*-type CD8+ T cell subsets share a unique TCR repertoire and are characterized by gene expression signatures characteristic of skin homing ability. Transcriptome comparison with diverse skin resident T cell subsets in dermis and epidermis identified a significant overlap with dermal CD8+ T cells irrespective of their CD103 signature. Moreover, we could show that CD8+ *helper*-type T cells contribute to the pathogenesis of psoriasis, a skin related autoimmune disorder. Altogether, our data highlights the importance to take CD8+ *helper* T cells into account when analyzing the contribution of CD8+ T cells to human health and disease.

Figure 1

DOI: 10.1002/eji.201970300



YI2

Reactive oxygen species modulate macrophage immunosuppressive phenotype through the up-regulation of PD-L1

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Introduction: The combination of immune checkpoint blockade with chemotherapy is currently under investigation as a promising strategy for the treatment of triple negative breast cancer (TNBC). A well-characterized mediator of chemotherapy-induced cytotoxicity is the accumulation of reactive oxygen species (ROS). Tumor associated macrophages (TAMs) are the most prominent component of the breast cancer (BC) microenvironment (TME) and they influence tumor progression and the response to therapies.

Objectives: We hypothesized that chemotherapy-induced ROS could affect tumor infiltrating-macrophage functions and the expression of programmed death ligand-1 (PD-L1).

Materials & methods: To assess ROS regulation of PD-L1 expression we treated bone marrow-derived macrophages with well-known ROS-inducers, buthionine sulphoximine (BSO) and paclitaxel. PD-L1 expression by RT-PCR and flow cytometry as well as the secretion of immunosuppressive cytokines by protein arrays were evaluated. To validate the *in vitro* results we exploited a mouse mammary tumor model carrying BRCA1/Trp53 deletion and resembling human TNBC (K14cre BRCA1$^{f/f}$p53$^{f/f}$, KBP). Finally, mice were treated with a combination of a neutralizing PD-L1 antibody and paclitaxel.

Results: We showed that macrophages acquired an immunosuppressive phenotype and increased the expression of PD-L1 when treated with ROS inducers such as BSO and paclitaxel. Mechanistically, these agents cause accumulation of ROS that in turn activated NF-κB signaling to promote PD-L1 transcription and the release of immunosuppressive cytokines. Systemic *in vivo* administration of paclitaxel promotes PD-L1 accumulation on the surface of TAMs in a mouse model of TNBC, consistent with *in vitro* results. Indeed, combinatorial treatment with paclitaxel and a neutralizing anti-PD-L1 antibody significantly improved the therapeutic efficacy of paclitaxel by reducing tumor burden and increasing the number of tumor-associated cytotoxic T cells.

DOI: 10.1002/eji.201970300

Conclusion: Our results provide a strong rationale for the use of anti-PD-L1 blockade in the treatment of TNBC patients. Furthermore, interrogation of chemotherapy-induced PD-L1 expression in TAMs is warranted to define appropriate patient selection in the use of PD-L1 blockade.

YI3

Distinct surface expression of activating receptor Ly49H drives differential expansion of NK cell clones upon murine cytomegalovirus infection

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Upon antigen exposure a small population of T cells, which harbors T cell receptors (TCRs) specific to the relevant antigen, is selected to rapidly expand. After antigen clearance this expanded T cell population contracts to 5–10% of its peak size. Importantly, the number of T cells remaining after this contraction phase is considerably larger than that of antigen-specific T cells available in the naive T cell compartment. This numerical increase of antigen-specific T cells, through clonal selection, clonal expansion and preferential clonal maintenance, is one of the key factors underlying adaptive immunity and immunological memory. However, the antigen-driven evolution of the TCR repertoire does not stop at the selection of antigen-specific T cell clones *per se*. It is instead characterized by the further selection of certain T cell clones within the antigen-specific T cell population. This phenomenon, which has been termed TCR repertoire focusing, is thought to be driven by the enhanced expansion and reduced contraction of T cell clones, whose TCRs bind to the relevant antigen with optimal affinity.

Question: Interestingly, an antigen-dependent enrichment of lymphocytes recognizing certain target structures has also been identified for Natural killer (NK) cells in the context of cytomegalovirus (CMV) infection. Whether these "antigen-specific" NK cells respond uniformly to CMV infection or show distinct clonal dynamics similar to those found in T cells remains unknown.

Methods: In our studies we used retrogenic color-barcoding and single-cell adoptive transfer to track clonal immune responses derived from individual Ly49H$^+$ NK cells during murine cytomegalovirus (MCMV) infection.

Results: We found that clonal expansion of single NK cells varied dramatically. The observed variability could not be attributed to the additional presence or absence of inhibitory Ly49 receptors in responding clones. Instead, NK cell clones showed distinct levels of Ly49H receptor expression that correlated with the degree of clonal expansion and persistence during the contraction phase. Furthermore, NK cell clones sorted for high or low expression of Ly49H clonally maintained their Ly49H expression levels.

In conclusion, akin to adaptive processes shaping an antigen-specific T cell receptor repertoire, the Ly49H$^+$ NK-cell population adapts to MCMV infection. This is achieved by preferential expansion and maintenance of NK cell clones expressing higher levels of Ly49H.

YI4

The short-chain fatty acid pentanoate suppresses autoimmunity by modulating the metabolic-epigenetic crosstalk in lymphocytes

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Short-chain fatty acids (SCFAs) have immunomodulatory effects, but the underlying mechanisms are not well understood. Here we show that pentanoate, a physiologically abundant SCFA, is a potent regulator of immunometabolism. Utilizing a combination of pharmacological and biochemical studies, we investigate the pentanoate-mediated IL-10 expression in lymphocytes of reporter mice.

Pentanoate enhances IL-10 production by reprogramming their metabolic activity towards elevated glucose oxidation. Mechanistically, this reprogramming is mediated by supplying additional pentanoate-originated acetyl-CoA for histone acetyltransferases and by pentanoate-triggered enhancement of mTOR activity. In experimental mouse models of colitis and multiple sclerosis, pentanoate-induced regulatory B cells mediate protection from autoimmune pathology. Additionally, pentanoate shows a potent histone deacetylase-inhibitory activity in CD4$^+$ T cells, thereby reducing their IL-17A production. In germ-free mice monocolonized with segmented filamentous bacteria (SFB), pentanoate inhibits

DOI: 10.1002/eji.201970300

the generation of small-intestinal Th17 cells and ameliorates SFB-promoted inflammation in the central nervous system.

Taken together, the SCFA pentanoate modulates the metabolic state of T and B cells, thereby impacting on epigenetic modifications and cellular function. By enhancing IL-10 production and suppressing Th17 cells, pentanoate might be of therapeutic relevance for inflammatory and autoimmune diseases.

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Luu *et al.* 2019 - Nature Communications

https://doi.org/10.1038/s41467-019-08711-2

YI5

Keratinocytes control skin immune homeostasis through de novo-synthesized glucocorticoids

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Glucocorticoids (GC) are known for their immunosuppressive actions and are widely used as clinical therapeutics against inflammatory diseases. Beside the main GC synthesis in the adrenal cortex, several extra-adrenal organs including the skin are known to produce functional GC as well. The de novo GC synthesis in the skin is orchestrated by a local endocrine network and is proposed to support the skin to cope with stress signaling from the outer and inner environment. Although the cutaneous GC synthesis is well described, the physiological role and function of skin-derived GC are yet still unexplored. Here, we addressed the epidermal-specific GC synthesis *in vivo* using a novel model with inducible deletion of the critical enzyme Cyp11b1 in keratinocytes. Cyp11b1 deletion decreased expression and abrogated keratinocyte-specific GC synthesis in the skin leading to epithelial cell activation and increased skin dendritic cell (DC) migration towards draining lymph nodes (dLN). Furthermore, we observed increased expression of pro-inflammatory Th1 and Th17 type cytokines in dLN of KO mice which, subsequently, leads to the development of spontaneous skin inflammation involving polymorphic immune cell infiltrates. Similarly, FITC-induced contact hypersensitivity reaction was remarkably enhanced with increased type 17 response in KO mice. Defective de novo GC synthesis in the skin also resulted in an exacerbated Aldara-induced psoriasiform skin inflammation and prevented remission whereas the pathogenesis of MC903-induced experimental atopic dermatitis (AD) was only mildly modulated by skin de novo GC. Importantly, expression of enzymes involved in the de novo GC synthesis was downregulated in human skin lesions of patients with inflammatory skin diseases such as AD and psoriasis indicating a defective de novo GC synthesis. Our finding indicates the importance of keratinocyte-derived de novo GC in skin immune homeostasis and reveals their regulatory nature in physiological and pathological conditions of the skin.

YI6

The IL17 ablation improves specific anti-tumor immune response

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Introduction: Pancreatic Ductal Adenocarcinoma (PDA) is considered an "immunologically-cold" tumor because of the immune-suppressive microenvironment and the absence of tumor neoantigens. It is characterized by a complex inflammatory response in which the role of the IL17 and T helper-17 is evident but remain controversial.

Objectives: Our lab has demonstrated that a DNA-based vaccination strategy targeting alpha-enolase (ENO1), a PDA-associated antigen, significantly prolongs survival in genetically engineered mice (GEM). Moreover, we have observed that the absence of IL17 deeply shapes the tumor microenvironment and increases the recruitment of immune cells. The aim of this study was to assess the role of IL17 in combination with the DNA-vaccination.

Methods: We crossed GEM with IL17$^{-/-}$ mice. GEM/IL17$^{+/+}$ and GEM/IL17$^{-/-}$ mice were vaccinated at 8 weeks of age every 2 weeks for a total of four vaccination rounds. Mice were monitored to obtain a Kaplan-Meier survival curve.

DOI: 10.1002/eji.201970300

Some mice were sacrificed 2 weeks after the last vaccination to collect serum and spleen for in vitro experiments: ELISA, ELISPOT and Flow Cytometry analysis. Moreover, PDA cells were orthotopically injected into the pancreas of syngeneic mice, which were treated or not with anti-IL17, in combination or not with ENO1-DNA vaccine. After one month, mice were euthanized; tumor masses were collected and analysed by immunohistochemistry and quantitative PCR analysis after RNA extraction.

Results: The absence of IL17 correlated with an increased fibrosis and intra-tumor T cell recruitment in KPC/IL17$^{-/-}$ mice. DNA vaccination prolonged survival of KPC/IL17$^{-/-}$ mice compared to the non-vaccinated or to vaccinated KPC/IL17$^{+/+}$ mice. The ENO1 vaccination in absence of IL17 elicited an increased production of anti-ENO1 antibodies and number of IFNg-secreting T cells. The analysis of the tumor infiltrating immune-cells revealed an increase of antigen-presenting cells and more effector/memory CD4^{+} and CD8^{+} T cells. Orthotopically injected mice treated with the combination displayed a significantly reduced tumor growth that paralleled the induction of cytotoxic T cells not observed in those receiving the ENO1-vaccine or anti-IL17 Ab alone.

Conclusions: Overall these results candidate the antibody-mediated depletion of IL17 as an effective combination with DNA-vaccination to design novel immunotherapeutic strategies in pancreatic cancer.

Infection I (O1-O5)

O1
Role of Otubain-1 (OTUB1) during inflammatory liver diseases

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Introduction: Immune responses to pathogens are regulated by the process of ubiquitination and deubiquitination. The deubiquitinating enzyme OTUB1 preferentially cleaves K48-linked ubiquitin chains from its substrates and thereby prevents their proteasomal degradation. OTUB1 has been shown to regulate NF-κB signalling by removing K48-linked ubiquitin chains from the cellular inhibitor of apoptosis (cIAP) and to inhibit TNF-induced cell death.

Objectives: To identify the hepatocyte-specific function of OTUB1 during bacterial infection

Results and Methods: To gain an insight into the hepatocyte-specific function of OTUB1, conditional OTUB1 knock-out mice lacking OTUB1 in the hepatocytes (Alb-Cre x OTUB1$^{fl/fl}$) were generated and infected with *Listeria monocytogenes*. Upon *Listeria*-infection, the Alb-Cre x OTUB1$^{fl/fl}$ mice succumbed to the infection within six days post infection while the OTUB1$^{fl/fl}$ control mice survived. The Alb-Cre x OTUB1$^{fl/fl}$ mice displayed enhanced liver damage as shown by increased serum Alanine transaminase (ALT) levels. In addition, mRNA levels of the proinflammatory cytokine TNF was significantly increased in the Alb-Cre x OTUB1$^{fl/fl}$ mice both *in vivo* as well as *in vitro* indicating that the enhanced hepatocellular death in the absence of OTUB1 might be TNF dependent. Interestingly, western blot analysis revealed reduced cleavage of caspases but enhanced necrosome formation in the *Listeria*-infected livers of the Alb-Cre x OTUB1$^{fl/fl}$ mice suggesting that OTUB1-deficient hepatocytes are prone to necroptotic cell death. Confirmatively, administration of Necrostatin-1s, a necroptosis inhibitor, but not zVAD, a caspase inhibitor, prior to *Listeria*-infection protected the Alb-Cre x OTUB1$^{fl/fl}$ mice.

Conclusion: Collectively, our study identifies OTUB1 as a regulator of hepatic necroptosis during listeriosis.

O2
Functional cooperation between complement factor H and the long pentraxin PTX3 in the immune response to *Aspergillus fumigatus*

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DOI: 10.1002/eji.201970300

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Introduction. *Aspergillus fumigatus* (AF) is the major etiologic agent of Invasive Aspergillosis (IA), a severe infection amongst immunocompromised individuals. A pivotal role in the host resistance to this fungal pathogen is played by polymorphonuclear neutrophils (PMNs) and complement, in a functional cooperation with the long pentraxin PTX3 [1]. This has opsonic activity, and enhances phagocytosis and killing of AF conidia by PMNs via complement pathways [2].

Objectives. Primary aim of this study was to characterize the molecular crosstalk between PTX3 and complement in the opsono-phagocytosis of AF.

Materials & methods. AF conidia were incubated with complement proteins and human sera depleted of selected complement components in the presence and absence of recombinant PTX3. Complement activation on AF was assessed by Western Blotting and ELISA. In parallel experiments, AF phagocytosis and killing by PMNs freshly isolated from human peripheral blood was analyzed by Flow Cytometry and Cytotoxicity Assays. Substitution/deletion mutants were made to dissect the structure/function relationships of PTX3 in the opsono-phagocytosis of AF. Phagocytosis was assessed *in vivo* using an animal model of AF infection in C57BL/6 mice.

Results. We found that PTX3 promotes the selective recruitment of C3b (from C3 cleavage) on the conidial wall, by exclusively targeting the alternative pathway (AP) of complement. To our surprise, factor H (main inhibitor of AP) is required for such process, thus pointing to a novel function (activating rather inhibitory) of this complement regulator when combined with PTX3. Consistent with this, in phagocytosis experiments with purified human PMNs, factor H was necessary to sustain the pro-phagocytic and pro-killing activities of PTX3. Furthermore, we made a tetrameric mutant of PTX3 (as opposed to the octameric wild type protein) that retained the opsono-phagocytic properties of the wild type protein both *in vitro* and *in vivo*.

Conclusion. Here we described a cooperation between factor H and PTX3 with an unexpected functional outcome: enhanced recruitment of C3b onto AF. Given the potent opsonic activity of C3b, we believe that this is a major mechanism of PTX3 in the promotion of AF phagocytosis and killing. Moreover, we generated a PTX3-derived protein with pro-phagocytic activity *in vitro* and *in vivo*.

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O3

Myd88-deficiency impairs clearance of *Coxiella burnetii* Nine Mile Phase II: a new mouse model of Q-fever resolution and chronicity

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The zoonotic disease Q-fever is caused by the obligate intracellular, Gram-negative bacterium *Coxiella burnetii* that replicates within phagocytes. Q-fever often occurs as a mild acute, self-limiting flu-like illness, but can also become chronic and develop into an endocarditis. Recognition of *C. burnetii* by macrophages requires TLR2 and triggers production of pro- and anti-inflammatory cytokines. A single nucleotide polymorphism in the TLR adapter molecule Myd88 is reported to be associated with the development of chronic Q-fever in patients. We observed that Myd88-deficient murine macrophages failed to clear *C. burnetii* and to produce the cytokines TNF, IL-6 and IL-10 after *in vitro* infection. Therefore, we employed mice deficient in Myd88 as an *in vivo* model for chronic Q-fever. The attenuated strain *C. burnetii* Nine Mile Phase II was injected intraperitoneally or intratracheally followed by kinetic analysis of bacterial burden and cytokine gene expression in lung, liver, spleen and heart tissue. *Myd88-/-* mice had significantly higher *C. burnetii* load on day 5 and day 20 after intraperitoneal infection, which was associated with reduced expression of *Ccl2*, *Ifng* and *Nos2*. Additionally, we observed increased bacterial dissemination from the lung to other organs in *Myd88-/-* mice after intratracheal injection of *C. burnetii*, which mimics the natural route of infection. Moreover, qPCR analysis of infected organ tissue revealed *C. burnetii* genome copies in *Myd88-/-* mice still on day 42 after intratracheal infection, whereas wild type mice cleared the infection already at day 27. However, increased

DOI: 10.1002/eji.201970300

bacterial loads in *Myd88-/-* mice were accompanied by reduced granulomatous inflammation as well as lower amounts of chemoattractants, inflammatory cytokines and IFNg-induced genes relevant for pathogen control. Together, interfering with innate immune recognition of *C. burnetii* by deletion of the TLR adapter Myd88 impairs the clearance of attenuated *C. burnetii* and results in a shift from acute to more chronic infection. In ongoing work, we address the role of Myd88-dependent and IFNg-induced genes in promoting bacterial survival and macrophage reprogramming during *Coxiella burnetii* infection.

O4

Presence of neutrophils in the skin enhances *S. aureus* colonization

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Introduction: Our skin is constantly exposed to a large number of pathogens while at the same time undergoing selective colonization by harmless commensal microorganisms such as *S. epidermidis*. Changes in the composition of the microbiome can promote colonization by the pathogen *Staphylococcus aureus* and can deteriorate barrier defects for instance in atopic dermatitis patients. Keratinocytes, as the most abundant cell type in the epidermis, are able to orchestrate specific immune responses in the skin in response to colonizing commensals as well as to pathogens. However, the mechanism how keratinocytes can discriminate commensals from pathogens is barely understood.

Objectives: In this work we investigate the role of different types of immune cells in *S. aureus* skin colonization and how skin commensals can modulate the recruitment of immune cells to the skin.

Materials and Methods: Using an *in vitro* co-culture model with primary human keratinocytes and PBMCs or neutrophils as well as by using an *in vivo* epicutaneous mouse skin infection model we analyzed the recruitment of specific immune cells subsets to the skin in response to commensals or pathogens as well as the effect of the recruited immune cells on *S. aureus* skin colonization.

Results: We show that skin inflammation induced by tape stripping induces a rapid recruitment of neutrophils which correlates with enhanced skin colonization with *S. aureus*. Consequently, depletion of neutrophils *in vivo* resulted in reduced *S. aureus* colonization. Moreover, *in vitro* co-culture of keratinocytes with neutrophils but not PBMCs led to enhanced *S. aureus* colonization. And finally, we show that *S. epidermidis* prevents excessive neutrophil recruitment to the skin.

Conclusion: In healthy skin *S. epidermidis*, as part of the skin microbiota, prevents excessive recruitment of neutrophils to the skin and by this protects the skin from *S. aureus* colonization. Further studies will provide deeper insight into the mechanism how neutrophils contribute to enhanced *S. aureus* skin colonization.

O5

STING induces early IFN-β in the liver and constrains myeloid cell-mediated murine cytomegalovirus dissemination

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Cytomegalovirus is a DNA-encoded beta-herpesvirus that induces STING-dependent type I interferon (IFN-I) responses in macrophages and uses myeloid cells as a dissemination vehicle. Here we report that *STING*$^{-/-}$ mice are as resistant to murine cytomegalovirus (MCMV) infection as WT controls, whereas mice with a combined Toll-like receptor/RIG-I-like receptor/STING deficiency do not mount IFN-I responses and succumb to the infection. Although STING alone is dispensable for survival, early IFN-β induction in Kupffer cells is STING-dependent and controls early hepatic virus propagation. Infection experiments with an inducible reporter MCMV revealed that STING constrains

DOI: 10.1002/eji.201970300

MCMV replication in and limits viral dissemination via myeloid cells. In contrast, restriction of viral dissemination from hepatocytes to other organs is independent of STING. Thus, during MCMV infection STING is relevant for early IFN-β induction in Kupffer cells and the restriction of viral dissemination via myeloid cells, whereas it is dispensable for survival.

Tumor immunology and microenvironment I (O7-O12)

O7
Immune regulation and tumorigenesis in the chronically injured gut are governed by the alarmin HMGB1.

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Sterile injury and pathogenic infection alike trigger inflammation in all vascularized tissues. While immune responses mainly serve to restore tissue homeostasis in the acute phase, they can also effectuate maladaptive wound healing when injury becomes chronic. The crosstalk between dying parenchymal cells, pathogens and leukocytes regulates immune responses and may be accessible for targeted therapeutic interventions. Following release, the nucleoprotein High-mobility group box 1 (HMGB1) interacts with several receptors to affect inflammatory responses, yet its specific roles in the immune regulation of the gut mucosa remain elusive.

Aim: To identify relevant cellular sources and targets of HMGB1 in acute and chronic models of intestinal injury, and to outline its role in inflammation-dependent and –independent colorectal carcinogenesis.

Methods: Conditional *Hmgb1*-knockout mice and control littermates were treated with dextran sodium sulfate (DSS)-mixed water or infected with *Citrobacter rodentium*, respectively, to induce acute colitis. Azoxymethane (AOM) with or without chronic DSS treatment, as well as the genetic *Apc*$^{+/min}$ model were employed to assess colitis-associated and spontaneous tumor development, respectively.

Results: Compared to control littermates, mice with *HMGB1* deletion in intestinal epithelial cells (*Hmgb1*$^{\Delta IEC}$) exhibit profoundly exacerbated colitis severity in the DSS- but not the *Citrobacter rodentium* colitis model, suggesting a context-dependent protective role for enterocyte HMGB1 during acute intestinal inflammation. By contrast, HMGB1 from myeloid cells (*Hmgb1*$^{\Delta LysM}$) or dendritic cells did not affect the inflammatory response in either scenario. In the *Apc*$^{+/min}$ model, *Hmgb1*$^{\Delta IEC}$ displayed profoundly accelerated colorectal tumor growth. Conversely, in the AOM/DSS model, tumor burden was comparable between *Hmgb1*$^{\Delta IEC}$ and controls, but significantly reduced in *Hmgb1*$^{\Delta LysM}$ animals. **Conclusion**: Epithelial HMGB1 context-dependently partakes in immune response to intestinal damage. Myeloid-cell derived HMGB1 promotes inflammation-associated intestinal tumorigenesis, whereas epithelial HMGB1 impedes tumor growth in spontaneous colorectal cancer models.

O8
HDAC7 controls CD8$^+$ T cell dependent anti-viral and anti-tumor immunity

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Introduction: Class II Histone deacetylases (HDAC) were shown to orchestrate T cell-dependent immune responses via the epigenetic control of genes and via the post-translational modification of cytoplasmic and nuclear proteins, but the contribution of single HDAC family members to T cell differentiation and function remain elusive.

Objectives: We aimed to elucidate the role of HDAC7 in T cells by using mouse models of acute and chronic infection as well as tumor models.

Materials & Methods: T cells from WT or *Hdac7flox/floxCd4-Cre* (Hdac7Ko) mice were analyzed by mass cytometry and flow cytometry. LCMV infection and tumor challenge experiments were performed in WT, *Hdac7flox/floxCd4-Cre* and *Hdac7flox/floxE8I-Cre* mice. Homing- and anti-tumor-capacities of CD8+ T cells were tested via adoptive CTL transfer experiments in tumor-bearing mice. RNA-sequencing and *in vitro* CTL differentiation experiments were

DOI: 10.1002/eji.201970300

performed to clarify the role of HDAC7 in T cells. Seahorse analysis was used to investigate the role of HDAC7 in the metabolism of CD8+ T cells.

Results: Hdac7Ko mice featured defects in the differentiation and maintenance of memory CD8+ T cells due to deregulation of the transcription factor Eomes and its target genes. During memory recall responses, LCMV-Clone 13 infected Hdac7Ko mice failed to clear viral infection due to decreased expansion and increased cellular exhaustion of virus-specific CD8+ T cells. In chronic antigen exposure models of lymphoma, Hdac7Ko mice failed to control tumor growth of syngenic EG-7 tumor cells, as Hdac7Ko mice harbored significantly lower numbers of tumor-infiltrating CD8+ T cells when compared to WT mice. Seahorse analysis and RNA sequencing show that deletion of HDAC7 reduced the survival of CD8+ T cells, due to deregulation of amino acid metabolism- and apoptosis-regulating genes as well as reduced protein expression of mTOR resulting in increased cell death and functional exhaustion of CD8+ T cells. Furthermore, Hdac7Ko CD8+ T cells harbored impaired production of IFNγ, which could be linked to defective Store-Operated Calcium Entry (SOCE) signaling.

Conclusion: Our data reveal that HDAC7 serves as a key regulator of T cell mediated anti-viral and anti-tumor immunity via controlling the memory differentiation, survival, metabolism and calcium homeostasis of CD8+ T cells.

O9

Microbial short-chain fatty acids promote T cell-mediated anti-cancer immunity

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Introduction: Some commensal bacterial species are able to enhance anti-cancer immune responses, however the underlying molecular mechanisms and the contribution of individual bacterial metabolites to anti-tumor effects remain unknown. Short-chain fatty acids (SCFAs) are generated by intestinal bacterial fermentation of nondigestible polysaccharides. Novel studies have revealed that SCFAs are able to modulate the metabolism and function of immune cells in the intestine and other organs.

Objectives: The interplay between diet, intestinal microbiota and the immune system considerably affects various biological functions and health status of the host. The aim of this study was to investigate the impact of specific microbial metabolites (SCFAs) on the eradication of established murine tumors.

Materials & Methods: Naïve CD8+ T cells were purified from OT-I mice and differentiated into cytotoxic T lymphocytes (CTLs) in the presence or absence of SCFAs. Mice received subcutaneous injection with B16-OVA tumor cells. The eradication of established tumors was analyzed after adoptive transfer of tumor-specific CTLs.

Results: We show that bacterial SCFAs elicit anti-cancer immunity by enhancing the function of murine and human CTLs. The improved capability of effector lymphocytes to kill established tumors was mediated via SCFA-triggered inhibition of HDAC activity and by modulation of the immunometabolism. Treatment of CTLs with SCFAs increased the expression of transcription factors Eomes and T-bet, as well as the production of IFN-g, TNF-a and granzyme B. ChIP experiments revealed a significant increase in histone H4 acetylation at the promoter regions of *Eomes*, *Tbx21* and *Ifng* genes after the treatment of CTLs with SCFAs butyrate and pentanoate. Furthermore, we found that the supernatant of the human bacterium *Megasphaera massiliensis*, which produces high levels of pentanoate, was also able to generate CD8+ T cells with high cytotoxic activity similar to SCFAs.

Conclusions: Several studies have reported that intestinal microbiota could be implicated in the cancer development. Our data suggest that SCFAs or commensal bacterial strains producing high amounts of SCFAs might be used as microbial therapeutics for targeting human tumors.

O10

The macrophage tetraspan MS4A4A enhances Dectin-1-dependent NK cell-mediated resistance to metastasis

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DOI: 10.1002/eji.201970300

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Introduction: The membrane spanning 4-domains A4A (MS4A4A) is a tetraspan-like molecule that belongs to the MS4A family. Although it has been reported that MS4A4A is part of the transcriptional signature of alternatively-activated macrophages (M2), the function of MS4A4A is totally unknown.

Objective: We aimed to characterize MS4A4A expression regulation, to identify MS4A4A receptor partners and to determine MS4A4A function on macrophages.

Materials and Methods: MS4A4A expression was evaluated in monocyte-derived macrophages stimulated with M1/M2 stimuli and in human tissue resident macrophages from healthy donors and rheumatoid arthritis or cancer patients. Through split-ubiquitin yeast two hybrid analysis, FLIM-FRET, immunofluorescence and high-resolution microscopy (STED) we identify MS4A4A partners on macrophages. We generated a mouse strain lacking MS4A4A specifically in macrophages (*Ms4a4a*$^{fl/fl}$ x *Lyz2*^{Cre}) and we analyzed MS4A4A function in *in vivo* tumor models.

Results: We demonstrated that MS4A4A expression is restricted to murine and human mononuclear phagocytes and is induced during monocyte-to-macrophage differentiation. We showed that MS4A4A is co-expressed with M2/M2-like molecules in subsets of human normal tissue resident macrophages, infiltrating macrophages from inflamed synovium, and tumor-associated macrophages. We identified Dectin-1 as a receptor partner for MS4A4A. We showed that MS4A4A interacted and co-localized with Dectin-1 in lipid rafts. MS4A4A also impacted on signalling downstream Dectin-1 by promoting the Syk-dependent pathway in macrophages and thus their production of pro-inflammatory cytokines and ROS. While MS4A4A deficiency in macrophages did not affect primary tumor growth, MS4A4A was essential for Dectin-1-mediated activation of macrophages in metastasis and it was required for the efficient activation of NK cell anti-metastatic functions.

Conclusions: We demonstrated that MS4A4A is a tetraspan molecule selectively expressed in macrophages during differentiation and polarization. We showed MS4A4A is essential for Dectin-1-dependent activation of macrophages and for their ability to induce NK cell-mediated resistance to metastasis. These results identified for the first time a functional role of MS4A4A in a pathological state, such as cancer.

O11

Complete workflows allow comprehensive tumor microenvironment analysis and culture of cell subsets of limited tumor patient samples

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Immunotherapy has proven clinical efficacy and tremendous potential. But clinical benefit is experienced by only a subset of patients, such that additional research is necessary to improve outcomes. In particular, analyze steady-state anti-tumor immunity and monitor the effects of therapy on the tumor microenvironement. However, TIL numbers can be very low. Flow cytometry phenotyping different cell populations requires dividing limited tumor material into multiple samples, reducing the number of cells available for analysis. Therefore, innovative tools and workflows are needed to maximize the quality of data obtained from limited tumor patient samples.

DOI: 10.1002/eji.201970300

We established complete workflows combining tissue storage, dissociation, cell isolation and analysis. Tissues were stored and shipped in a solution that was shown to maintain cell viability and phenotype up to 48h after collection (Tissue Storage Solution™). Tumor dissociation was automated and optimized for epitope preservation using a tissue dissociator (gentleMACS™ Octo). We developed new enrichment reagents for magnetic cell sorting, incorporating novel technology enabling the removal of both superparamagnetic beads and antibody fragments (REAlease®). Using MACS® and REAlease technology, tumor-infiltrating T-, B-, Myeloid cells, cancer-associated fibroblasts and tumor cells were isolated. The isolated cell populations were characterized by flow cytometry, multi-parameter imaging and transcriptomics. Isolated tumor and T cells were cultured and expanded *in vitro*, allowing identification of tumor cell-specific T cells. Importantly, purification of tumor-infiltrating T cells with high viability was essential for high-resolution immune profiling by single cell transcriptomics.

In conclusion, we have optimized workflows that allow the comprehensive analysis of the tumor microenvironement, including T-, B-, Myeloid cells, cancer associated fibroblasts and tumor cells. Sequential isolation of the different cell types maximized the number of cells of each cell type available for analysis and cultureThese workflows greatly reduce experimental time and allow the performance of more complex experimental setups. We believe the use of these innovative tools and workflows can significantly increase the quality of the data obtained in immuno-oncology and immunotherapy research.

O12
Prussian blue nanoparticle-based antigenicity and adjuvanticity trigger robust antitumor immune responses against neuroblastoma

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Introduction: Neuroblastomas are most remarkable for their broad spectrum of clinical behavior, which can range from spontaneous regression, to aggressive disease with metastatic dissemination leading to death. Therefore, the ideal neuroblastoma therapy effectively eradicates primary tumors, as well as disseminated and metastatic cancers by activating an immune response cascade.

Objectives: Given the high prognosis of disseminated disease in neuroblastoma patients, we engineered a nanoimmunotherapy comprised of CpG-coated Prussian blue nanoparticle-based photothermal therapy (CpG-PBNP-PTT). We studied the effects of this therapy in eliminating primary tumors as well as upregulating an antitumor immune response that treated distal or metastatic disease.

Materials & Methods: We assembled CpG on PBNPs using a layer-by-layer methodology. Neuroblastoma-bearing mice (Primary and synchronous models) were treated with a nanoimmunotherapy consisting of CpG-PBNPs for PTT+/-aCTLA-4. Tumor growth, survival, and immune responses elicited after treatment were measured. Upregulation of an antitumor immune response was studied using flow cytometry to determine the immune cell populations in the tumors, spleens, and draining lymph nodes

Results: CpG-PBNP-PTT triggers tumor cell death and increases tumor antigenicity. Simultaneously, the CpG coating functions as an exogenous adjuvant that complements the endogenous adjuvants released by the PTT (ATP, calreticulin, and HMGB1), increasing adjuvanticity. When administered in a Neuro2a model, CpG-PBNP-PTT results in complete tumor regression in 70% of treated animals relative to controls. Further, the long-term surviving, CpG-PBNP-PTT-treated animals reject Neuro2a rechallenge suggesting that our nanoimmunotherapy generates immunological memory. When we treat a synchronous model of neuroblastoma, wherein the primary tumor is nanoimmunotherapy-treated and the secondary tumor remains untreated, 55% of mice treated with our nanoimmunotherapy show complete eradication of both the primary and secondary tumors compared to controls, which showed no survival or memory efficacy.

Conclusion: We describe a novel nanoimmunotherapy using CpG-PBNPs that leverages the ablative properties of PBNPs and the immunostimulatory properties of CpG. Our findings show the importance of simultaneous cytotoxicity, antigenicity, and adjuvanticity using CpG-PBNP-PTT in generating robust and persistent antitumor immune responses against neuroblastoma.

DOI: 10.1002/eji.201970300

Figure 1

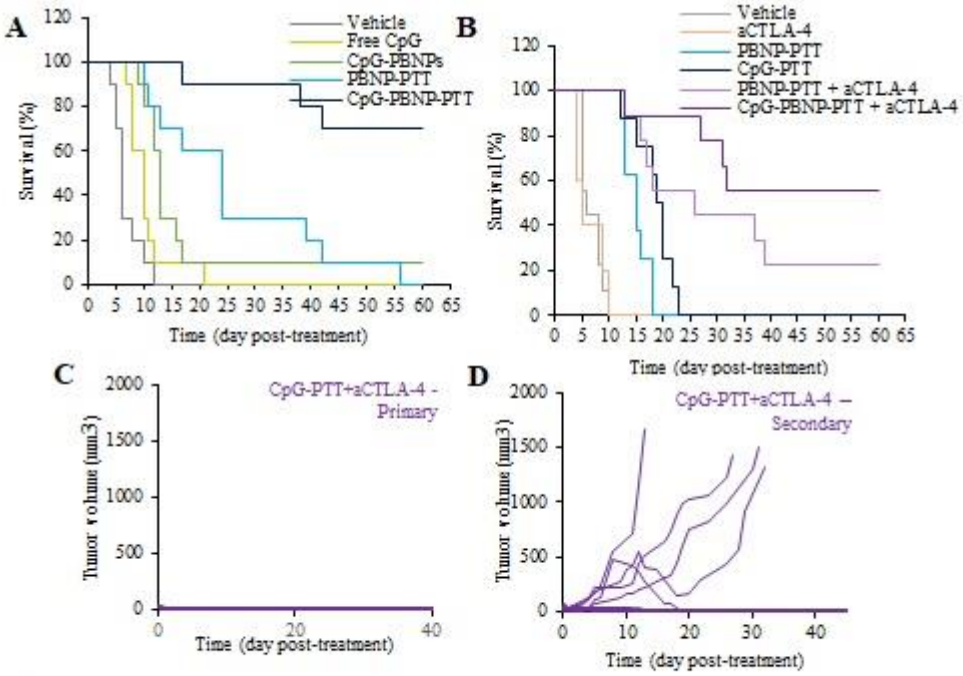

Figure 1: Mice treated with CpG-PBNP-PTT show an increased tumor regression and survival compared to controls in primary (A) and synchronous (B-D) models of neuroblastoma

Natural killer and innate lymphoid cells (O13-O18)

O13
The natural cytotoxicity receptor NKp44 interacts with a subset of HLA-DP molecules

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Natural killer (NK) cells represent a major component of the human innate immune system with the ability to recognize and lyse virus-infected and tumor cells. Their activity is regulated by the expression of activating and inhibitory receptors, many of which interact with HLA class I. Although early studies suggested a functional impact of HLA class II (HLA-II) on NK-cell activity, NK-cell receptors that specifically recognize HLA-II have never been identified.

DOI: 10.1002/eji.201970300

We investigated whether two major families of NK-cell receptors, killer-cell immunoglobulin-like receptors (KIRs) and natural cytotoxicity receptors (NCRs), contained receptors that bound to HLA-II.

To identify interactions between NK-cell receptors and HLA-II we determined the binding ability of different NK-cell receptor Fc-constructs to an array of HLA-II coated beads. Remarkably, NKp44 Fc-constructs displayed binding to a subset of HLA-DP molecules, including HLA-DP401, one of the most frequent allotypes in Caucasians, but not to any of the HLA-DR- or HLA-DQ–coated beads tested. Using SPR, we confirmed a direct interaction between NKp44 and HLA-DP401 with an affinity of 42.6 ± 16.2 µM. In cell-based assays, NKp44ζ+ Jurkat reporter cells upregulated CD69 after co-incubation with HLA-DP401, while less reporter cell activity was observed upon incubation with HLA-DP601 and HLA-DP301 monomers, which displayed low binding to NKp44 Fc-constructs. Similar to NKp44ζ+ reporter cells, NKp44-expressing primary human NK cells upregulated CD107a upon co-incubation with HLA-DP401 monomers, which was abrogated by pre-incubation with an α-NKp44 blocking antibody (median percentage of CD107a+ cells: 9.2% vs. 2.02%). To investigate the interaction of NKp44 with membrane-bound HLA-DP we generated HLA-DP-expressing Jurkat cell lines and co-incubated them with NKp44ζ+ reporter cells. Interestingly, we observed the same hierarchy in CD69 expression as for plate-coated HLA-DP monomers, with HLA-DP401-expressing cells inducing significantly higher reporter cell activity than HLA-DP601- and HLA-DP301-expressing cells.

In conclusion, this study identified a previously unknown interaction between a subset of HLA-DP molecules and the NK-cell receptor NKp44 that has a functional impact on NK-cell activity. It also provides a potential mechanism for the described associations between HLA-DP subtypes and several disease outcomes, including hepatitis B virus infection, graft-versus-host disease and inflammatory bowel diseases.

O14

The frequency of tissue-resident donor T and NK cells in peripheral blood after lung transplantation is modulated by normothermic ex vivo lung perfusion

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Question: The appearance of passenger donor lymphocytes in recipient blood after double lung transplantation has been described decades ago. However, neither the distribution of lymphocyte subsets, nor the early kinetics and their clinical relevance have been addressed in detail. Therefore, we investigated phenotype and frequencies of donor T and NK cell subsets within the first 24h to 3wk after lung transplantation (Tx) and correlated them to clinical parameters.

Methods: Blood and perfusion solutions of 59 lung recipients (30 male, 29 female, median age 51) were analysed pre Tx, at T0, T24 and at 3 weeks regarding T and NK cell subsets. In a subset of 20 patients with standard cold donor lung preservation and 9 preserved with portable ex vivo lung preservation (OCS), donor T and NK cells were identified in blood by staining of donor HLA epitopes combined with lineage markers. Frequencies of donor lymphocytes were correlated to cold ischemic time (CIT), primary graft dysfunction (PGD) and chronic lung allograft dysfunction (CLAD).

Results: In all lung recipients, the frequency of CD4+ and CD8+ T and NK cells was significantly increased at T0, T24 ($p=0.04$). At 3wk, T cells disappeared while NK cells were still detectable. Donor NK cells comprised 18.8% at T0, 17.1% at T24 and 7.8% at 3 wk ($p<0.001$) of circulating NK cells. Frequencies were for donor CD8+ T cells 8.3%, 6.6% and 2.6%, and for CD4+ donor T cells 6.4%, 4.6% and 1.3% of the respective subset. At T0, significantly less donor NK cells were detected in recipients of OCS lungs ($p<0.008$), while T cells were reduced but not significantly. No correlation between donor NK or T cell frequencies was observed for CIT or PGD. In the limited number of patients at risk, a trend towards higher early donor T cell frequencies in recipients not developing CLAD at two years after Tx was observed ($p<0.05$).

Conclusion: Donor T and NK cells are detectable in blood of all lung recipients during the first 3 weeks after Tx and did not correlate with CIT or PGD. Preservation with portable EVLP resulted in decreased NK cell frequencies which might be explained by their ex vivo mobilization. Furthermore, transient donor chimerism might have a protective effect against CLAD development.

DOI: 10.1002/eji.201970300

O16

Human ILC2 are induced to produce type 1 or type 3 cytokines and are inhibited by Treg cells

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Introduction: Human group 2 innate lymphoid cells (ILC2) represent the innate counterpart of Th2 cells and cooperate with them both in the protection against helminths and in the pathogenesis of allergic diseases. Some reports mainly in mouse model described the plasticity of ILC2 and very few studies investigated so far the cellular and molecular mechanisms mediating the inhibition of ILC2.

Objective: To define if the mechanisms of immune deviation and immune regulation described for Th2 cells regards also ILC2 immune response.

Materials & methods: Circulating ILC2 were isolated via immunomagnetic and FACS sorting and then expanded in vitro. Then ILC2 were cultured in presence of IL-12 or IL1b plus IL-23 ILC2 for two weeks and their plasticity were evaluated in terms of cytokines production and trscritpion factor expression. Modulated ILC2 were co-cultured with B cells to investigate Ig production. Additional experiments were performed co-colturing ILC2 in presence of circulating CD4+CD25highFoxp3+Treg cells and their proliferation rate was evaluated by 3H thymidine incorporation and/or by CFSE dilution. Finally the immunosuppressive effects of inhibitory cytokines such as IL-10 and TGFb on ILC2 were investigated.

Results: We found that human circulating ILC2 in presence of IL-12 acquired the ability to produce IFN-g and upregulated the expression of Tbet whereas in presence of IL1b and IL-23 acquire produced IL-22 and upregulated RORC expression. In all these conditions, ILC2 did not reduce the typical type 2 cytokines and transcription factors expression. Moreover, "modulated" ILC2 have reduced ability to induce IgE producing when co-cultured with autologous B cells. ILC2 proliferation, cytokines production and CD154 expression were inhibited by CD4+CD25highFoxp3+ Treg cells.

Conclusion: This study defines possible cellular and molecular mechanisms responsible for modulation and inhibition of ILC2 activity in terms of proliferation, cytokines production, and CD154 expression. These results may be useful in the development of strategies aimed to dampen ILC2 function in type-2 mediated diseases.

O18

ILC2 phenotype and plasticity is determined by the tissue microenvironment

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Introduction: Group 2 innate lymphoid cells (ILC2s) are important regulators of type 2 immune responses in helminth infection and allergic diseases. ILC2s are equipped with a wide array of receptors to sense, integrate and respond to local cues provided by other immune cells, epithelial cells, and stromal cells of the tissue niche they reside in. Accordingly, recent studies have demonstrated that ILC2s in different non-lymphoid organs are phenotypically and functionally distinct.

Objective: The present study investigates how tissue-specific factors shape phenotype, function and plasticity of ILC2s.

Material & methods: ILC2s from kidney, lung, and small intestinal lamina propria (siLP) were isolated from naïve mice and characterized by flow cytometry and bulk RNA sequencing. FACS-sorted ILC2s of kidney, lung, and siLP from IL-33 treated wild type mice were transferred into alymphoid *Rag2-/-Il2rg-/-* mice and various tissues were analyzed 8 weeks after transfer. In addition, ILCs from kidney, lung, and siLP of IL-13 fate-mapping (IL-13fm) mice were analyzed by flow cytometry. ILC2s FACS sorted from kidney of IL-33-treated mice were cultured under various conditions for up to 7 days and analyzed for their cytokine production via intracellular cytokine staining and detection of cytokines in the supernatant.

DOI: 10.1002/eji.201970300

Results: Here, we show that murine ILC2s from different anatomical locations (kidney, lung, small intestine) exhibit tissue-specific transcriptional and phenotypic differences. However, when ILC2s from different locations were transferred into alymphoid *Rag2-/-Il2rg-/-* mice, they were equally effective in reconstituting ILC2 populations in all tissues examined and adapted the tissue-specific phenotype of target organs.

Moreover, fate mapping of ILC2s in cytokine reporter mice showed that especially in the siLP, ILC2s can switch to an ROR-γt+ ILC3 phenotype. We could further demonstrate in in vitro experiments with sorted ILC2s that a combination of TGF-β-, retinoid acid-, and Notch-signaling is able to induce a functional switch of ILC2s towards an ILC3 phenotype.

Conclusion: In summary, we show here that ILC2 phenotype and plasticity is determined by the tissue microenvironment. Ongoing studies are aimed at identifying the factors that drive this tissue-specific ILC2 adaptation.

B-cell biology (O19-O24)

O19
TNFR2 positivity identifies B cells with regulatory function

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B cells with regulatory function are thought to play a major role in the control of the immune responses in infection and autoimmunity. However, to date no reliable marker has been identified that would allow purification of human Breg cells. Recently, we showed that stimulation of human B cells with TLR9 ligand CpG ODN induces the upregulation of Tumor Necrosis Factor Receptor 2 (TNFR2) expression, which was further found to correlate with production of IL-10, the hallmark of suppressive B cell function. Here, we investigated the influence of TNFR2+ B cells on T cell proliferation. To understand the functional impact of TNFR2+ B cells, we sorted TNFR2-positive and -negative B cells after a two day stimulation with CpG and co-cultured these cells with autologous CFSE-labelled CD19-depleted PBMC that were subsequently stimulated with anti-CD3/CD28 beads. Proliferation of CD4 and CD8 T cells was measured via CFSE dilution in flow cytometric analysis. The results obtained revealed that proliferation of CD4 and CD8 T cells was decreased in the presence of TNFR2+ B cells but was unaffected in the presence the TNFR2-negative B cell subpopulation. Further sorting of TNFR2 positive B cells revealed that all TNFR2+ B cell subpopulations, e.g. switched memory (IgM-CD27+), IgM memory (IgM+CD27+) and naïve (CD27-) B cells displayed suppressive capacity on T cell proliferation. Notably, suppressive function was most pronounced in the presence of TNFR2+ IgM+ memory B cells and had stronger impact on CD4+ T cell proliferation than on CD8+ T cells. Well in line with these findings IL-10 concentrations in the co-culture supernatants correlated with suppression in co-cultures with IgM+ B cells. By contrast, IL-10 levels of were low in co-cultures containing switched memory B cells, indicating that other mechanisms could be responsible for T cell suppression in these cultures.

Conclusions: Altogether, our results demonstrate that TNFR2+ B cells exert suppressive activity on the human T cell cultures and confirm that TNFR2 could serve as a marker for enrichment of B cells with regulatory function.

O20
Distinct subsets of human memory B cells define the strategy of the human memory response throughout life

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Two populations of memory B cells (MBCs), that execute different and non-interchangeable functions, have been described: switched and IgM MBCs. We have shown that, whereas switched MBCs are mostly generated in the germinal centers (GC) at all ages, IgM MBCs can be distinct in three types with different developmental history: innate, remodelled and GC-derived IgM MBCs. In the mouse, based on the expression of surface markers, but

DOI: 10.1002/eji.201970300

independently of the expressed immunoglobulin (Ig) isotype, different subsets of MBCs have been identified that are either more naïve- or more memory-like. MBCs of the first subset are poorly mutated and able to form secondary GC whereas those of the second group are highly mutated and immediate precursors of Ab secreting cells (ASCs).CD27 is the cell surface marker able to identify most MBCs in humans. The intensity of CD27 expression changes with age. We show that, independently of the expressed Ig isotype, in infants MBCs express low levels of CD27 (CD27dull). In the adult most MBCs are CD27bright. Thus, the most used definition of human MBCs, as either IgM or switched, may be insufficient to describe the complexity of human B-cell memory responses and functions.We prove that CD27dull MBCs are T-, GC- and CD40L-independent. CD27bright MBCs, in contrast, are exclusively generated in individuals with functional GCs. Based on VH usage, frequency, type and distribution of SM, we show that CD27dull and CD27bright MBCs represent two distinct and sequential MBC developmental stages and that stringent Ag-driven pressure selects CD27dull into the CD27bright MBC pool. The MBC developmental program is massively implemented in the first 2-3 years of life, and it is impaired in the elderly. In women, pregnancy is associated to shrinkage of the MBC pool. From the second to third trimester a substantial proportion of CD27dull MBCs convert into CD27bright. During the first months after the delivery, the expansion of pre-existing CD27dull MBCs leads to the replenishment of MBC numbers and to the re-constitution of the maternal MBC repertoire. For the first time we show that highly selected and specific MBCs derive from less refined MBC precursors, thus challenging the postulate that high affinity MBCs always derive from naïve B cells recruited in the GC response. The stability of the human MBC repertoire throughout life is ensured by the existence of CD27dull MBCs that have a unique capacity to expand and differentiate in response to change.

O21

Gene editing in primary B cells to study the role of m^{6}A RNA methylation in lymphocyte development and activation

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N^6-methyladenosine (m^{6}A) is the most common internal modification of messenger RNAs and it regulates different aspects of mRNA metabolism, such as alternative splicing, decay and translation. In mammals, the addition of this modification is catalyzed by the methyltransferase METTL3 and removed by the demethylase FTO. Recent studies have highlighted the importance of m^{6}A in embryonic stem cells, where it controls the expression of pluripotency genes and regulates lineage differentiation. In T cells, the depletion of the methyltransferase METTL3 negatively affects the IL-7 signaling response due to an accumulation of the SOCS family transcripts, which normally require the m^{6}A modification in order to be degraded. Here, we study the role of METTL3 and of FTO in primary B cells undergoing the germinal center reaction, which leads to the generation of high-affinity antibody-secreting plasma cells (PCs). To achieve this goal, we used a *Rosa26* knock-in mouse constitutively expressing the Cas9 nuclease together with the GFP reporter. Cas9-expressing naïve B cells are activated *in vitro* by CD40L and BAFF stimulation and then complemented with retrovirus-delivered small guide RNAs targeting *Mettl3* or *Fto* gene. Next, mutant B cells are subjected to the induced germinal center-like B cell (iGB) culture system in the presence of IL-21, to study the effects of m^{6}A modulation on B cell terminal differentiation. Our data indicate that *Mettl3* mutant iGB cells show impaired proliferation and are outcompeted when placed in competition with *wild-type* B cells. Moreover, *Mettl3* mutant iGB cultures express higher levels of PC-associated markers, whereas *Fto* mutant cultures display increased proliferation and fail to downregulate B cell identity markers. Altogether, these data suggest that *Mettl3* and *Fto* could exert opposite functions in PC differentiation, pointing to a critical role of m^{6}A regulation in IL-21 induced B cell activation. Further investigations will include the mapping of m^{6}A targets in *wild-type* iGB cultures through RNA immunoprecipitation as well as the transcriptomic profile of *Mettl3* or *Fto* mutant iGB cells. The combination of these results will help to elucidate for the first time the molecular mechanism underlying the role of m^{6}A in B cell terminal differentiation.

O22

Hetero-oligomerization of BAFFR and TACI regulates BAFFR and TACI signaling and ADAM-dependent shedding.

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DOI: 10.1002/eji.201970300

Binding of the B-cell-activating factor of tumor necrosis factor family (BAFF) to its receptor BAFFR activates essential pro-survival functions in B cells. However, it can also induce proteolytic shedding of BAFFR from the surface of marginal zone and germinal center B cells by the proteases ADAM10 and ADAM17. Shedding of BAFFR limits BAFF-mediated pro-survival signals. Recruitment of ADAM10 protease to BAFFR requires in addition to BAFF binding the co-expression of TACI as TACI associates with ADAM10 thereby catalyzing the constitutive cleavage of TACI resulting in its release as decoy receptor. Here we show that BAFFR and TACI spontaneously associate into hetero-oligomers and that the heteromerization of the receptors in enhanced by BAFF binding. Binding of BAFF to BAFFR-TACI heteromers changes the recruitment of TRAF molecules and the association of BAFFR and TACI with other binding partners which were identified by proteomic studies. BAFF-induced signals of BAFFR-TACI heteromers are transmitted via NIK, TRAF2 and the activation of Jun-kinases. They are required to render the structure of BAFFR in such a way that the receptor is accessible to ADAM-dependent proteolytic cleavage.

Our data show that the signaling cascades -induced by BAFF binding to BAFFR are far more complex than previously anticipated. They activate in addition to protein synthesis, metabolic changes and mitochondrial functions also a complex series of metalloprotease activities. By limiting BAFFR signaling and by inducing the release of TACI decoy receptors which modulate the biological activity of BAFF, ligand-dependent processing of BAFFR and TACI forms a regulatory circuit controlling B cell homeostasis.

O23

Generation of GFP-Env fusion tagged endogenous retroviruses transgenic mice reveals a Toll-like receptor dependent breakage of B cell self-tolerance against a germline encoded (neo-) antigen

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The generation of autoantibodies is both the diagnostic and pathogenic hallmark for a number autoimmune diseases. However, for most B cell associated autoimmune diseases, we still do not know, which molecular pathways are compromised, or if exogenous infections contribute to overt pathogenesis. From in vivo model systems it became clear that expression of foreign antigens through the germline in transgenic mice results in B cell specific tolerance against neo self-antigens. Here we report that the expression of GFP as Env-GFP fusion protein in Moloney-MuLV (Mo-MulV) transmitted through the germline of a transgenic mouse, results in the spontaneous production of (neo)-autoantibodies against GFP. The GFP is encoded in the murine germline in the context of Moloney-MuLV which represents a replication competent form of an endogenous retrovirus (ERV). In EGT-315 (Endogenous retrovirus-GFP-Tagged) mice backcrossed to TLR3-TLR7-TLR9 triple deficient mice no antibodies against GFP are detectable. These mice do suffer a very strong reactivation of the transgenic ERV and succumb early in life to leukemia. Our model demonstrates that a replication competent ERV induces a retrovirus specific antibody response, which is inversely correlated with retrovirus spread/reactivation or replication. The expression of GFP (Env-GFP fusion) in the activated endogenous retrovirus implies that this could lead to breakage of B cell tolerance and autoantibody formation. This could be the first step towards pathological autoimmunity.

O24

"Two B or not two B?" The Potency of Notch2 in Follicular vs Marginal Zone B Cell Identity

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Introduction: Notch2 signaling has been shown to be essential for the peripheral differentiation of bone marrow-derived immature B cells to mature marginal zone B cells (MZBs). It is currently considered that either follicular B cells (FOBs) or MZBs develop from transitional B cells depending on a signal-strength interplay of B cell receptor-, BAFF receptor- and Notch2-signaling.

Objectives: In this study, we want to investigate the capability of mature follicular B cells (FOBs) to convert to marginal zone B cells (MZBs) through the induction of a constitutively active Notch2 signaling pathway.

DOI: 10.1002/eji.201970300

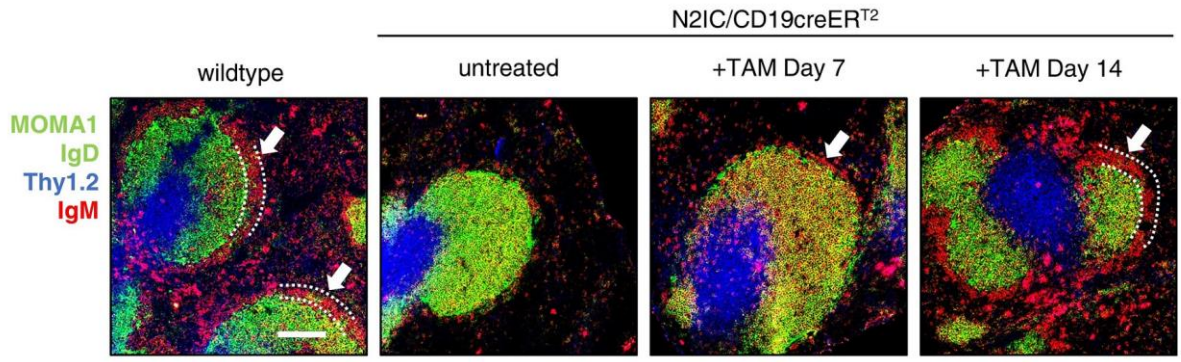
Materials & Methods: Our lab developed a mouse model carrying a floxed Notch2IC protein (N2IC) - a constitutively active intracellular signaling domain of the Notch2 receptor - combined with a B cell-specific, inducible Cre-line to be able to introduce a strong Notch2 signal in mature B cells.

N2IC expression was induced in FOB cells via tamoxifen treatment. The phenotypes and localization of N2IC-expressing cells were followed up over time by FACS and histology. Functional testing of N2IC-expressing B cells was performed by *in vitro* stimulation experiments and *in vivo* immunizations. Moreover, N2IC-expressing B cells were sorted at different time points after tamoxifen treatment for whole transcriptome RNA-Seq.

Results: We found that induction of Notch2 signaling in FOB cells causes an identity conversion to *bona fide* MZB cell within two weeks. Newly generated MZBs were indistinguishable from their wild-type counterparts regarding the FACS phenotype and splenic localization. Moreover, Notch2IC-expressing B-cells rapidly differentiated to plasmablasts *in vitro* and *in vivo* after challenge with LPS - a MZB-cell-specific immunological function. RNA sequencing confirmed the shift from FOB to MZB gene expression signature and revealed the potency of Notch2 in orchestrating a plethora of transcriptomic alterations.

Conclusions: The current view of B cell lineage commitment and dead-end cell fate decision to either FOB or MZB type has to be broadened. We provide evidence that strong activation of the Notch2 pathway induces conversion of fully differentiated mature FOB to genuine MZB cells.

Figure 1



T cell development (O25-O30)

O25
Characterization of the thymic vascular specialization and its impact on lymphocyte progenitor immigration and T cell egress
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Introduction: The recruitment of lymphocyte progenitor cells into the thymus is essential for T cell development and induction of central tolerance and is therefore key to a functional immune system.

Objectives: In order to understand lymphocyte progenitor recruitment into the thymus it is important to understand the cellular and molecular components that are involved in the endothelial cell mediated cell recruitment from the blood vessels into the thymic stroma. We characterize the molecular and functional differences of venular endothelial cells (VECs) compared to arteries and capillaries (non-venular endothelial cells, NVECs). We aim at identifying the signaling cascades and transcriptional regulators defining venular endothelial cell physiology and function in cell recruitment and cell egress.

Materials & methods: A newly developed antibody, recognizing DARC (Duffy Antigen Receptor for Chemokines, e.g. Ackr1) allows for venular-specific endothelial staining, isolation and functional characterization compared to NVECs.

DOI: 10.1002/eji.201970300

We used this antibody to perform whole thymus 3D analysis of the vascular spatial distribution and analyzed VEC and NVEC localization and positioning within the thymus. Further, we used this antibody to generate a multi-tissue comparative expression atlas for VECs and NVECs using population RNA-seq and SC RNA seq.

Results: We observe a highly dense vascularization of the thymus with venules distributed throughout the tissue. The 3D analysis of the whole thymic vasculature allows us to quantify vessel specialization with respect to different thymic regions such as the outer cortex and the inner medulla. The gene expression patterns of venules differ from non-venules and we were able to identify sets of genes unique for different tissues and genes specific for venules and non-venules as well as common endothelial cell expressed genes and common VEC genes.

Conclusion: This comprehensive analysis helps to identify the molecular components important for vasculature specialization and function. We can use this to address the proposed gate keeper function of VECs in lymphocyte progenitor recruitment into the thymus and to revisit the assumption of a preferential recruitment at the cortex-medullar junction in a comprehensive manner.

O26

Identification of NFATc1 target genes in thymocyte development

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Introduction: During thymocyte development CD4-CD8- double-negative (DN) cells differentiate to the CD4+CD8+ double-positive (DP) stage, which undergoes the process of positive and negative selection to finally give rise to CD4+ or CD8+ single positive (SP) T cells. During this development, we observed increasing expression of the transcription factor NFATc1 in the nucleus. We described previously that hematopoietic cells-specific ablation of NFATc1 activity resulted in an arrest of thymocyte differentiation at the very early stage of development. On the other hand, over-expression of a constitutively active version of NFATc1 resulted in an impaired transition of DN cells to further stages, suggesting that a certain threshold level of NFATc1 activity is critical at this point.

Objectives: Molecular analysis will elucidate the role of NFATc1 in the regulation of genes controlling thymocyte development.

Materials & methods: To identify the genes regulated by NFATc1, we generated mice in which a chimeric NFATc1/A-Bio protein and the biotin-ligase BirA are co-expressed. These mice allow the purification of NFATc1-DNA complexes by Streptavidin beads with high specificity in ChIP experiments with thymocytes.

Results: ChIP-seq analysis with transgenic freshly isolated thymocytes allowed us to identify 1636 NFATc1-binding peaks nearby 2321 genes expressed during thymocyte development. Several of these genes are known to be involved in normal thymocyte maturation.

We also performed ChIP-seq analysis with thymocytes stimulated by TPA and Ionomycin for 4h to identify all potential NFATc1 binding sites. The combination of these results with RNA-seq analysis revealed change in gene expression of 365 genes by NFATc1 overexpression out of which 171 contain peaks, thus being potential NFATc1 target genes.

Conclusions: The identification of NFATc1-regulated genes will thus give the opportunity to clarify which of these genes are involved in the coordination of thymocyte development. Further analysis will reveal also how dysregulation of these genes or of NFATc1 expression and activation, can lead to severe diseases as lymphomas or leukemia.

O27

Steering MHC-I restricted T cells towards the NKT developmental pathway

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Introduction: Conventional T cells interact with MHC-I and MHC-II molecules on thymic epithelial cells for positive selection. NKT cells, on the other hand are positively selected by interaction with CD1d on DP thymocytes, where

DOI: 10.1002/eji.201970300

SLAMr homotypic interactions are critical. One reason that conventional T cells do not typically develop an NKT-like program may be that MHC-I and MHC-II are not expressed by DP thymocytes, owing to the absence of key transactivating factors in DP. This was suggested to be the case for MHC-II because forced expression of the class II transactivator (CIITA) on thymocytes, which led to MHC II upregulation on DP, resulted in selection of NKT-like (PLZF+) MHC II restricted CD4 T cells.

Objectives: The objective of this project is to test if upregulation of MHC I expression on DP thymocytes would induce the same effect by provoking selection of PLZF+ MHC I restricted CD8 T cells.

Materials & methods: Mice are analyzed with the use of flow cytometry for frequencies and characteristics of developing iNKT cell subsets.

Results: The overall approach was to create a gene-modified mouse overexpressing the MHC class I transactivator (CITA) (encoded by the *Nlrc5* gene) in DP thymocytes. To do this, we generated aRosa26-floxed stop CITAtg mouse crossed to lck-Cre mice to allow MHC I overexpression on thymocytes. We will study the impact of DP thymocyte self-peptide presentation on deletion of autoreactive T cells utilizing CITA and CIITA transgenic mice crossed to Act-2W mice (expressing OVA and 2W peptides under the ubiquitous actin promoter). OVA-Kb and 2W-IAb tetramer enrichment will be performed to determine the number and NKT-like state of MHCII and MHC I restricted T cells.

Conclusions: With the results obtained from this study we will be able to answer two main questions: Can MHC I expression on DP thymocytes steer MHC I restricted T cells towards the NKT cell developmental pathway? Will autoreactive T cells selected on DP thymocytes be spared from clonal deletion?

O28

Single-cell RNA sequencing identifies that Scart1+ Vγ6+ T cells adapt as highly activated and IL-17A producing effector cells to their skin habitat

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Introduction: γδ T cells take a small yet important part in the immune system. While they are less frequent in peripheral blood, they are highly enriched tissues. γδ T cells producing the pro-inflammatory cytokines IL-17A and IL-17F (γδT17) were ascribed pleiotropic functions from mediating local immune responses against infections, contributing to tissue-homeostasis during anti-viral responses, to regulating body thermogenesis. They are further characterized by expression of (semi)-invariant Vγ4+ or Vγ6+ T cell receptors, mainly develop in the prenatal thymus and later persist as long-living cells in all kind of tissues.

Objective: In this project we explored the exchange of IL17-producing γδ T cells between tissues and the underlying transcriptional mechanisms mediating their tissue-specific functional adaptation.

Methods: We apply single-cell RNA sequencing (sc-seq) in combination with flow cytometric analysis using different mouse models of IL17-producing γδ T cells within different tissues.

Results: Parabiosis demonstrated that IL-17-producing Vγ6+ T cells were fairly tissue-resident in thymus, peripheral lymph nodes (pLNs) and skin. Transcriptome analysis of these Vγ6+ T cells indicated the formation of tissue-specific homogenous cell populations. In pLNs, resting Vγ6+ T cells were featured by expression of *Slfn2* and *Klf2*, while a small fraction of proliferating Vγ6+ T cells (*Top2a* and *Mki67* expression) was identified. BrdU uptake of Vγ6+ T cells further pointed out that Vγ6+ T cells in pLN and thymus have a higher proliferation rate than their skin counterparts. In the skin, tissue-resident γδT17 cells showed TCR activation profiles (e.g. high *Cd69*, *Cd44*, *Nr4a1* expression), presumably resulting in high expression of *Il17a* and *amphiregulin*. Further in vivo analysis identified skin Vγ6+ T cells as IL17A-producing cells under steady-state conditions. Our data further suggested that Bcl2a1 proteins might mediate the long-term maintenance of the functionally active skin Vγ6+ T cells. Final analysis of other skin-resident IL17-producing γδ T cell subsets, namely Vγ4+ T cells, showed that Vγ4+ and Vγ6+ T cells have highly similar phenotypes, but can be separated by Scart-2 and Scart-1 expression, respectively.

Conclusion: Together, using sc-seq as well as other approaches, this study demonstrates how mouse Scart1+ Vγ6+ T cells undergo tissue-specific functional adaptation to persist as IL-17-producing effector cells in the skin.

DOI: 10.1002/eji.201970300

Figure 1

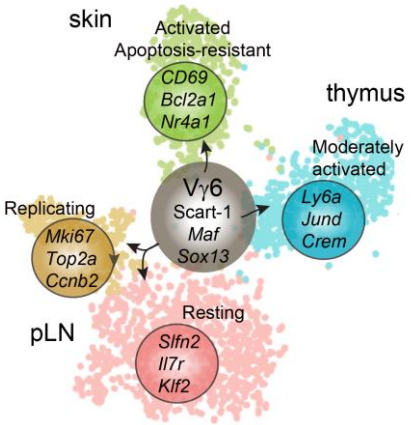
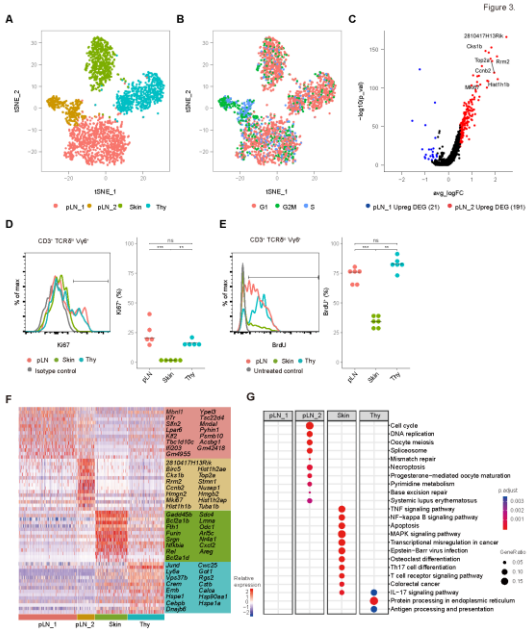


Figure 2



O29

Human FCH domain only 1 *(FCHO1)* deficiency reveals an essential role for clathrin-mediated endocytosis for the development and function of T cells

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DOI: 10.1002/eji.201970300

Clathrin-mediated endocytosis (CME) is critical for internalisation of molecules across the cell membranes. The FCH domain only 1 (FCHO1) protein is one of the key molecules involved in early stages of CME formation, acting as a nucleating and sorting agent during formation of clathrin-coated pits (CCP). Although the function of FCHO1 was intensively studied, the consequences of mutations in *FCHO1* in humans are unknown.We identified ten patients from seven unrelated pedigrees with variable T and B cell lymphopenia, who are homozygous for six distinct mutations in *FCHO1.* All variants segregated with the disease and have not been reported in genetic databases. Using a heterologous system, CRISPR/Cas9 genetic editing and high-resolution confocal microscopy we demonstrated that the identified mutations either led to mislocalisation of the protein at the plasma membrane or prevented its interaction with chief binding partners, EPS15 or EPS15R. Live-cell imaging of cells expressing mutant variants of FCHO1 further corroborated impaired formation of CCP. Patient T cells were unresponsive to re-stimulation upon TCR triggering as shown by decreased proliferation and production of cytokines. The internalisation of the T-cell receptor and release of calcium was severely perturbed in FCHO1 deficient Jurkat cells but could be reconstituted upon expression of wild-type FCHO1. Thus, we discovered a previously unrecognised critical role of FCHO1 and CME during T-cell development and function in humans.

O30

Heteromeric interference, a novel pathogenesis for human immunodeficiency.

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Immune system is a higher-order complexed biological system that is essential to protect our body from pathogenic organisms. Before the birth when individual begins to be exposed to pathogen-rich outer environment, essential components of immune system must be generated by developmental program embedded on genome. In other words, perturbation of this process appears as primary immune deficiencies (PID) resulting in suffering from recurrent infection. Given central roles of transcription factors (TFs) to govern immune cell development, many genetic variations that impairs TFs function have been identified as causal for PID. In some PID cases, heterozygous variation also causes PID through mechanisms such as gain-of-function, negative dominance, or haploinsufficiency. Here we show a family case for B-cell deficiency and isolated a heterozygous missense mutation in *IKZF3* gene that results in a glycine to arginine replacement within the DNA binding domain of its encoded AIOLOS protein. Using a mouse model that harbors the corresponding mutation, we unravel that the mutant AIOLOS not only did not bind the canonical AIOLOS DNA sequence, but also altered the DNA binding specificity of heterodimers with IKAROS. Thus, the B-cell deficiency is caused by the missense AIOLOS mutation hijacking IKAROS function. Recently, the *de novo* heterozygous missense variant in the *BCL11B* gene, which generates a BCL11BN441K mutant protein, was reported to be causal for T-cell deficiency. In this case, early thymocyte development is more severely inhibited in *Bcl1bD/B441K* mice than in Bcll1b-deficient mice, suggesting that BCL11BN441K mutant acts as dominant-negative against other proteins than Bcl11b. We unraveled that Bcl11b make heterodimer with Bcl11a, the most related family member, in T-cell progenitors, suggesting that the Bcl11bN440K mutant protein interferes with Bcl11a function. Our analyses of two PID cases prompt us to propose that heteromeric interference is a novel mechanism of autosomal dominance that causes disease by impairing protein function via mutation of its heterodimeric partner.

Infection II (O31-O35)

O31

Platelets and tuberculosis: small cells, not so innocent bystanders.

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DOI: 10.1002/eji.201970300

Introduction: In addition to their classic role in hemostasis, platelets modulate innate and adaptive immune responses. Platelets may interact with the immune system in tuberculosis (TB) to regulate human inflammatory responses that lead to morbidity and spread of infection.

Objectives: We have investigated the absolute count of platelets and the platelet/lymphocytes (P/L) ratio among Healthy Donors (HD), subjects with latent TB infection (LTBI), active and cured TB patients to identify an additional tool to differentiate the various clinical conditions. Moreover, we have also evaluated platelets activation/maturation state and their localization in the lung, the site of *M. tuberculosis* replication in pulmonary TB, using autoptic lung tissues.

Patients and Methods: The absolute platelet count and the P/L ratio was evaluated in 184 subjects: 30 HD, 52 LTBI subjects, 66 active TB and 36 cured TB patients. The evaluation of platelet phenotype was performed by flow cytometry using anti human CD61, CD62p, CD42b, CD41a, CD42a, PAC-1 and CD45 mAbs. We performed a Luminex Multiplex Immunoassay to measure plasma cytokines associated with activated status of platelets and bone marrow platelets differentiation. Platelets were localized in the autoptic lungs of TB patients by immune-hystochemistry (IHC), using anti-FVIII/von Willebrand (VWF) factor mAb.

Results: Patients with active TB had a high platelet/lymphocyte (P/L) ratio and elevated numbers of platelets in the circulation, which expressed an activated phenotype. The basal serum concentrations of different mediators promoting platelets differentiation and associated with active platelets were found increased in active TB patients. IHC studies showed that in active TB patients platelets were localized around the granuloma lesions, unlike control patients where platelets were detected in the alveolar interstitium.

Conclusions: The absolute count of platelets and the P/L ratio were increased in active TB compared to other groups. The platelets of active TB patients showed an activated phenotype and this was associated to an increased concentration of sera mediators which are released by activated platelets. Finally, there was a higher proportion of areas positive to the staining of FVIII/VWF at the site of disease in pulmonary TB. Altogether these results suggest that platelets are involved in the pathogenesis of human TB.

O32

IL-18R depended protection against bacterial pneumonia by pulmonary epithelial cells

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Introduction: The epithelial barrier of the lung is the first line of defence against pulmonary pathogens. Although it is known that epithelial cells produce cytokines that elicit strong immunity, little is known about immune cytokines promoting protective responses by pulmonary epithelial cells.

Objectives: Our aim is to elucidate the mechanisms by which lung epithelial cells and myeloid cells protect against pulmonary *Legionella longbeachae*, whichcauses a serious and often fatal community-acquired pneumonia known as Legionnaires" disease in humans.

Material & Methods: Our work is based on a murine model of respiratory *Legionella longbeachae*infection. We have used wild-type and genetically-modified strains to investigate anti-bacterial defense mechanisms.

Results: During infection with *L. longbeachae,*we have observed a strong infiltration of neutrophils into the lung tissue. A significantly higher fraction of those neutrophils contains *L. longbeachae* as compared to other myeloid cells, and they are necessary for bacterial clearance.Additionally, we found that production of the pro-inflammatory cytokine IL-18 is required for efficient clearance of *L. Longbeachae*. IL-18 has been shown to promote anti-bacterial defences by promoting IFNγrelease from lymphoid cells. Surprisingly, expression of the IL-18 receptor on immune cells did not contribute to *L. longbeachae*clearance. Instead, IL-18 receptor expression by pulmonary epithelial cells was required and sufficient for efficient clearance. Finally, we will discuss our data on the effect of IL-18 on pulmonary epithelial cells and how those cells modulate microbicidal fuctions of neutrophils during infection with *L. longbeachae*.

DOI: 10.1002/eji.201970300

Conclusion: Our results show a novel IL-18-dependent bacterial defence mechanism, in whichIL-18 receptor expression by epithelial cells, but not by immune cells, is required and sufficient for bacterial clearance in the lung.

O33

Lymphotoxin β receptor: A crucial role in *Toxoplasma gondii* infection

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Introduction: Infection with the obligate intracellular protozoan parasite *Toxoplasma gondii* (*T. gondii*) causes toxoplasmosis, a global problem, since about 30 % of the population is infected worldwide. Acute toxoplasmosis causes no or only mild flu-like symptoms, but immunocompromised patients can be severely affected. Primary infection of pregnant women can lead to congenital toxoplasmosis and may cause miscarriage. After infection the production of cytokines induces potent cell autonomous effector mechanisms such as the production of murine guanylate-binding proteins (mGBPs) which are essential for controlling the parasite. Lymphotoxin β receptor (LTβR) mediated signalling is known to play an important role in the efficient initiation of innate and adaptive host responses to a variety of pathogens.

Objective: The aim of this project is to further elucidate the role of the LTβR in the adaptive immune response to *T. gondii* infection. New insights into the pathology of *T. gondii* could provide new therapeutic strategies for the treatment of human toxoplasmosis.

Material & methods: Therefore, immune responses, such as cytokine, mGBP production and T cell responses were analysed in a *T. gondii* infection model *in vivo* and *in vitro* in LTβR$^{-/-}$ and WT mice.

Results: Compared to WT mice, LTβR$^{-/-}$ mice showed an increased parasite burden and a markedly increased mortality in the acute phase of *T. gondii* infection. FACS analysis revealed that while these mice are generally able to generate *T. gondii* specific CD8^{+} T cells this does not seem to be sufficient for clearing the infection. Tissue and serum of LTβR$^{-/-}$ mice revealed deregulated cytokine expression patterns and production, particularly regarding interferons and interleukins. Since Interferon γ (IFNγ) mediated upregulation of mGBPs is essential for parasite clearance, expression and localization of mGBPs was assessed. *In vivo*, delayed/reduced up-regulation of mGBP expression was observed, whereas initial *in vitro* experiments in LTβR$^{-/-}$ fibroblasts demonstrated that mGBPs can be upregulated after IFNγ treatment and are able to localize to the parasitophorous vacuole. Furthermore, RNA-Sequencing was performed to identify potential target genes for LTβR signalling.

Conclusion: These data indicate a link between IFNγ and LTβR signalling pathways and suggest that defects in cytokine signalling, especially IFNγ deregulation, may contribute to the decreased survival rates of LTβR$^{-/-}$ mice in *T. gondii* infection.

O34

Malaria-induced CD4^{+} and CD8^{+} regulatory T cells suppress effector T cells

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Malaria, a life-threatening disease caused by *Plasmodium* parasites, is still a global health threat. The majority of the 500.000 yearly casualties are children under the age of 5, falling victim to severe disease progression. However, neither various vaccination attempts nor primary infections establish long-term protection against re-infection. Conversely, people infected multiple times tend to show reduced T cell-induced immune pathology.

The overwhelming activation of cytotoxic T cells during *Plasmodium* infection can lead to severe malaria. We assume that the induction of different subsets of regulatory T cells may limit the pathological potential of the immune-response during blood-stage malaria.

We employ a well-established animal model to mimic severe malaria induced by *Plasmodium falciparum* infection by infecting C57BL/6J mice with *Plasmodium berghei* ANKA (PbA). To further differentiate between immune responses to the liver- or blood-stage of infection, mice were infected with mosquito-generated sporozoites or infected red blood

DOI: 10.1002/eji.201970300

cells. T cells phenotype was acquired by multi-color flow cytometry and partially analyzed with Hierarchical Stochastic Neighbor Embedding (HSNE). Cytokine levels in blood were measured with bead-based immunoassays.

While classical FoxP3$^+$ regulatory T cells are not upregulated during infection, we could observe the emergence of CD49b$^+$ Lag3$^+$ CD4$^+$ T cells (Tr1). Furthermore, we are the first to observe the induction of CD49b$^+$ Lag3$^+$ CD8$^+$ T cells during malaria. Both T cell subsets are induced by the blood-, not the liver-stage of the disease. Elevated levels of the anti-inflammatory cytokine IL-10 in the blood correlate with the induction of regulatory T cells. Additionally, in vitro suppression assays revealed the capacity of both these malaria-induced T cell subsets to suppress CD4$^+$ and CD8$^+$ effector T cells. Despite the expression of the memory marker CD27 on a big fraction of these regulatory T cells, the numbers decline rapidly after withdrawal of antigen. Moreover, we could not observe a re-induction of these regulatory T cells during memory challenge.

The induction and suppressive capacity of PbA induced regulatory CD8$^+$ und CD4$^+$T cells was shown. Their capacity to suppress CD4$^+$ and CD8$^+$ T cell function indicates their role to resolve inflammation. Our findings give new insights in disease progression of severe malaria and suggest CD4$^+$ and CD8$^+$ regulatory T cells as a mode of treatment against immune pathology.

O35

***Salmonella* SiiE prevents an efficient humoral immune memory by interfering with IgG-secreting plasma cell persistence in the bone marrow**

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Question: *Salmonella enterica* serovar Typhimurium is an intracellular bacterial pathogen and kills 155,000 persons per year. The bacteria are repeatedly infected in animal and human and no vaccine against the bacteria is available yet. It remains unknown how *Salmonella* escapes from humoral immune memory and prevents efficient vaccination against itself.

Methods: Effects of *Salmonella* infection on humoral immune response and memory were analyzed by ELISpot assay, flow cytometry and confocal microscopy. To identify the responsible molecule, chromatography and mass spectrometry were performed.

Results: *Salmonella* specifically reduced the number of IgG-secreting plasma cells in the bone marrow (BM), which are the main source of whole IgG in the body, by secreting a *Salmonella*-specific protein SiiE. SiiE-deficient *Salmonella* induced high and lasting titers of protective *Salmonella*-specific IgG, and qualifies as a novel efficient vaccine against *Salmonella*. A SiiE-derived peptide with homology to laminin β1 binds to integrin β1 and ablates IgG-secreting plasma cells from the BM in competition with laminin β1, identifying laminin β1 as a novel and specific component of niches for IgG-secreting plasma cells in the BM.

Conclusion: *Salmonella enterica* serovar Typhimurium specifically reduces the main source of protective IgG and escapes from humoral immune memory and vaccination.

Tumor immunology and microenvironment II (O37-O42)

O37

Discovery of new chemotherapy-associated antigens induced by immunogenic cell death in NSCLC

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DOI: 10.1002/eji.201970300

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Introduction: Immunogenic cell death (ICD) has emerged as one of the main topics of therapy-induced antitumor immunity and constitutes an important pathway for the activation of the immune system against cancer. Infact, ICD of tumor cells induced by platinum based-chemotherapeutic drugs affects antigen presentation leading to an improved anti-tumor T-cell response.

Objectives: Here, we aim to i) identify new immunogenic antigens from non-small cell lung cancer (NSCLC)cells that result from chemotherapy-induced ICD, ii)assess the role of new antigens in survival and immune response improvement.

Material and methods: A primary NSCLC cell line was metabolically labeled for the Stable Isotope Labeling by Amino acids in Cell culture (SILAC).After, a labeled cell line was treated with cisplatin (CDDP) inducing apoptosis, maintaining the labeled counterpart in untreated condition. After a cell sorting approach, viable and late apoptotic fractions were submitted to LC-MS to identify the differentially expressed proteins.

Peripheral blood mononuclear cells (PBMCs) from 16 NSCLC patients in CDDP and CDDP+Nivolumab treatment were collected for immunologic validation. Thus, the immunogenicity was evaluated after *in vitro* stimulation of NSCLC patients-derived memory T cells with apoptotic epitopes, analyzing pro-inflammatory cytokines production in $CD4^+$and $CD8^+$T cells with multiparametric flow-cytometry analysis.

Results: Using a SILAC proteomic approach we characterized the proteome of apoptotic and viable cells with more than 800 identified proteins. Most importantly, 2% of total proteins were classified as up-regulated fragments in late apoptotic fraction compared to viable cells. After, we described these new ICD-derived fragments as consequence of caspase-dependent cleavage activity.

Immunologic validation of selected fragments showed an increased percentage in IFN-γ and TNF-α production in patients after CDDP and nivolumab treatment, compared to before treatment. Lastly, a major survival in Nivolumab-patients with higher IFN-γ and TNF-α production demonstrated an important role of chemotherapy-associated epitopes to predict the cancer progression.

Conclusion: These data confirm our strategy to unveil new ICD-associated epitopes. In addition, these antigens may be validated as new prognostic tumor biomarkers or used as a direct pharmaceutical target for cancer vaccines.

O38

Role of the atypical receptor CCRL2 in lung cancer immune surveillance

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Introduction: Lung cancer is the leading cause of cancer-related deaths worldwide and NSCLC subtype accounts for the approximately 85% of all lung cancers. Cancer-related inflammation is a key aspect of tumor growth and dissemination. The inflammatory process is sustained by the local recruitment of immune cells and the secretion of pro-inflammatory cytokines, including chemokines.

CCRL2 is a poorly characterized seven transmembrane domain receptor related to the family of the "Atypical Chemokine Receptors"(ACKRs). ACKRs are known to regulate inflammation by their ability to bind and scavenge their chemotactic ligands. Some members of this family were shown to regulate also cancer-related inflammation.

CCRL2 binds chemerin, a protein that promotes chemotaxis of selected leukocyte subsets including macrophages and NK cells. Moreover, CCRL2 controls the inflammatory response in different pathological settings.

Objectives: Investigate the role of CCRL2 in the regulation of cancer-related inflammation focusing on lung cancer.

DOI: 10.1002/eji.201970300

Materials and methods: To dissect the role of CCRL2 receptor in lung carcinogenesis, the genetic mouse model of *Kras*G12D/+;*p53*LoxP(TK) mice, the urethane chemically-induced model and the transplantable LG1233 cell line were used as experimental models of lung cancer with molecular and histopathological similarities with human *Kras*-driven lung carcinomas. Histological and FACS analysis were performed on lungs of tumor bearing mice.

Results: The genetic deletion of *Ccrl2* promoted tumor progression in urethane-induced and in *Kras*G12D/+/*p53*LoxP lung tumor models. Similarly, *Ccrl2* deficient mice showed enhanced growth of a *Kras* mutant lung tumor cell line. This phenotype was associated with a reduced inflammatory infiltrate characterized by the impaired recruitment of several leukocyte populations including NK cells. Bone marrow chimeras highlighted the role of CCRL2 expression by non-hematopoietic cells. In human and mouse lung, CCRL2 is expressed by a fraction of CD31+ endothelial cells, where it can control NK cell infiltration. Elevated CCRL2 expression in biopsies from human lung adenocarcinoma positively correlated with clinical outcome.

Conclusions: These results provide evidence for a crucial role of CCRL2 in shaping immune response against lung tumor growth.

O40

The soluble protein GPNMB/Osteoactivin produced by tumor-conditioned macrophages promotes cancer cell stemness and metastasis via production of IL-33

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Introduction: Myeloid cell in the tumor micro-environment support tumor cell survival and their invasive ability through a variety of mechanisms. We previously reported that tumor-conditioned macrophages co-cultured with cancer cells produce the soluble form of the Glycoprotein non-metastatic melanoma protein B (GPNMB), also named Osteoactivin, a highly glycosylated transmembrane protein that can be cleaved by proteases.

Question: In this study we addressed the question of the biological role of soluble GPNMB in the cross-talk between tumor-associated macrophages and cancer cells.

Materials & methods: To study the effect of this protein in mouse tumor models *in vivo*, we used DBA2J mice, which lack a functional Gpnmb gene due to a spontaneous mutation, and the reconstituted strain DBA2J/Gpnmb+ mice. We used primary methylcolantrene-induced sarcoma generated in DBA/2J mice (MCA-1 cells) and the syngeneic P815 mastocytoma, both deficient for GPNMB, and we transduced MCA-1 cells with Gpnmb cDNA (MCA-1-GPNMB)

Results: MCA-1 and P815 cells showed tumor incidence and metastatic ability when transplanted in DBA2J/Gpnmb+ mice, where the protein was expressed by macrophages in the tumor microenvironment. A single inhalation of macrophage-derived soluble GPNMB in mice significantly augmented tumor cell seeding in the lungs after i.v. injection of GPNMB-deficient tumor cells. GPNMB-transduced MCA-1 cells grew remarkably earlier and had higher metastatic capacity compared to Mock-cells. *In vitro*, MCA-GPNMB cells were able to survive in conditions of nutrient deprivation and spontaneously formed spheroids able to self-renew. Tumor spheres expressed typical cancer stem cell markers: CD199, CD117, Sca1, drug resistance and tumor-forming ability. The same phenotype was reproduced when MCA-mock cells were exposed to macrophage-derived GPNMB or to the recombinant protein. Tumor spheres strongly upregulated the cytokine IL-33 and its receptor IL-1RL1. Notably, recombinant IL-33 alone was able to induce sphere formation MCA-1-Mock cells.

Conclusion: Our results identify a new molecular axis whereby macrophages influenced by cancer cells release GPNMB, which in turn triggers in tumors the production of IL-33, ultimately promoting stem cell expansion and the acquisition of a metastatic phenotype in vivo.

DOI: 10.1002/eji.201970300

O41

Tumor immunoevasion by high glycolytic tumors through an acidosis-dependent induction of non-inflammatory tumor-associated macrophages

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Introduction: Aggressive types of skin cancer have become more common over the last 25 years. Immune checkpoint inhibitors have revolutionized melanoma treatment. However, only less than 40% of patients benefit from these immune checkpoint inhibitors since tumors evolve sophisticated strategies to evade the immune system. To develop new innovative therapeutic strategies, detailed understanding of tumor- and micro milieu-specific mechanisms contributing to inefficient anti-tumor immune responses is essential.

Objectives: We focused our work on identifying signaling pathways and molecules involved in melanoma formation and anti-tumor immune responses.

Material & Methods: By comparative database (TCGA atlas), histological, Seahorse and PET analyses of primary human and murine tumors we investigated the metabolism of different tumor entities. Moreover, using the preclinical B16 melanoma and MC38 colon adenocarcinoma mouse model and by transcriptome and proteome analyses we analyzed the phenotype of tumor-associated macrophages (TAM).

Results: Hereby, we could show that primary human melanomas in comparison to colon adenocarcinomas are transcriptionally privileged to produce energy by high-rate glycolysis resulting in tumor acidification. These results could be as well confirmed for B16 melanoma and MC38 colon adenocarcinoma mouse tumors. Using the B16 model we could further demonstrate that this tumor acidosis induced a G protein-coupled receptor- and cAMP-mediated expression of the transcriptional repressor ICER in TAM, leading to functional polarization of TAM towards a non-inflammatory M2 phenotype promoting tumor growth. In contrast to that, B16 melanoma-infiltrating macrophages of *Icer*-deficient mice possess an inflammatory anti-tumor M1 phenotype which results in a spontaneous rejection of high glycolytic tumors by *Icer*-deficient mice, as well as mice with a conditional deficiency of *Icer* in macrophages. To test the "drugability" of cAMP modulation in melanoma treatment we conducted *in vivo* experiments with therapeutic application of the Adenylate cyclase inhibitor MDL-12. Inhibition of *de novo* cAMP synthesis lead to a rejection of B16 melanomas in C57BL/6J mice.

Conclusion: Taken together, our findings identify an evolutionary conserved mechanism of physicochemical communication between non-lymphoid tissue and the immune system that is exploited by high glycolytic tumors for immune evasion.

O42

View of the tumor microenvironment through a different lens: *In silico* immune cell clusters

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Introduction. *In situ* information provides clues as to the spatial relationships and the role of cells in the tissue microenvironment. We observed melanoma positive cells in the spleen of the YUMMER1.7 mutagenized melanoma mouse model as early as 24 hours post-tumor cell implantation in the rear flanks of these mice (**Figure 1**). Simple 2-color histology revealed that there is a spontaneous immune response to melanoma tumor cells. However, we needed a method to characterize and highlight complex immune cell phenotypes and clusters in histology tissue *in situ*.

Objectives. To develop a method to phenotype, quantitate, and visualize cells and cell clusters *in silico* utilizing multiplexed fluorescence histology and the TissueFAXS imaging system with a conventional microscopy setup.

DOI: 10.1002/eji.201970300

Materials & Methods. Spleens from melanoma mice were formalin-fixed and paraffin-embedded. CD4 Cy5, biomarkers of CD4 activity (IL-6 Cy3, Granzyme B Cy5, Ki-67 Cy5, T-bet Cy5, RORγ(t) Cy3, Foxp3 Cy3), and DAPI nuclear marker were combined in multiple staining rounds on the same microscope slide. TissueFAXS (TissueGnostics, Vienna, Austria) was used for image acquisition. Images were acquired in multiple sequential tiles at 20× magnification for analysis. Image processing and analysis was performed using StrataQuest software (TissueGnostics) and compared with manual ground truth (Fiji software). An image stitching algorithm was used on the tiled images to reconstruct the whole image. Algorithms were created to align the stitched images, generate a composite image containing all biomarkers from all staining rounds, isolate each cell in the composite image, and identify the positive cells in the composite image. Tissue cytometry coupled with backgating into the tissue images was used to visualize, quantitate, and validate the *in silico* data.

Results. Through *in silico* analysis, we found three clusters of CD4 expressing cell populations: intermediate, high, and mixed. Each cell cluster was composed of different combinations of the biomarkers of CD4 activity (e.g. **Figure 2**). The biomarkers had a precision range of 0.44–0.88, recall/sensitivity range of 0.85–0.99, specificity range of 0.89–0.99, and a F1 score range of 0.60–0.89.

Conclusion. Overall, we found that *in silico* analysis coupled with flow cytometry-like abilities such as gating, backgating, and histogram/dot scatterplot outputs were powerful tools for phenotyping cells and uncovering distinct cell clusters.

Microbiome and mucosal immunity (O44-O48)

O44
Microbial-derived metabolites drive protective type-I interferon responses in models of gut epithelial damage and limit graft-versus-host disease

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Background: Graft-versus-host disease (GVHD) is a dreaded complication after stem-cell transplantation (SCT). Standard treatment relies on immunosuppressants but is associated with an increased risk of infection and relapse. Up 50% of patients develop steroid-refractory GVHD, with dismal impact on SCT outcomes.

Recently, we reported that induction of type-I interferon (IFN-I) signalling or activation of IFN-I inducing pathways such as cGAS/STING or RIGI/MAVS can promote gut barrier integrity and limit GVHD. However, the endogenous ligands that drive this "protective" IFN-I response are still poorly defined. New data in mice and humans suggest that microbial-derived metabolites such as small-chain fatty acids or indoles can decrease GHVD mortality. Here, we describe a IFN-I inducing metabolite that improves outcomes in mouse models of gut epithelial damage and acute GVHD.

Methods: To investigate which cell types mediate protection and how, we generated intestinal organoids and bone marrow-derived antigen presenting cells (APC) of WT or genetically deficient mice ($STING^{-/-}$, $IFN\alpha R^{-/-}$) under steady state conditions versus chemotherapy, total body irradiation and after allogeneic SCT in the presence or absence of bacterial metabolites. Analysis was performed by microscopy, immunoblotting, qPCR, ELISA and flow cytometry. Outcomes of gut injured mice were assessed by clinical scoring, flow cytometry and histopathology.

Results: Metabolite treatment promoted regeneration of intestinal organoids as assayed by organoid numbers as well as proliferation. These effects were dependent on IFN-I and STING signalling. In addition, we found activated pro-inflammatory NFκB signalling and decreased apoptosis as evidenced by reduced caspase-3 cleavage. In APCs, IFN-I responses were enhanced in the presence of metabolites including increased IFN-β production and upregulation of

DOI: 10.1002/eji.201970300

IFN stimulated genes. Metabolite-treated mice showed improved recovery of intestinal stem cells following gut injury and increased survival in acute GVHD.

Conclusions: Our findings uncover a mechanism by which microbial metabolites amplify IFN-I signals, limit gut damage and thereby prevent allo-activation and GVHD. Perhaps the poor prognosis of GVHD patients exhibiting a loss of microbiota diversity can be explained in part by an absence of "protective" metabolites able to amplify IFN signalling. We are currently studying whether metabolite levels correlate with severity and outcome of GVHD in humans.

O45

Long-lasting consequences of neonatal infections on the intestinal immune system

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The neonatal window of opportunity was first revealed by epidemiological studies highlighting correlations between postnatal environmental exposures and susceptibility to diverse diseases. Our own studies also had identified the first ten days after birth as a critical neonatal window of opportunity for the stromal cells of gut-draining mesenteric lymph nodes (mLN) to be stably imprinted by microbiota with the capacity to foster an efficient *de novo* induction of regulatory T cells (Treg), which are crucial for the establishment of intestinal tolerance.

Objective: We aimed to study the long-lasting consequences of transient gastrointestinal infections during the neonatal period on the intestinal immune system.

Methods: Neonatal BALB/c mice were infected intragastrically with enteropathogenic *Escherichia coli* (EPEC) and *Yersinia pseudotuberculosis* (*cnfY* knock-out strain YP147). Different time points post infection (p.i.), the intestinal immune cell composition was analyzed by flow cytometry and Treg-inducing properties of the gut-draining LNs were studied by adoptive transfer of transgenic $CD4^+$ T cells followed by systemic T cell priming. LNs from previously infected mice were transplanted to dissect the impact on the LN stromal cell compartment. Low-input RNAseq and 16S rDNA analyses were performed to unravel global changes on transcriptomes of stromal cell and microbiome, respectively.

Results: Twelve weeks p.i. with EPEC or YP147 a clear shift in the Treg/Th17 balance favoring RORgt-expressing Th17 cells accompanied by elevated frequencies of ILC3s could be observed in the colon, suggesting a long-lasting impact of the neonatal infections on the innate and adaptive mucosal immune system. In addition, the *de novo* Treg induction capacity was substantially impaired in liver-draining celiac lymph nodes (celLN), while surprisingly mLN was not negatively affected by the neonatal infections. These findings were supported by LN transplantation experiments, which demonstrated a strong, long-lasting negative effect of neonatal infections on the tolerogenic properties of celLN stromal cells. Low-input RNAseq allowed the identification of up/down-regulated genes in celLN fibroblastic stromal cells as a consequence of the neonatal infections, and neonatally infected mice also showed an altered microbiota composition lasting until adulthood.

Conclusion: Together, our data suggest that transient gastrointestinal infections during the neonatal window can have long-lasting consequences that result in a disturbed microbiota composition, an altered intestinal immune cell homeostasis and impaired functional properties of the liver-draining LN, finally affecting the *de novo* induction of Tregs as key players of peripheral tolerance.

O46

Epigenomic landscape defines immuno-modulatory properties across lymph node stromal cell subsets

DOI: 10.1002/eji.201970300

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Gut-draining mesenteric lymph nodes (mLN) provide the infrastructure and microenvironment to tissue-specifically shape adaptive immune responses. We previously delineated transcriptional signatures in mLN fibroblastic stromal cells (FSC) that lastingly modulate the dendritic cell compartment to promote peripheral tolerance by fostering their capacity to *de novo* induce Foxp3+ regulatory T cells.

Here, we dissect the tissue-specific epigenetic DNA accessibility and CpG methylation landscape of LN FSC from both colonized and germ-free mice, and identify a microbiota-independent core epigenetic signature of mLN stromal cells.

By aligning transcription factor (TF) binding sites with the transcriptional profiles of FSCs, we delineated TFs poising skin-draining peripheral LN (pLN) stromal cells for pro-inflammatory responses and determine TFs that regulate the expression of dormant anti-inflammatory accessible genes in mLN stromal cells. Furthermore, using scRNA-seq we dissected the developmental trajectory of mLN stromal cells derived from postnatal to aged mice, identifying two distinct progenitors, namely fibroblastic adventitial and reticular stromal cell progenitors, which both feed the FSC compartment rapidly expanding early after birth. Finally, we excerpted key regulatory TFs derived from the epigenetic signature to the respective progenitors along development and delineated TF networks required to shape the epigenomes of FSCs within the developing mLN.

Our data constitute a comprehensive map of mLN development and describe the epigenetic landscape of location-specific, microbiota-independent properties of mLN stromal cells, providing a valuable resource to delineate environmental stimuli that impinge on the developing mLN early in life, thereby long-lastingly shaping adaptive immune responses.

O48

Changes in pH can modify the ability of a simplified human intestinal microbiota to stimulate MAIT cells

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Introduction: With this study we aim to determine if environmental changes alter the intestinal microbiota and hence affect their capacity for stimulating MAIT cells.

Materials & Methods: Previously, we established the simplified human intestinal microbiota (SIHUMIx) community as an *in vitro* model for bioreactor systems. We simulated the human colonic environment and cultivated the SIHUMIx community for 14 days. Since it has been reported that some patients with active ulcerative colitis show a low colonic pH, we perturbed community growth by a pH drop from 6.5 to 5.5 on day 5 to mimic these environmental conditions and with the aim to introduce a severe perturbation. Microbial community structure was analyzed by flow cytometric fingerprinting and metaproteomics, community metabolism was evaluated by short chain fatty acid (SCFA) analysis and riboflavin (vitamin B2) measurement. Harvested bacteria from the bioreactor system were used for *in vitro* MAIT cell stimulation assays.

Results: We observed that the pH drop altered the microbial community structure and metabolism. The acidification of the environment promoted better growth conditions to the acidophilic bacterium *L. plantarum* and the aciduric bacteria *C. butyricum* and *B. longum,* even though the community composition did not change dramatically. Due to the environmental changes the bacteria produced less riboflavin and lost their ability to stimulate MAIT cells: the numbers of CD69+/TNF+ MAIT cells dropped dramatically when stimulated with bacteria from day 5 but increased after the bacteria were cultivated at colonic pH again. **Conclusion**: Our results show that changes in the microbial environment can alter the intestinal community and hence affect MAIT cell stimulation downstream.

DOI: 10.1002/eji.201970300

Antigen presentation and vaccination (O49-O54)

O49
Sensing of phosphorylated isoprenoid metabolites (Phosphoantigens; PAg) by human γδ T cells: Lessons from the alpaca

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All jawed vertebrates possess Ig, γδ-, and αβTCR genes. The major subset of human blood gd T cells uses Vγ9 and Vδ2 TCR genes. These Vg9Vd2 T cells kill tumor cells and expand massively in infections like malaria. This response is initiated by TCR-mediated sensing of phosphorylated isoprenoid metabolites (phosphoantigens: PAgs) accumulated in cells. These PAgs bind to an intracellular domain of the B7-like molecule butyrophilin 3A1 (BTN3A1) which induces changes at the cell surface recognized by the Vγ9Vδ2 TCR. These effects are strongly enhanced by cooperation of the PAg-binding BTN3A1 with the PAg-insensitive isoforms BTN3A2 and BTN3A3. Here, we demonstrate trans-molecular interactions of protein domains by co-expressing modified BTN3 isoforms in BTN3-deficient cells. A deletion of the extracellular IgV-like domain of BTN3A1 abolishes cell surface expression and stimulatory potential. Nevertheless, both features are rescued by co-expression with BTN3A2 or BTN3A3 suggesting a cooperation of protein domains in BTN3 heteromers. A BTN3 molecule uniting all features required for efficient PAg sensing is expressed by the camelid species alpaca (*Vicugna pacos*), which we present as the first non-primate species possessing PAg-sensing cells. Newly developed monoclonal antibodies against alpaca Vδ2 and BTN3 allow the demonstration of expansion of Vδ2-positive T cells upon stimulation with the PAg HMBPP, which is abolished by the BTN3-specific mAb. Murine transductants expressing alpaca Vγ9Vδ2 TCR show a remarkable PAg-induced reactivity to alpaca as well as to human BTN3-expressing cells. Furthermore, analysis of BTN3 chimeras demonstrates that chimeras expressing the human BTN3A1 extracellular domain and the alpaca BTN3 transmembrane/intracellular domain mediate PAg-induced activation of Vγ9Vδ2 TCR transductants even more efficiently than natural human BTN3A heteromers. These findings will be used to identify the minimal structural requirements for efficient PAg-sensing by the γδTCR.

O51
Helicobacter pylori dampens MHC-II expression on macrophages *via* the up-regulation of miRNAs targeting CIITA

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Macrophages have a major influence on inflammation, pathology and disease and the available data suggest that *H. pylori*persistence can be explained in part by the failure of the bacterium to be killed by professional phagocytes. Macrophages are cells ready to kill the engulfed pathogen, through oxygen-dependent and -independent mechanisms; however, their killing potential can be further augmented by the intervention of T helper (Th) cells upon the specific recognition of MHC-II-peptide complexes on the surface of the phagocytic cells. As it pertains to *H. pylori*, the bacterium is engulfed by macrophages, but it interferes with the phagosome maturation process leading to phagosomes with an altered degradative capacity, and to megasomes, wherein H. pylori resists killing. We recently shown that macrophages infected with H. pylori strongly reduce the exposure of MHC-II molecules on the plasma membrane and this compromises the bacterial antigen presentation to Th lymphocytes. In this work, we demonstrate that *H. pylori*hampers MHC-II expression in macrophages, activated or non-activated by IFN-gby down-regulating the expression of the class II major histocompatibility complex transactivator (CIITA), the "master control factor" for the expression of MHC class II genes. We evidenced that this effect relies on the up-regulation of Let-7f, let-7i, miR-146b and -185 targeting CIITA and that the high expression of let-7i, miR-146b and -185 is associated with the early neoplastic conditions and gastric cancer in H. pylori-infected patients.

O52
Antigen-specific CD8 T cells in cell cycle circulate in the blood after vaccination

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DOI: 10.1002/eji.201970300

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Introduction: Although clonal expansion is a hallmark of adaptive immunity, the location(s) where antigen-responding T cells enter cell cycle and complete it have been poorly explored. This lack of knowledge stems partially from the limited experimental approaches available. Clonal expansion of antigen-responding CD8 T cells has been assessed by a few methods, including dye-labelling of proliferating cells, and staining for the intranuclear protein Ki67. To date, available dyes that label cells proliferating over time (e.g. CFSE; BrdU) do not assess whether the labeled cells found in a particular location proliferated locally or rather migrated into that organ after dividing elsewhere. Furthermore, though Ki67 is generally considered to label dividing cells, it actually labels all cells not in G0. Thus, it does not distinguish actively cycling cells committed to mitosis (those in S-G2/M) from those in G1, which may quickly proceed into S, or stay in a prolonged G1, or even revert to G0 without dividing.

Objectives: Our aim was to exploit a combination of Ki67 plus DNA staining and flow cytometric analysis to track antigen-specific CD8 T cells in different phases of cell cycle in spleen, lymph nodes (LNs) and blood of vaccinated BALB/c mice.

Methods: BALB/c mice were vaccinated intramuscularly with antigen-expressing viral vectors. Rare naïve CD8 T cells clonally expanded, and we examined the resulting polyclonal population by Ki67/ DNA content analysis. This assay distinguishes cells in active proliferation (i.e. in S-G2/M phases of cell cycle) from those in G1 and in G0. We observed that most proliferating cells had high scatter profile and were undetected by current criteria to evaluate flow cytometry data. We thus developed a novel strategy for data analysis.

Results: We set up a new highly sensitive method that turned out to be a breakthrough in antigen-specific CD8 T cell analysis. For example, we found that current criteria underestimated up to 6-times antigen-specific cell frequency in LNs at early times of response. By using our method, we found a significant number of antigen-responding CD8 T cells actively cycling in LNs, spleen and —surprisingly— in the blood, which is an unexpected site for cycling of normal non-leukemic cells.

Conclusion: Taken together, our results challenge the current flow cytometry guidelines for CD8 T cells at early times of response and open new directions for investigation and intervention in the T cell field.

O53

Immunogenic properties of single component adjuvants for vaccine formulations

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Introduction: An appropriate adjuvant is of pivotal importance for vaccine efficacy. Hence, adjuvants should be selected on the basis of their immunogenic modes of action (MOA) since they are the key inducer of a directed immune response against the antigen. However, several adjuvants' immunogenic MOA remain still elusive, making the arising immune response difficult to predict.

Objectives: In this study we aim to link specific innate and adaptive immunogenic MOA to the adjuvant's properties to facilitate the rational design of adjuvant-based vaccines.

Materials & methods: We investigate the immunogenic MOA of ten different single component adjuvants *in vitro*, comprising TLR agonists, aluminium salt, an oil-in-water emulsion and saponin. We focus on side-by-side comparison of the adjuvants using a human primary cell-based assay composed of monocyte-derived dendritic cells (DCs) and autologous peripheral blood lymphocytes (PBLs).

Results: By investigating maturation markers and endocytosis capacity by flow cytometry, we observed that Alum, Poly I:C, Addavax and Quil-A do not maturate DCs, whereas the other adjuvants tested induce a strong DC maturation. To assess the effect on the adaptive immune response, the ability of the adjuvants to induce PBL proliferation within the co-culture was examined. We found that Pam3CSK4, Gardiquimod, R848, synthetic MPLA,

DOI: 10.1002/eji.201970300

natural-derived MPLA and TDB induced antigen-independent proliferation of PBLs to varying degrees, with R848 being the strongest stimulator. More detailed examination of B-, NK-, NKT-, $CD4^+$ and $CD8^+$ T cells within the proliferated PBL population revealed that each adjuvant promoted the proliferation of different cell types. In antigen-specific T cell experiments we observed that the adjuvants' effect on the expansion of FluM1-specific $CD8^+$ T cells is highly donor variable. However, Alum and synthetic MPLAs significantly increased the proportion of FluM1-specific $CD8^+$ T cells significantly by 1.7- and 1.9-fold respectively. These expanded cells are still polyfunctional by secreting IFNγ, TNFα and IL-2 upon restimulation with the FluM1 peptide.

Conclusion: We found that the tested adjuvants induce different immunogenic profiles. This work may contribute to improve the selection of candidate adjuvants for a certain indication.

O54

Antigen targeting of Fc receptors induces strong and functional relevant T cell responses *in vivo* independent of ITAM signaling but dependent on dendritic cell subsets

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Dendritic cells (DCs) are important antigen presenting cells (APCs) and induce immune responses, but also preserve peripheral tolerance. We showed the preferential induction of either CD4+ or CD8+ T cell responses by DC subpopulations *in vivo* by targeting antigens to endocytic C-type lectin receptors. The also highly endocytic active Fc receptors (FcRs) enable APCs to take up antigens in form of immune complexes. As they are expressed on various APCs, we aimed to identify responsible APCs for primary and secondary immune responses by using our antigen delivery by recombinant antibodies to activating and inhibitory FcRs. This targeting induced CD4+ and CD8+ T cell responses independent the receptor's type. Moreover and in contrast to DEC205 and DCIR2 targeting, especially antigen delivery to FcγRIV was superior in inducing simultaneously CD4+ and CD8+ T cell responses., not only in a transgenic setting, but also in naïve mice. As FcγRIV is expressed on both splenic cDC subsets, we used it to verify the subset intrinsic preferences to trigger either CD4+ or CD8+ T cell responses. Thereby we could clearly show the induction of CD4+ T cell responses by splenic CD8- DCs, whereas the CD8+ DCs induced CD8+ T cell responses.

The naïve CD8+ T cell responses were of functional relevance, as we demonstrated the effective dose-dependent killing of peptide loaded target cells *in vivo*. Therefore, we suggest antigen targeting to FcRs as useful tool to induce *de novo* as well as the modulation of immune responses for future therapeutic applications. Additionally, we could demonstrate the responses to be effective in a murine melanoma model (in a preventive as well as a therapeutic setting). We now further investigate, which mechanisms play a role after antigen targeting to CD11b+CD8- DCs, which adjuvant is most promising, and if the concomitant induction of a CD4+ T cell response is beneficial to the anti-tumor CD8+ T cell response in our system.

This project was partly funded by the DFG (RTG1660, RTG1962, CRC1181-TPA7, CRC1054-TPA6, and SO1149/1-1), BayGENE, and the Emerging Fields Initiative (BIG-THERA). This work was further supported by intramural funding (IZKF-J54, IZKF-A65, and IZKF-A68).

DOI: 10.1002/eji.201970300

Cytotoxic T cells (O55-O60)

O55
Activated CD8+T cells extensively bind extracellular vesicles during acute viral infections

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The *in vivo*detection of dead cells remains a major challenge. Besides technical hurdles also efficient removal of dead cells by phagocytes hampers a reliable quantification of apoptotic cells. Here we present a novel method, where administration of fluorescent Mfge8 (Mfge8-eGFP) *in vivo*combined with imaging flow cytometry and deep learning allows the identification of dead cells based on their exposure of phosphatidylserine (PS). During acute viral infection, apoptosis increased mainly among central memory T cells and B cells. Unexpectedly, these analyses revealed that most PS^+cells were not apoptotic, but rather live cells associated with PS^+particles, which were identified as extracellular vesicles (EVs) strongly associating with activated $CD8^+$T cells in infected mice. EVs bound by these cells carried markers of exosomes and antigen-presenting cells. Here we present the first *in vivo*characterization of naturally occurring EVs and their target T cells, allowing to study immunomodulatory effects of EVs during infections, autoimmunity and cancer.

O57
Dynamic changes in 3D spatial chromatin organization underpin virus specific CD8+ T cell differentiation

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Infection triggers large-scale changes in the phenotype and function of virus-specific CD8+ T cells ensuring that they acquire the necessary lineage specific functions critical for immune clearance of the pathogen. Whilst the molecular mechanisms that control these changes are becoming apparent, how they combine and contribute to regulate CD8+ T cell differentiation is still unclear. Genome wide mapping of chromatin interactions (HiC), histone PTMs (ChIP-seq) and chromatin accessibility (ATAC-seq) combined with high resolution confocal microscopy (dSTORM) demonstrate that maturation of higher order chromatin structures occurs upon differentiation of CD8+ T cells from an immature to mature state. Interestingly, the chromatin structure within naïve CD8+ T cells appear to be pre-configured in a lineage-specific way, both at the level of histone PTMs and higher order chromatin contacts. This genomic pre-configuration is then associated with targeted epigenetic maturation of lineage-specific genomic elements upon T cell activation, thus implying that the outcome of CD8+ T cell differentiation is largely pre-determined. These data have implications better understanding of the molecular events, and their regulation, that occur during the generation of effective T cell responses and establishment of immunological memory.

O58
CEACAM1 promotes $CD8^+$ T cell responses and improves control of a chronic viral infection

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Dysfunction of $CD8^+$ T cells can lead to the development of chronic viral infection. Identifying mechanisms responsible for such T cell dysfunction is therefore of great importance to understand how to prevent persistent viral infection. Here we show using lymphocytic choriomeningitis virus (LCMV) infection that carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1) is fundamental for recruiting lymphocyte-specific protein kinase (Lck) into the T cell receptor complex to form an efficient immunological synapse. CEACAM1 is essential for activation of $CD8^+$ T cells, and the absence of CEACAM1 on virus-specific $CD8^+$ T cells limits the antiviral $CD8^+$ T cell response. Treatment with anti-CEACAM1 antibody stabilizes Lck in the immunological synapse, prevents $CD8^+$ T cell exhaustion, and improves control of virus infection in vivo. Treatment of human virus-specific $CD8^+$ T cells with anti-CEACAM1 antibody similarly enhances their proliferation. We conclude that CEACAM1 is an important regulator of virus-specific $CD8^+$ T cell functions in mice and humans and represents a promising therapeutic target for modulating $CD8^+$ T cells.

DOI: 10.1002/eji.201970300

O59

Development of virus-specific memory CD8 T cells during persistent liver infection results in functional adaptation

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Introduction: Tissue-resident CD8 memory T cells (T_{RM}) are described to populate parenchymal organs and to be essential for an effective immune response against pathogen re-challenge. However, the role of T_{RM} cells in viral infections of the liver, like chronic infection with Hepatitis B Virus (HBV), is largely unknown.

Objectives: We aimed at monitoring and characterizing the formation of liver-resident T cell populations upon acute-resolving or chronic viral infection of the liver for OVA-based infection models as well as for chronic Adeno-HBV infection in mice.

Materials & methods: C57BL/6 mice were infected with liver-targeting adenoviral vectors encoding ovalbumine under the CMV-, or the hepatocyte-restricted TTR-promoter to achieve acute-resolving or chronic viral infection. At different time points, virus-specific T cells from spleen and liver were analysed with respect to phenotypic and functional parameters. Similarly, mice were infected with a chronic dose of Adeno-HBV after having received naïve HBVcore-antigen-specific CD8 T cells and analysed accordingly.

Results: Upon resolved infection, remaining virus-specific CD8 T cells in the liver subdivided into a CX_3CR1^+ effector-memory and a $CXCR6^+$ $CD69^+$ liver-resident memory population that could be clearly distinguished by phenotypic and functional analysis. CX_3CR1^+ cells were also found in the spleen. In contrast, during chronic liver infection, virus-specific T cells were expanded and maintained exclusively in the liver. These cells exhibited a $CXCR6^+$ $CD69^+$ T_{RM}-like phenotype along with a co-existing functional adaptation characterized by low Granzyme B levels, inability to secrete cytokines and high expression of inhibitory receptors. First results for persistent Adeno-HBV infection confirmed this establishment of a virus-specific CD8 T cell population in the liver that shares T_{RM}-characteristics, but exhibits functional impairment.

Conclusion: Our results suggest that the development of $CXCR6^+$ liver-resident T cells occurs not only in the course of resolving, but also during chronic viral infection of the liver. In a chronic setting, however, $CXCR6^+$ cells do not represent a functional T_{RM} population, but exhibit attenuated effector functions that presumably reflect adaptation to the persistent viral infection. On-going work is dedicated to define the mechanisms of T_{RM} development during chronic infection and to find ways for reverting T cell attenuation through adequate stimulation.

O60

Interleukin-1β activates essential checkpoints in the formation of lung-resident memory T cell

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Introduction: Tissue-resident memory T cells (TRM) are specialized front line immune cells with superb effector functions. They are essential in the protection against localized infections at barrier tissues like lung or skin, but their unique features are also critical in anti-tumor immunity. However, specific approaches to establish TRM are scarce, partly due to incomplete understanding of the mechanistic framework. In previous studies, we developed a highly effective adenoviral vaccination strategy against Influenza A viruses (IAV), which specifically enhanced lung TRM responses by the inclusion of vector-encoded IL-1β in the vaccine.

Objective: Here, we elaborate the mechanistic relationship between IL-1β and TRM induction.

Materials and methods: Mice were immunized intranasally with recombinant Adenoviruses (Ad) encoding IAV antigens in combination with Ad-IL-1β or Ad-empty. The vaccine-induced inflammation and immunogenicity was

DOI: 10.1002/eji.201970300

analyzed in depth. Immunization of bone marrow chimeras with IL-1R deficiency in stromal or hematopoietic cells allowed the determination of IL-1β-responsive cell types.

Results: IL-1β activates several essential checkpoints in the formation of lung TRM. The adjuvant induces a broad lung inflammation characterized by the production of cytokines, chemokines, and adhesion molecules. At the same time, an immediate infiltration of immune cells into the lung is observed including TRM-priming CD103+ DCs. Infiltration of CD8+ and CD4+ T cells with characteristic expression of TRM markers like CD69, CD103 and CD11a is detectable as early as day two post immunization. However, TRM induction is completely dependent on the availability of local antigen. Bone marrow chimeras showed that optimal TRM-development requires IL-1R signaling in both stromal and hematopoietic cells but with stronger contribution of the latter ones. In particular, CD4+ T cell attraction into the lung and their activation is directly induced by IL-1R signaling, while formation of CD8+ TRM seems to be influenced only indirectly by IL-1β.

Conclusion: Our data reveal the multifaceted effects of IL-1β on the induction of TRM and contribute to the basic understanding of localized T cell responses. These insights will help to define requirements for vaccine-induced inflammation to induce local immunity.

Autoimmunity and chronic inflammation I (O61-O66)

O61
Cd101 is a type 1 diabetes susceptibility gene in NOD mice

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Introduction: Type 1 diabetes (T1D) is a multi-factorial disorder characterized by an immune-mediated destruction of the insulin-producing pancreatic beta cells. Variations at a large number of genes influence also the susceptibility to T1D in non-obese diabetic (NOD) mice, one of the most frequently studied animal models for human disease.

Objectives: The genetic analysis of NOD mice allowed the identification of many insulin-dependent diabetes (*Idd*) loci and candidate genes, one of them being *Cd101*. Thus, we elucidated the mechanism(s) by which CD101 mediates protection from T1D using congenic NOD.B6 *Idd10* mice in which the susceptible NOD region had been replaced by T1D-resistant B6 genes including *Cd101*.

Materials & Methods: NOD.B6 *Idd10* and NOD *scid* mice were intercrossed to develop the NOD.B6 *Idd10 scid* strain. B6 CD101−/− mice were backcrossed onto the NOD background to develop the CD101−/− NOD.B6 *Idd10* strain. T1D was assessed by the evaluation of urinary glucose. Single-cell suspensions were prepared and evaluated for the distribution of CD101 expression by flow cytometry. Spleen cells enriched in TCRb-positive T cells from donor mice were transferred intraperitoneally into NOD *scid* or NOD.B6 *Idd10 scid* recipients

Results: The genotype-dependent expression of CD101 correlates with a decreased susceptibility to T1D in NOD.B6 *Idd10* congenic mice compared to parental NOD controls. The knockout of CD101 within the introgressed B6-derived *Idd10* region increased T1D frequency to that of the parental NOD strain. The loss of protection from T1D was paralleled by decreased Gr1-expressing myeloid cells and FoxP3+ regulatory T cells and an enhanced accumulation of CD4-positive over CD8-positive T lymphocytes. As compared to CD101+/+ NOD.B6 *Idd10* donors, adoptive T cell transfers from CD101−/− NOD.B6 *Idd10* mice increased T1D frequency in NOD *scid* and NOD.B6 *Idd10 scid* recipients. Increased T1D frequency correlated with a more rapid expansion of the transferred CD101−/− T cells and a lower proportion of recipient Gr1-expressing myeloid cells. Fewer of the Gr1+ cells in the recipients receiving CD101−/− T cells expressed also CD101.

DOI: 10.1002/eji.201970300

Conclusion: Thus, our results connect the *Cd101* haplotype-dependent protection from T1D to an anti-diabetogenic function of CD101-expressing Tregs and Gr1-positive myeloid cells and confirm the identity of *Cd101* as *Idd10*. The frequency of polymorphisms in the *Cd101* gene in T1D patients is subject of current investigations.

O63

The HLA-C*06:02-restricted autoimmune response against melanocytes in psoriasis is differentially controlled by ERAP1 variants

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Introduction By the analysis of a pathogenic psoriatic Vα3S1/Vβ13S1 TCR we have previously shown that in psoriasis vulgaris, HLA-C*06:02 mediates an autoimmune response against melanocytes through presentation of an autoantigenic peptide from ADAMTS-like protein 5 (ADAMTSL5) to CD8+ T cells as the underlying pathogenetic mechanism. The genetic risk for psoriasis involves epistasis between the main psoriasis risk allele, HLA-C*06:02, and variants of the gene encoding endoplasmic reticulum aminopeptidase I (ERAP1). ERAP1 trims peptides to the appropriate size for binding to HLA-class I molecules.

Objectives To investigate the functional impact of the gene-gene interaction between ERAP1 and HLA-C*06:02 on the psoriatic autoimmune response against melanocytes and ADAMTSL5.

Materials & methods Our experiments were based on a TCR activation assay that integrated the five key components of the psoriatic autoimmune response - HLA-C*06:02, melanocytes as autoimmune target cells, the psoriatic autoantigen ADAMTSL5, the melanocyte-directed ADAMTSL5-specific, HLA-C*06:02-restricted Vα3S1/Vβ13S1 TCR, and different ERAP1 variants. ERAP1-/- clones (MCC) were generated by CRISPR targeted genome editing from two HLA-C*06:02-positive melanoma cell lines (MCL), WM278 and WM793, which can replace normal human melanocytes for analyzing the melanocyte-specific autoreactivity.

Results In psoriasis skin lesions, the expression of both, ERAP1 and HLA-C were strongly increased on melanocytes as compared to normal skin. ERAP1 knockout significantly reduced the expression of HLA-C and the antigenicity of MCCs for the pathogenic psoriatic Vα3S1/Vβ13S1-TCR. Reconstitution with an ERAP1 psoriasis risk haplotype restored HLA-C expression and the antigenicity of ERAP1-/- MCCs to a significantly greater extent than a ERAP1 haplotype protective for psoriasis. In a similar way, the psoriasis risk haplotype of ERAP1 generated the autoantigenic HLA-C*06:02-presented ADAMTSL5 epitope from NH2-extended precursors to a much higher degree than the protective ERAP1 variant.

Conclusion The joint activities of HLA-C*06:02 and ERAP1 haplotypes on the autoimmune potential of melanocytes and the psoriatic autoantigen, ADAMTSL5 may represent the principle mechanism by which ERAP1 variants modulate the risk of HLA-class I-associated immune-mediated diseases.

O64

Ablation of beta2 Integrins (CD18/CD11) in Foxp3 expressing T regulatory lymphocytes results in generalized inflammation accompanied by psoriasis-like skin lesions.

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Beta2 integrins are heterodimers consisting of one of four variable alpha subunits (CD11a-CD11d), paired with a constitutive CD18 beta subunit. In contrast to all other integrins, the beta2-integrin family is exclusively expressed on leukocytes. These integrins are essential for intercellular interactions, transendothelial migration toward inflamed tissues and uptake of opsonized pathogens. Moreover, cytosolic tails of beta2 integrins, which may undergo phosphorylation, convey inside-out and outside-in signaling involved in cytoskeletal rearrangements, intercellular communication and leukocyte activation. Complete ablation of the gene locus *Itgb2*, coding for CD18, results in an immuno-compromised state, termed leukocyte-adhesion deficiency type 1 (LAD1). LAD1 patients suffer from recurrent infections and are at higher risk of developing autoimmune diseases. So far only mice with a complete CD18

DOI: 10.1002/eji.201970300

deficiency or CD18 hypomorphic mice (expressing CD18 at a low level) were available to study the relevance of beta2 integrins for leukocyte function. We have generated mice that allow for the tissue-specific deletion of *Itgb2 locus*, enabling us to disentangle the importance of beta2 integrins for specific leukocyte populations.To investigate the relevance of β2-integrins for CD4+Foxp3+regulatory T (Treg) cell function, we generated mice lacking CD18 in FoxP3-expressing Treg. Interestingly, even though CD18ΔFoxp3 mice display unaltered frequencies and organ distribution of Treg cells, they spontaneously develop psoriasiform skin inflammation and present with a systemic immune activation involving splenomegaly as well as lymphadenopathy. Ongoing experiments are focused to delineate the involvement of beta2 integrins in Treg cell differentiation, migration and function. Moreover, we aim to identify the molecular mechanisms that account for a CD18-dependent switch between a suppressive and a T helper cell (TH)17-biased Treg response.

O66

T helper lymphocytes of chronic inflammation are maintained by fatty acid oxidation and adapt to it through Twist1

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Inflamed tissue is characterized by low availability of oxygen and nutrients. Yet, CD4+ T helper lymphocytes persist over time in such tissues ans probably contribute to the chronicity of inflammation. Here we show that these cells undergo specific metabolic adaptations to the inflamed environment. We have analyzed the dependency on particular metabolic pathways of ex vivo isolated synovial and blood CD4+ memory/effector T cells of patients with juvenile idiopathic arthritis and of once and repeatedly stimulated CD4+ Th1 cells using pharmacological inhibitors. We have also investigated the role of the transcription factor Twist1 in regulating the metabolism of the CD4+ T cells. We could show originally that PD1+ Th cells of JIA synovia survive on fatty acid oxidation, and express the E-box binding transcription factor *TWIST1*. Repeatedly restimulated murine Th cells, expressing *Twist1* as well, require *Twist1* to survive on fatty acid oxidation. In addition, *Twist1* protects the cells against reactive oxygen species. Our studies demonstrate that *TWIST1* is a master regulator of metabolic adaptation of Th cells to chronic inflammation and a target for their selective therapeutic elimination.

Dendritic and myeloid cells I (O67-O72)

O67

Ontogenetic diversity of dendritic cells in early life

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Introduction: Early life immune balance is essential for survival and establishment of healthy immunity in later life. Dendritic cells (DCs) are versatile controllers of immunity that in neonates are qualitatively distinct from adults. Why such age-dependent differences exist is unclear but newborn DCs are considered underdeveloped and functionally immature.

Objectives: We aim to understand how DCs develop in different tissues of mice in early life. Additionally, we investigate whether ontogenetic aspects contribute to the functional differences between DCs in early and adult life. We further want to define the cell extrinsic and intrinsic signals that contribute to the functional differences between DCs in early and adult life.

Materials & methods: We use mouse models that allow for lineage tracing and visualization of DCs and other mononuclear phagocytes based on their ontogenetic descendance from committed precursors. Coupled to single cell technologies, transcriptomics and innovative imaging techniques we define cell type specific and age-dependent transcriptional programs and investigate the functions of DCs.

Results: Ontogenetic tracing of conventional DC precursors in Clec9a^{Cre}Rosa^{YFP} mice allows for faithful identification of conventional DCs across tissues in steady state and inflammation based on YFP labelling. When analyzing these mice, we have found that splenic cDC2, defined as CD11c^{+}MHCII^{+}CD11b^{+} cells, which strongly label with YFP in adult

DOI: 10.1002/eji.201970300

mice, exhibit a paucity in labelling in neonates. These data suggest an ontogenetic diversity of $CD11c^+MHCII^+CD11b^+$ cells in early life. Transcriptional profiling and fate mapping has revealed that YFP^- $CD11c^+MHCII^+CD11b^+$ cells derive from hematopoietic stem cells and, surprisingly, are related to the lymphoid branch of hematopoiesis. These cells also exist in other organs and preliminary data indicate that YFP^+ and YFP^- $CD11c^+MHCII^+CD11b^+$cells in early life exhibit functional differences.

Conclusion: We find an unappreciated ontogenetic heterogeneity of splenic $CD11c^+MHCII^+CD11b^+$ cells in early life. We hypothesize that DCs in early life may not be functionally immature but rather that DC poiesis is developmentally regulated to help create essential age-dependent immune balance.

O68

Identification of a DC precursor population with pDC and cDC potential responding to endosomal TLR stimulation with increased pDC output

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Introduction: Plasmacytoid DCs (pDCs) are a subset of DCs highly specialized in recognition of viruses and rapid production of large amounts of type I interferons (IFN). Being short lived and non-proliferative, these cells are continuously replenished from both myeloid and lymphoid progenitors in the bone marrow (BM). We have identified a population of pDC-like precursor cells in murine BM, which respond to TLR-7 and -9 ligands and produce type I IFNs, but retain plasticity to differentiate into conventional DCs (cDCs).

Objectives: We investigated whether this DC precursor plasticity is a result of oligopotency, whereby individual cells can have different cell fates instructed by external stimuli, or subset-specific commitment established at earlier stages of differentiation.

Materials & Methods: Murine DC precursor subsets were characterized by transcriptome analysis. Their cell fate commitment was investigated by differentiation assays *in vitro* and *in vivo* under steady state and inflammatory conditions. DC precursor and progenitor populations were subjected to single cell RNA-sequencing and analyzed by cell clustering and trajectory inference methods.

Results: Within the Siglec H+ CCR9low pDC-like precursor population, two subsets were distinguished by differential gene expression (DEG) and enrichment of specific transcription factor binding motifs in DEG modules. scRNAseq analysis confirmed the relationship of the B220hi subset with pDCs and of the B220lo subset with pre-cDCs, revealing further heterogeneity within the latter population. Whereas the B220hi subset was largely committed to pDCs, the more proliferative B220lo subset gave rise to both pDCs and cDCs *in vitro* and *in vivo*. Addition of GM-CSF favoured cDC generation, while TLR9 stimulation promoted pDC differentiation of these subsets *in vitro*. The effect of CpG-A was dependent on MyD88 expression in the precursors, but not stromal cells, and could be mimicked by IFN-a.

Conclusion: In line with previous findings, we observe a high degree of pre-commitment in DC precursors. However, a subset of proliferative precursors with pDC and cDC fate is responsive to endosomal TLR activation which promotes pDC output. This could be a mechanism for rapid replenishment of pDCs in viral infections.

O70

The C5a/C5a receptor 1 axis controls pulmonary tolerance at the level of pulmonary $CD11b^+$conventional dendritic cells.

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DOI: 10.1002/eji.201970300

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Introduction: Pulmonary CD11b+conventional dendritic cells (cDC) are the main drivers of CD4+ T cell activation in allergic asthma. The mechanisms controlling this activation are still elusive. Previously, we found a protective role for C5a-mediated C5a receptor 1 (C5aR1) activation during allergen sensitization. Here, we determined the impact of locally-produced C5a from pulmonary CD11b+cDCs on allergen-induced T cell proliferation.

Materials and methods: House-dust-mite and ovalbumin (OVA) were intratracheally administered to Balb/c mice. After 24h, lin-CD11c+CD11b+MHC-II+CD64-cDCs were sorted based on the expression of C5aR1 and used for functional and RNAseq analyses. **Results**: We found two distinct CD11b+cDC populations that were either C5aR1+ (75%) or C5aR1- (25%). Both CD11b+cDCs were co-cultured with CD4+ T cells from DO11.10RAG2-/- mice. Proliferation was assessed after 4d of co-culture. Importantly, C5aR1- but not C5aR1+cDCs induced strong T cell proliferation in response to in vitro OVA exposure. MHC-II and CD40 costimulatory molecule expression was significantly lower in C5aR1+ than in C5aR1-cDCs which was associated with a lower frequency of interactions between C5aR1+cDCs–CD4+ T cells in comparison to the C5aR1-cDCs-CD4+ T cells. Further, we observed strong intracellular protein expression of C5a in both CD11b+cDCs upon ex vivo OVA pulsing. RNAseq analysis revealed 94 genes that were 2-10-fold higher expressed in C5aR1+ than in C5aR1-cDCs affecting complement activation and gene regulation among others. Impaired T cell proliferation induced by C5aR1+cDCs was significantly rescued in response to in vitro C5aR1 blocking, which was associated with increased CD40 expression. The *in vitro* blocking of both C5aR1 and CD40-CD40L interactions, on the C5aR1+cDCs resulted in weak T cell proliferation. *In vitro* blockade of CD40-CD40L interactions on the C5aR1-cDCs, resulted in weak CD4+ T cell proliferation only when MHC-II was limited in vitro or low concentration of OVA323-339 was used. **Discussion**: Thus, we propose a model in which OVA-induced paracrine C5a generation in pulmonary CD11b+cDCs controls tolerance towards aeroallergens. Activation of C5aR1 in CD11b+cDCs promotes a pathway that downregulates expression of CD40 keeping OVA-specific T cell proliferation by CD11b+cDCs low. Suppression of C5aR1 signaling on CD11b+cDCs releases the break and results in CD40 upregulation and strong OVA-driven T cell proliferation and allergic asthma development.

O71
BATF dampens type I interferon expression in plasmacytoid dendritic cells
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Introduction: BATF (Basic leucine zipper transcription factors, ATF-like) coordinates multiple aspects of B and T cell function including differentiation, proliferation, metabolism, and effector functions in infection and immunity. In recent transcriptome analyses we found *Batf* differentially expressed in interferon (IFN) β-producing plasmacytoid dendritic cells (pDCs). In pDCs, so far, no expression or function has been described for BATF.

Objectives: Characterizing the expression and functional implications of BATF in pDCs.

Materials & methods: We applied microarray hybridization and quantitative RT-PCR to identify differentially expressed genes in IFNβ-producing pDCs. Different cell populations in bone marrow and lymphoid organs of wild type or *Batf* knockout mice were characterized by FACS. Expression of Type I IFN was measured by ELISA or flow cytometry. Next generation sequencing (NGS) and quantitative RT-PCR were performed respectively for the identification and confirmation of BATF induced gene expression in pDCs. BATF ChIP-Seq was used to characterize the BATF target gene sequences in pDCs.

Results: Using IFNβ/YFP reporter mice we found that BATF is highly expressed in IFNβ-producing splenic and bone marrow (BM) derived pDCs. Upon CpG stimulation maximum *Ifnb* expression precedes the maximum *Batf* expression in pDCs. In order to study the effect of BATF on expression of type I IFN we employed *Batf* deficient mice. In comparison to wildtype littermates BM-derived pDCs from *Batf*- deficient mice produce increased amounts of IFNα and IFNβ at mRNA and protein levels after CpG stimulation. Our data suggest a modulatory role of BATF in the pDC dependent IFN response. In agreement with the *in vitro* data *Batf*$^{-/-}$ mice show higher serum levels of type I IFN after LCMV infection as compared to WT animals. Therefore, we hypothesize that BATF regulates the type I IFN response in innate immune defense. Multiomics data suggesting the molecular mechanisms underlying the BATF mediated regulation of type I IFN expression will be presented.

DOI: 10.1002/eji.201970300

Conclusion: We define the differential expression of *Batf* in IFNβ-producing pDCs with a modulatory role in the regulation of type I IFN expression in pDCs. Our data point to a so far unrecognized role of BATF in pDC dependent type I IFN responses suggesting an importance of BATF in anti-infectious immune responses and IFN mediated autoimmunity

O72

Assessment of escape from X chromosome inactivation on the single cell level in human pDCs

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Introduction: Following Toll-like receptor (TLR)7 stimulation plasmacytoid dendritic cells (pDCs) of females have a significantly higher percentage of Interferon(IFN)α-producing pDCs than males. This higher IFNα production in females has been suggested to contribute to sex-specific differences in the manifestations of infectious diseases and autoimmune diseases (e.g. SLE). Factors underlying these sex dimorphisms in immunity include genetics, sex hormones and exposure to environmental factors. Genetic differences between women and men are mainly due to a different distribution of chromosomes, with females possessing two X chromosomes. *TLR7* escapes X chromosome inactivation (XCI) in some female pDCs, leading to biallelic expression of TLR7 in those cells. The functional consequences of *TLR7* escape from XCI in pDCs remain unknown.

Question: The aim of this project was to investigate on the single cell level whether pDCs with escape from XCI in *TLR7* have a superior capacity of transcribing IFNα mRNA.

Methods: Healthy female donors were typed for heterozygosity of the *TLR7* SNP rs3853839. Freshly isolated PBMCs of heterozygous females were collected and stimulated with the TLR7/8 agonist CL097. Subsequently pDCs were isolated via FACS and cDNA of single pDCs was obtained using the Fluidigm C1 technology. The expression of mRNA with or without the SNP enabled determination of mono- or biallelic expression pattern of TLR7 within the single pDC. In the same pDCs, mRNA expression levels of TLR7, IFNα subtypes and other genes were assessed via qPCR.

Results and Conclusion: Studies in three females heterozygous for *TLR7* SNP rs3853839 showed that 19 - 37% of pDCs display biallelic expression of *TLR7*, using data from 176 single cells. The levels of TLR7 mRNA were higher in cells with biallelic compared to monoallelic TLR7 expression. In addition, multiple IFNα mRNA subtypes were expressed at higher levels in pDCs with biallelic compared to monoallelic expression of TLR7 following CL097 stimulation. These data demonstrate that on the single cell level escape from XCI can contribute to stronger IFNα transcription by female pDCs.

Regulatory and helper T cells I (O74-O78)

O74

Tissue-specific and tissue-unspecific mechanisms on memory T helper cell maintenance

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Question: Memory T helper (Th) cells are critical for long-lasting protective immunity. In a systemic immune response, memory Th cells are maintained mainly in the bone marrow (BM) and also in the spleen. However, it remains unknown how, by which survival signals, they are maintained in the BM and spleen. Here we show tissue-specific and tissue-unspecific mechanisms on the maintenance of memory Th cells *in vivo*.

Methods: The effect of serological and genetic ablation/neutralization of cell populations, cytokines and adhesion molecules on the maintenance of memory Th cells in the BM and spleen were assessed by flow cytometry and confocal microscopy.

DOI: 10.1002/eji.201970300

Results: BM but not splenic memory Th cells are depleted by neutralization of integrin and inhibition of integrin-associated kinase, while splenic but not BM memory Th cells are depleted by ablation and neutralization of IL-7 signal. The maintenance of memory Th cells in both tissues was commonly organized by regulatory T cells via BM stromal cells. Furthermore, an injection of anti-CD20 antibody expands the number of memory Th cells in the BM and spleen in an antigen-independent manner, due to not depletion of B cells but bystander activation by macrophages.

Conclusion: Memory Th cells are maintained by tissue-specific mechanisms; adhesion-dependent maintenance in the BM and cytokine-dependent maintenance in the spleen. A certain activation signal in macrophages provides the expansion of memory Th cell pool in the BM and spleen.

O75

Identification of nonlymphoid-tissue regulatory T cell precursors

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Regulatory T cells (Treg cells) are important mediators of peripheral tolerance, and their absence leads to catastrophic autoimmunity in men (IPEX) and mice (Scurfy). Treg cells are characterized by both expression of the hallmark transcription regulator Foxp3 and a unique epigenetic profile. Treg cells are critical to maintain self-tolerance. They modulate the functions of different immune cells, thereby affecting a variety of conditions, including autoimmunity, cancer, allergy, infection and inflammation. In recent years it became evident that in addition to these immunoregulatory properties, highly specialized Treg cells in non-lymphoid tissues are important to promote organ homeostasis and tissue repair. In particular, the release of the EGF-like growth factor Amphiregulin by tissue-resident Treg cells during wound repair suggested such a function. But how and where do Treg cells specialize into this tissue regenerative population found in non-lymphoid tissues? Recently, we identified a common population of tissue-homeostasis promoting Treg cells via genome-wide epigenetic and transcriptional profiling (Delacher et al., *Nature Immunology* 2017). In addition, we could describe the transcription factor dependency of this population (Delacher et al., *Nature Communications* 2019). Now, using state-of-the-art molecular tools like scRNA and scTCR sequencing as well as ATAC profiling and other methods, we identified progenitors of these non-lymphoid tissue Treg cells in spleen and lymph nodes and revealed their molecular path of differentiation. We identified critical transcription factor profiles and performed developmental kinetics *in-vivo*. Our study fills a critical gap in understanding nonlymphoid-tissue Treg cell differentiation and provides means to harness their potential for regenerative medicine in future applications.

O76

Influenza A virus-induced thymic atrophy results in an enhanced differentiation of regulatory T cells with a reduced TCR repertoire diversity

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Foxp3$^+$ regulatory T cells (Tregs) are crucial for the maintenance of self-tolerance and regulation of inflammatory responses against pathogens. Influenza A virus (IAV) infection has been reported to cause transient thymic involution. However, the impact of IAV-induced thymic atrophy on thymic Treg development is not known. Here, we generated kinetic data to describe the impact of IAV infection on thymic Treg development. While the absolute number of all major thymocyte subsets decreased significantly in thymi from IAV-infected mice compared to PBS-treated controls, the thymic Treg population was hardly affected, resulting in significantly increased Treg frequencies upon IAV-induced thymic atrophy. The high-resolution data were used to generate a mathematical model, which suggested several mechanisms accounting for the relative Treg increase. While a lower susceptibility of Tregs to apoptosis could be experimentally excluded, we demonstrated using RAG^{GFP} reporter mice that the increase in thymic Treg frequency

DOI: 10.1002/eji.201970300

maps to newly produced thymocytes, a process that correlated with significantly increased TGF-β levels within atrophied thymi. Surprisingly, the IAV-induced increase in thymic Treg frequencies was associated with a reduced TCR repertoire diversity not only within the newly produced Tregs, but also within their conventional counterparts. Taken together, our data suggest that IAV-induced thymic atrophy fosters an increased generation of thymic Tregs with a limited TCR repertoire, which preserves their absolute numbers within the atrophied thymus.

O77

Functional CRISPR dissection of gene networks controlling human regulatory T cell identity

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Regulatory T cells (Tregs) play a critical role in human immune homeostasis by suppressing inflammation and autoimmunity. Tregs must maintain suppressive functions even in pro-inflammatory microenvironments, and this maintenance is in large part controlled by transcriptional regulation. The transcription factor (TF) FOXP3 is known to be crucial for establishment and maintenance of Treg identity. The complete set of critical transcription factors in human Tregs and their downstream transcriptional targets remain unknown. Here we used both novel pooled as well as arrayed Cas9 ribonucleoprotein (RNP) screens in primary human Tregs under pro-inflammatory conditions to identify TFs that regulate expression of key Treg and effector T cell markers. We then deeply profiled a subset of these TFs by single cell RNA sequencing (scRNA-seq) of edited human Tregs, revealing distinct gene modules that preserve Treg transcriptional identity. These modules highlighted key genes of Treg functions regulating cytokine secretion, transcriptional regulation and metabolism in Tregs. We find that FOXP3 and PRDM1 individually regulate independent gene modules, while FOXO1 and IRF4 co-repress their own. We have also discovered that HIVEP2—which has not been previously implicated in Treg functions—participates with SATB1 in co-activating yet another gene module. By identifying key genetic programs controlled by individual TFs that shape Treg identity, we gain knowledge that could be used to ultimately engineer Treg-based cell therapies.

O78

Accumulation of tissue Tregs due to dendritic cell specific ablation of RelB protects from autoimmune inflammation

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Introduction: Foxp3+ regulatory T cells (Tregs) are well-known immune suppressors in various settings. Lately, more and more evidence is provided for the existence of several subtypes of Tregs that differ in their transcription factor profile as well as in their functionality. But little is known about the signaling pathways in dendritic cells (DCs) that are crucial to prime such different Treg qualities.

Objectives: We thought to elucidate whether and how the alternative NF-κB pathway in DCs can influence Treg quality and quantity. Furthermore, we aimed to prove functional consequences of altered Treg phenotypes in vivo and analyze underlying mechanisms in vitro.

Materials & methods: To address those questions, we are using a CD11c-Cre x RelB flox mouse model, multi-color FACS, RNA-seq and the experimental autoimmune encephalomyelitis (EAE) model.

DOI: 10.1002/eji.201970300

Results: Deletion of RelB in DCs results in an impaired oral tolerance induction and a marked type 2 immune bias among accumulated Foxp3+ Tregs reminiscent of a tissue Treg signature. Those Tregs are fully functional, expand independently of IL-33 and lead to an almost complete protection from experimental autoimmune encephalomyelitis.

Conclusion: RelB-dependent pathways in DCs are an attractive target for treatment of autoimmune diseases but may come at risk for reduced immune tolerance in the intestinal tract.

Complement (O79-O84)

O79
Functional Consequences of Dual Gene Deletion of C5aR1 plus C5aR2 during Bacterial Pneumonia

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Introduction: Bacterial pneumonia is a life-threatening infection with the risk of acute respiratory failure and subsequent death. The complement system participates in the clearance of encapsulated bacteria such as *Streptococcus pneumoniae,* which is a common pathogen for pneumonia. Yet, it is not entirely clear whether C5 and its activation product C5a have beneficial or detrimental effects on the outcome of *S. pneumoniae* infection. C5a is viewed as a potent chemoattractant. It binds to its two homologous C5a receptors (C5aR1 and C5aR2).

Objectives: The extent of functional overlap, role distribution and synergisms between C5aR1 and C5aR2 remain enigmatic. A critical barrier in the field has been the absence of mice with dual deletion of both receptors, which are encoded as adjacent genes on chromosome 7. C5aR1/C5aR2 double-knockout mice could help to close some critical knowledge gaps.

Materials And Methods: We used CRISPR/Cas9 guided gene editing combined with zygote/pronucleus microinjections in C57BL/6J mice to generate a C5aR1/C5aR2 double-knockout strain. As functional studies, we performed intra-tracheal injections of recombinant mouse C5a or intranasal injections of *S. pneumoniae TIGR4*.

Results: More than 50 offspring mice were obtained and PCR-based screening identified one male founder mouse. This founder mouse was backcrossed for several generations. Sanger sequencing confirmed a 12.6 kB deletion of the coding regions of C5aR1 (Exon 2) and C5aR2 (Exon 1+2). Absence of gene expression was confirmed by RT-qPCR and flow cytometry. This new mouse strain [C57BL/6J-Del(7C5ar2-C5ar1)1Bosm; or C5aR1/2-/-] is viable and fertile. The airway influx of Ly6G+SiglecF- neutrophils by recombinant C5a was abrogated in C5aR1/2-/- double-knockout mice. Surprisingly, the dual genetic absence of C5a receptors was associated with a much higher build-up of neutrophil numbers in alveolar spaces after *S. pneumoniae TIGR4* infection.

Conclusion: The C5aR1/2-/- double-knockout mice provide a powerful new tool to study putative synergisms and redundancies of C5a receptors in immunology. The requirement of C5a as a chemoattractant for promoting neutrophil migration to the site of bacterial infection may be different than previously assumed.

O80
C5a activates inflammatory eosinophils to drive house dust mite-mediated experimental allergic asthma

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DOI: 10.1002/eji.201970300

Introduction : Severe asthma is frequently associated with high eosinophil numbers. In the naive mouse lung, resident eosinophils (rEOS) occur. In experimental allergic asthma, an additional subset of inflammatory EOS (iEOS) is recruited. Previously it was shown that the complement cleavage product C5a can activate EOS in different ways.

Objectives : We aimed to determine the role of the C5a/C5aR1 axis in iEOS recruitment and activation in the context of experimental allergic asthma development.

Materials & methods: We used models of house-dust-mite (HDM)-induced allergic asthma and interleukin (IL)-33-induced airway inflammation that recapitulates several features of allergic asthma, including airway hyperresponsiveness (AHR), mucus secretion and inflammatory cell infiltration. Further, we adoptively transferred sorted in vitro HDM-pulsed iEOS from wildtype and *C5ar1*$^{-/-}$ mice to assess their impact on asthma development

Results: Upon repeated i.t. HDM or IL-33 exposure, iEOS accumulated in large numbers in the lung tissue and the airways. Further, some iEOS migrated to draining mediastinal lymph nodes (mLN). Our data suggest that lung iEOS derive from SiglecF^{+}CD125^{lo} EOS in the bone marrow which already transform into CD101^{+}CD11c^{lo} iEOS in the blood. Importantly, iEOS took up antigen more efficiently than rEOS, localized in the airways close to the T cells, and increased costimulatory molecules expression while homing to the mLNs. These data suggest that iEOS could serve as antigen presenting cells (APC). Clearly, HMD-induced iEOS triggered T cell proliferation *in vitro* and induced Th2-cytokine driven allergic asthma *in vivo*. In addition, iEOS expressed higher levels of C5aR1 compared to rEOS in allergen-induced allergic asthma. In *C5ar1*$^{-/-}$ mice, the recruitment of iEOS to mLN was significantly reduced and the upregulation of costimulatory molecules was abrogated. Further, adoptive transfer of sorted, HDM-pulsed *C5ar1*$^{-/-}$ iEOS into wt recipient mice resulted in lower AHR and airway inflammation when compared to transfer of wt iEOS.

Conclusion: Our findings demonstrate that iEOS can drive maladaptive Th2 cell-mediated experimental asthma. C5aR1 is highly expressed on iEOS and controls their migration from the lung to draining mLNs and the upregulation of costimulatory molecules. Also, C5aR1 activation on iEOS promotes the development AHR and airway inflammation in vivo suggesting that C5aR1 may serve as a novel target in eosinophilic asthma treatment.

O81

Complement terminal pathway activation in absence of C5 convertases

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C5 activation is the initiation point of the terminal pathway and occurs by proteolytic processing of C5 into C5a and C5b. The latter recruits the components C6-C9 to assemble the lytic pore complex C5b-9 (termed membrane attack complex, MAC) that inserts into cell membranes. A requirement of C5 activation is the forceful activation of the complement cascade leading to high levels of C3b deposition to facilitate the assembly of C5 convertases (C4b2a3b, C3bBb3b, C3bBb3bP).

Recently, we demonstrated that several C5 inhibitors either approved or trialled for clinical use reduce but fail to completely inhibit terminal pathway activity. Here we set out to uncover the mechanism of this residual C5 activity in presence of excess amounts of stoichiometric C5 inhibitors (*e.g.* Eculizumab, Coversin) and elucidated several novel features of C5 activation with important implications for our fundamental understanding on C5 activation as well as its clinical inhibition.

Using haemolytic assays with purified components we show that the fluid phase convertase C3bBb is sufficient for C5 cleavage as long as C5 is captured and primed on surfaces containing C3b deposition. On surfaces with dense C3b deposition C5 activation occurs even in complete absence of complement convertases (or other enzymes) indicating a conformation reorientation of C5 that is induced purely by interaction with the surface fixed C3b molecules. This implies that C5 can adopt a C5b-like conformation that resembles C3(H2O) and thus may be termed C5(H2O). We proved the existence of C5(H2O) on cell surfaces by flow cytometry: adding C5, C6 and C7 to C3b opsonised erythrocytes allowed to detect surface fixed C5 on the cells with the highest C3b density via fluorescently labelled Eculizumab or via anti-C5a antibodies. After addition of C8 and C9 to these latter cells, the population positive for C5 and C5a lysed demonstrating that the C5(H2O) fixed on the C3b opsonised surface remains functional. Importantly, conformational activation of C5 by C3b clusters could not be inhibited by single stoichiometric C5 inhibitors explaining the mechanism of residual C5 activity.

DOI: 10.1002/eji.201970300

In conclusion C5 activation on highly dense C3b surfaces occurs in absence of proteolytic cleavage via conformational changes which cannot be inhibited by single stoichiometric C5 inhibitors.

O82

Complement and microvesicle interaction influences the inflammatory response during polytrauma

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Introduction: It is well established that polytrauma (PT) leads to an early and excessive activation of the complement system. The resulting complementopathy and impairment of cellular defense systems are associated with a poor outcome. Previously described as platelet dust, microvesicles (MVs) have gained emerging importance in the research field of inflammation. Besides cellular communication, MVs can transfer cargoes consisting of lipids, peptides, RNA, and sugars, and thereby are able to influence recipient cell functions. Current data indicate that MVs are also involved in complement activation with a dramatic influence on inflammation after PT.

Objective: The objective of our study was to characterize MVs from PT patients. We hypothesized an interaction between MV shedding and complement activity, and we aimed to identify the involved molecular mechanisms especially after PT.

Patients and Methods: A prospective clinical study was conducted in patients after PT (ISS≥ 32) and healthy volunteers. The study was approved by the Independent Local Ethics Committee of the University of Ulm, no. 94/14. Blood was obtained upon admission to the ER and 4h, 12h, 24h, 48h, 120h and 240h after trauma. For MV studies, flow cytometry was used by defining a MV-specific gate. PMNs and PBMCs were isolated from healthy donors by Ficoll density centrifugation.

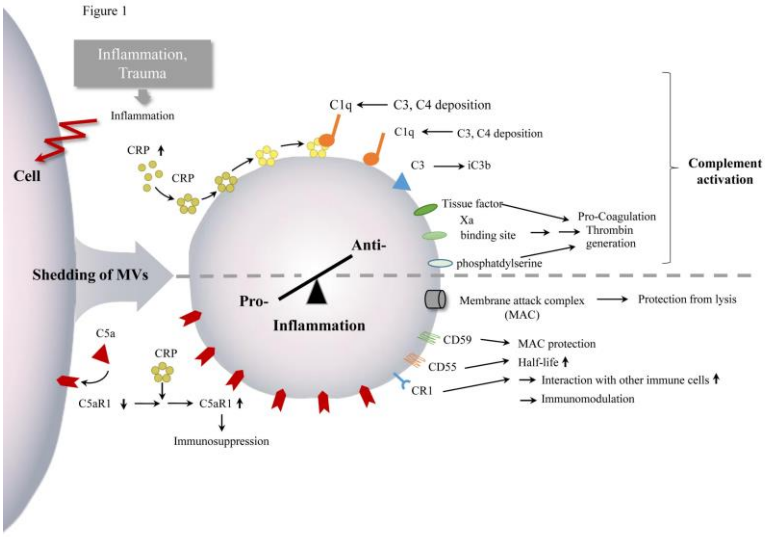
Results: Polytrauma patients showed a significantly altered MV shedding pattern and exhibited increased numbers of platelet-, PMN- and PBMC-derived MVs compared to healthy donors. Furthermore, complement C5aR and C3aR were significantly present on shed MVs. In concordance, the C5aR was time-dependently decreased on human granulocytes after PT, whereas C5aR expression was significantly increased on MVs. *In vitro* assays with PMNs confirmed that stimulation with C5a significantly increased PMN-derived MVs harboring the C5aR, which abolished in the presence of an antagonist for C5aR. Moreover, MVs from PT patients contain significantly more CRP on their surfaces. *In vitro* assays confirmed that CRP-bound MVs are able to bind complement C1q.

Conclusion: The data are indicative of a so far unknown interaction between the complement system and MV shedding. Complement can activate MV shedding and vice versa. Therefore, targeting the complement-MV interaction may represent a promising therapeutic approach to restore the imbalanced inflammation in PT.

DOI: 10.1002/eji.201970300

Fig.1: Complement-MV interaction during inflammation.

Figure 1



O83

Of mice and men: a novel "humanized" mouse model reveals evolutionary functional preservation of immune adherence

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Introduction: The immune system has multiple mechanisms in place to quickly and efficiently clear foreign, unwanted or infectious materials from the bloodstream. One such mechanism is "Immune Adherence" (IA), which targets complement-opsonized circulating (antigenic) materials to platelets in rodents or erythrocytes in humans. This fundamental difference raised the question whether mice are an appropriate system to model human blood clearance processes. We previously showed in mice that IA to platelets creates "dual track" clearance of systemic bacteria, targeting the majority of circulating Listeria monocytogenes to liver Kupffer cells for efficient killing, while diverting a small porting of bacteria-associated antigenic material to splenic DC for the induction of antibacterial cytotoxic T cell responses. Our identification of GPIb as critical receptor for murine IA enabled us to replace GPIb-α on platelets though an AI receptor on mouse erythrocytes, effectively producing a novel "humanized IA" mouse model to study blood clearance processes in mice as they proceed in humans.

Objectives: We here use a novel "humanized IA" mouse model to study the role of human IA on blood clearance processes and on the induction of adaptive immune responses to systemic bacterial infection.

Materials & Methods: We combine intravital microscopy with flow cytometry and classical microbiology to qualitatively and quantitatively assess IA complexes and correlate bacterial localization to antibacterial immune responses.

Results: As in wild-type (WT) mice with platelet IA, we find that "humanized IA" prolongs bacterial circulation and affects its distribution across phagocytes in liver and spleen. As in WT mice, complement receptor CRIg on Kupffer cells remains the main facilitator of hepatic clearance in the "humanized IA" model. In the spleen, IA ensures efficient bacterial targeting to CD8α+ dendritic cells and the generation of robust antibacterial cytotoxic T cell responses, regardless whether it is mediated though erythrocytes or platelets.

DOI: 10.1002/eji.201970300

Conclusion: We describe a novel "humanized IA" mouse model and identify that systemic clearance and immunity are guided by IA, regardless whether it is mediated via platelets (mice) or erythrocytes (humans). Thus, our model revels functional preservation of IA throughout evolution, cell-types and across species.

O84

The expression of complement component C3 is specifically localized in endometrial ectopic tissue and is involved in the endometriotic lesion formation

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Introduction: Endometriosis is a chronic condition that affects about 5-10% of women in fertile age and is characterized by pain and infertility; it is defined as the presence of functional endometrial tissue outside the uterine cavity. The most common locations for the ectopic implants are the ovaries, peritoneum and the utero-sacral ligaments. The hormonal cyclical bleeding of this tissue induces a condition of chronic inflammation. There are several theories about the pathogenesis of EM, but current evidence indicates that immune dysfunctions, in particular the upregulation of inflammatory pathway, help the implantation and survival of endometriotic lesions. Furthermore, numerous studies showed the pivotal role played by peritoneal mast cell in this disease.

Objectives: Several groups demonstrated that the glandular epithelial cells found in endometriotic implants produce and secrete the complement component C3. The aim of this work was to confirm the presence of C3 in the ectopic tissue compared to the eutopic one, and investigate the role of C3 in the pathogenesis of endometriosis.

Materials & Methods: We investigated by immunofluorescence, the expression of C3 on sections of endometriotic cysts and healthy uterus; we performed RT-qPCR experiments to highlight the synthesis of this C component at local level. We set up a murine *in vivo* model of endometriosis based on the injection of minced uterine tissue from a donor mouse, into the peritoneum of a receiving animal. We investigated the formation of endometriotic lesions in WT and C3 KO animals.

Results: We confirmed the presence of C3 selectively in the ectopic and not in eutopic endometrium, and the local synthesis of C3 in endometriotic tissue. We observed a greater amount of cyst formation in the peritoneum of WT mice compared to C3 KO mice.

Conclusion: We concluded that C3 can actually be considered a marker of endometriosis and that the local synthesis of this C component can promote the engraftment of the cysts.

Neuroimmunology (O86-O90)

O86

Synaptic proteins and neuronal circuits involved in brain plasticity are modulated during relapsing-remitting experimental autoimmune encephalomyelitis.

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Cognitive decline (CD) is an emerging feature of Multiple Sclerosis (MS) patients, which worsens their quality of life and makes them more vulnerable to psychiatric illness than individuals with a purely physical disability. Impaired brain plasticity is emerging as an underlying cause of CD in both MS and its preclinical models. On this basis, we explored the impact of the disease in two brain structures deeply involved in cognitive processes, namely the hippocampus and

DOI: 10.1002/eji.201970300

the prefrontal cortex (PFC), in the relapsing–remitting model of experimental autoimmune encephalomyelitis (RR-EAE), in which inflammatory and demyelinating lesions extend also to rostral areas, closely resembling human MS lesions. Histological examination of samples from RR-EAE SJL mice sacrificed in the acute phase of the disease confirmed the presence of inflammatory infiltrates in both regions, accompanied by a concomitant increase of activated microglia. Furthermore, quantitative PCR (qPCR) analysis performed on total mRNA extracted from PFC and hippocampus of RR-EAE and CTRL animals demonstrated induction of genes encoding for inflammatory cytokines (*Il1beta*, *Tnf* and *Il6*) in RR-EAE mice ($p<0.05$, $n=6$). At the molecular level, we investigated the impact of EAE on the expression and splicing of synaptic proteins whose deregulation is associated with neurological diseases, including Neurexins (*Nrxn1-3*) genes, by PCR analysis. Notably, we observed selected splicing modulation of some presynaptic and postsynaptic genes in both brain regions of RR-EAE mice in the acute phase of the disease. Furthermore, qPCR analysis and immunocytochemistry followed by unbiased stereology revealed, in both PFC and hippocampus, that RR-EAE caused an increased expression of Parvalbumin (PV), marker of a subset of GABAergic neurons crucially involved in establishment of local circuitry required for cognitive functions. Interestingly, the increased expression of the PV gene, as well as the increased number of PV+- interneurons, were positively correlated with the expression of inflammatory cytokines (Pearson correlation, $p<0.05$). Our data reveal a complex and multilayered impact of EAE and inflammation on neuronal circuitry and highlight new possible targets for neuroprotective strategies in MS.

O87

CNS associated DCs sample and present myelin antigens in the steady-state to allow parenchymal T-cell entry

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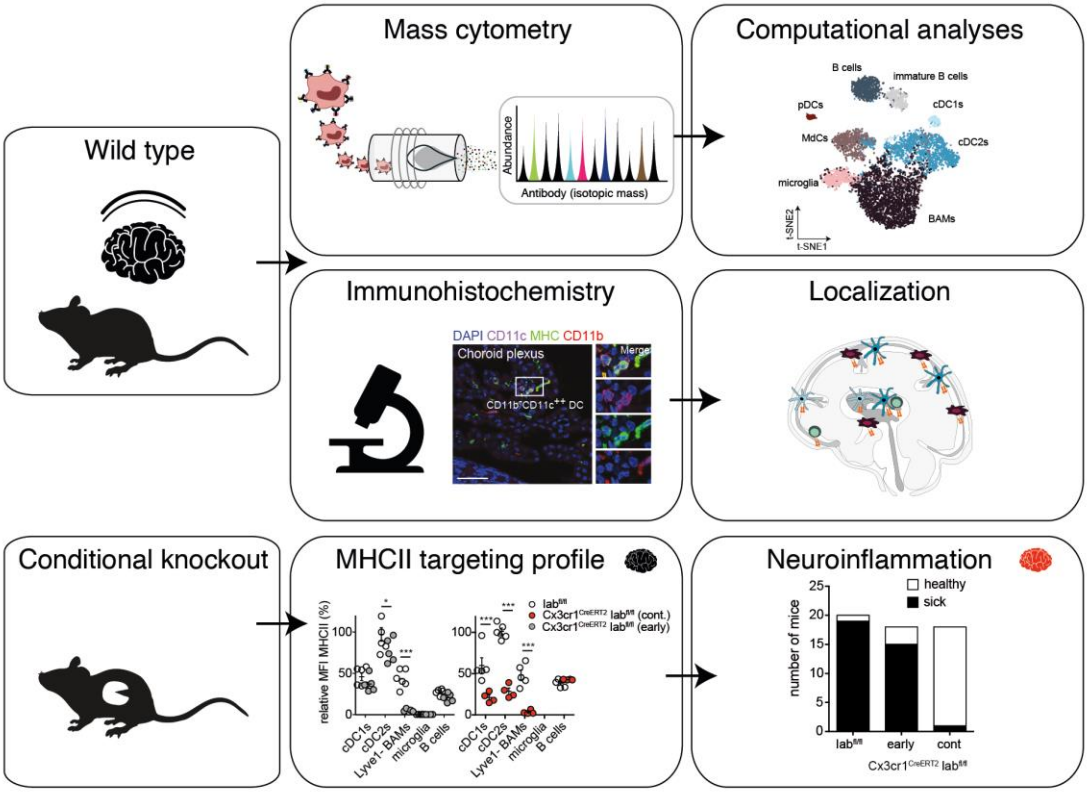
The central nervous system (CNS) is under close surveillance by immune cells, which mediate tissue homeostasis, protection and repair. Dysregulated T-cell invasion as seen in neuroinflammatory diseases such as multiple sclerosis, leads to immunopathology and neurological disability. To invade the brain parenchyma, even activated T cells must encounter their cognate antigens (Ags) presented via local Ag-presenting cells (APCs) that reside at the border regions of the steady-state CNS. The nature of the cell type that can sample, process and present neuro-Ag to auto-aggressive T cells is unknown. Here, we used a combination of high-dimensional single cell cytometry and conditional ablation of MHC class II molecules across potential CNS APCs to systematically characterize and interrogate each population for its ability to reactivate encephalitogenic T helper cells *in vivo*. We found that, in contrast to other CNS APCs, conventional dendritic cells (cDCs) are essential for licensing T cells to infiltrate the CNS parenchyma and to initiate the inflammatory cascade leading to neuroinflammation.

DOI: 10.1002/eji.201970300

Figure 1



O88

Dietary tryptophan is required for homing and encephalitogenicity of auto-reactive T cells in a murine multiple sclerosis model

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Multiple sclerosis (MS), the most common neurological disorder among young adults, is thought to be mainly driven by auto-reactive T cells that infiltrate the central nervous system (CNS). Recent data from preclinical studies suggest that impaired host-microbiota interaction in the gut may result in dysregulated systemic T cell responses contributing to autoimmune diseases in distant organs such as the CNS. The gut microbiota composition is highly influenced by specific dietary constituents.

Here, we demonstrate that deprivation of a single amino acid – tryptophan (trp) - from the diet is sufficient to abrogate CNS autoimmunity using the murine experimental autoimmune encephalomyelitis (EAE) model. The essential amino acid trp has previously been identified as a potent modulator of local and systemic immune responses. Yet, we show that protection from neuroinflammation by dietary trp deprivation (DTR) is independent of classical trp-sensing pathways controlled by aryl hydrocarbon receptor (AHR) and general control non-derepressible 2 (GCN2). Abrogation of CNS autoimmunity was accompanied by impaired encephalitogenic T cell responses, as well as profound alterations in gut microbiota composition and expression of marker genes involved in immune activation locally in the colon. In line with this, protective effects of DTR were abrogated in germ-free (GF) mice.

In conclusion, our data provide an important insight into the regulation of autoimmune neuroinflammation by a single essential dietary constituent and subsequent perturbations of gut microbiota homeostasis. This link may offer novel therapeutic strategies for protection from CNS autoimmunity.

DOI: 10.1002/eji.201970300

O90

Fate mapping reveals a *Cxcr4*-dependent immune response of monocytes to experimental stroke

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Tissue-resident and monocyte-derived macrophages are ontogenetically distinct components of the innate immune system. However, the assessment of their respective roles during inflammation is complicated by dynamic changes of the macrophage phenotype. We show that Cxcr4 is expressed in the hematopoietic stem cell (HSC) lineage, but is absent in most tissue-resident macrophages, including microglia. Exploiting this distinct expression pattern, we generated a novel Cxcr4-CreER mouse model allowing us to dissect the spatio-temporal and molecular responses of HSC-derived monocytes and microglia after stroke. Using conditional Cxcr4 ablation in HSC-derived cells, we found that Cxcr4 regulates monocyte recruitment to the infarct area and the expression of monocyte-specific pattern recognition and defense response genes after photothrombosis and transient middle cerebral artery occlusion (tMCAO). Cxcr4-deficiency affected the microglial response indirectly in both stroke models and deteriorated outcome after tMCAO, suggesting that monocytes are important for the initiation of tissue repair and that Cxcr4 is essential for the local innate immune response after brain injury. This ontogeny-based dissection of microglia and monocyte functions should help investigate the molecular responses and targeted therapies in inflammatory brain disorders.

Autoimmunity and chronic inflammation II (O92-O96)

O92

Autoimmune hepatitis in mice is driven by local activation of autoreactive CD4 T cells in the liver and formation of ectopic lymphoid tissue

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Introduction: Autoimmune hepatitis (AIH) is a chronic, inflammatory disease of the liver, associated with an adaptive immune response to liver antigens.

Objectives: Our aim was to elucidate the mechanisms behind the loss of tolerance to liver antigens in AIH.

Methods: We generated a mouse model of AIH that is characterized by hepatocellular expression of an MHC class II-restricted CD4 T cell epitope of lymphocytic choriomeningitis virus (GP61-80) under control of the albumin promoter, and by abundance of cognate Smarta1 CD4 T cells recognizing GP61-80.

Results: In these Alb-iGP_Smarta mice, autoreactive T cells were not deleted in the thymus, due to antigen ignorance, but accumulated in the liver. Accordingly, these mice spontaneously developed hepatitis with typical disease features of human AIH, including elevated IgG (4.0 vs. 0.9 mg/ml, p=0.0159), elevated serum transaminases (593.3 vs. 60.8 U/l, p=0.0325), antinuclear autoantibodies in 76% of the mice (titer>1:80, p=0.005), and lymphocytic periportal infiltrates with interface hepatitis (mHAI score 14/18, p=0.0079). AIH development was facilitated by pathogenic maturation of autoreactive CD4 T cells towards IFNg and TNF co-producers (38.3% vs. 4.2% of hepatic CD4), which seemed to occur within ectopic lymphoid structures in hepatic portal fields that transiently formed during the preclinical disease stage, showing the typical segregation of T and B cell zones, dendritic cell networks and high endothelial venules. In advanced disease, these lymphoid structures had disaggregated and merged into large, dispersed infiltrates.

DOI: 10.1002/eji.201970300

Conclusions: Our findings indicate that AIH pathogenesis is driven locally in the liver by pathogenic maturation of previously ignorant autoreactive CD4 T cells. Transient formation of ectopic lymphoid tissue in the portal fields may be a relevant feature of early pathogenesis also in human AIH.

O93

Staphylococcus aureus-induced tissue resident memory T helper 17 cells (Trm17 cells) drive renal autoimmune disease

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Introduction: Tissue-resident memory T (TRM) cells comprise CD4+ and CD8+ memory T cells that reside in peripheral organs without recirculating. They provide rapid on-site immune protection against previous encountered pathogens. TH17 cells are important for the control of extracellular pathogens such as *Staphylococcus aureus*. However, TH17 cells are also associated with several autoimmune diseases like glomerulonephritis (GN)

Objectives: We aim to clarify the contribution of TRM cells to renal autoimmunity. For this, we analyse the phenotype and function of kidney resident TH17 cells in human kidneys as well as in a mouse model for *Staphylococcus aureus* infection which we combine with models for renal autoimmune diseases.

Materials & methods: To study resident renal T cells, we used a combined approach of flow cytometry, histology and single cell RNA-Sequencing. Human kidney tissue was obtained from tumour-nephrectomies. In mice, renal Th17 cells were induced by S. *aureus* infection. GN was induced with the nephrotoxic sheep serum or by immunisation with a fragment of the α3 chain of type IV collagen. In addition, we used *Listeria monocytogenes* as second infection model.

Results: We found a substantial population of CD4+ CD69+ TRM cells of the TH17 phenotype both in mouse and human kidneys, and operationally named them TRM17 cells. In the mouse model of *S. aureus* infection, we could induce accumulation of long-term persisting TH17 cells in the kidney. Induction of GN in mice which had recovered from *S. aureus* infection resulted in an enhanced renal TH17 response and development of a severe course of crescentic GN. In contrast, prior infection with *Listeria monocytogenes*, which causes a TH1 and CD8+ immune response, did not alter renal autoimmunity. By labelling renal cells, we were able to demonstrate that *S. aureus* induced TRM17 cells contributed to the enhanced local TH17 response in GN. In addition, depletion of TH17 cells after *S. aureus* infection or neutralization of IL-17A ameliorated GN in mice, demonstrating the major impact of resident TH17 cells in renal autoimmune disease.

Conclusion: Our data shows the presence of tissue-resident TH17 cells in the kidney of humans and mice. Bacterial infection induced these TRM17 cells which subsequently contribute to renal autoimmune diseases. Thus, our results suggest that TRM cells have a previously unknown role in the organ-specific autoimmunity.

O94

Tissue resident T cells arise during chronic infection

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Introduction: Until recently, it was believed that during a chronic infection effector cells solely enter a terminally differentiatded state of dysfunction known as exhaustion. A recent study has highlighted the presence of a small subpopulation that behave with memory characteristics and to which we refer as "memory like" population. This population is capable of persisting in the absence of antigen and can be recalled. Based on this study, we became interest in addressing if not only circulating but also tissue resident memory T cells (TRMs) are formed in chronic infections. Alongside, we are interested in characterizing the extend to which populations of resident CD8 T cells populations become are lodged in tissues. Tissue Resident populations have been shown to mediate long-lived

DOI: 10.1002/eji.201970300

protective immunity at sites of pathogen exposure, and may represent a pool of cells that are contributing to the persistence of virus and tissue damage.

Question: Do tissue resident populations form during chronic infection?

Methods: To examine the differentiation of cells during a chronic infection we infect C57BL6 mice with Clone 13 LCMV (Cl13) and compare to Armstrong LCMV (Arm). Additionally, we assess the effect of the level of antigen using a modified strain of Clone 13, in which less antigen is present but the viral load is unchanged (mixed). To prove the residency status of cells we use the well accepted methods of FTY720 administration, IV labeling and comparing the cells transcriptome to previously published datasets.

Results: High frequencies of presumably resident population have been found in the liver, salivary glands and kidneys following each of the aforementioned infections. After Cl13 infection, these cells are phenotypically similar to those seen in the acute infection, and decreases are seen in this population when the amount of antigen is decreased in the mixed infection.

Conclusions: TRM-like cells are produced during a chronic infection, which has warranted additional studies into the function, transcription and epigenetics of these cells.

O96

Roquin deficiency in T cells induces early stages of pancreatic cancer

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Introduction: Our immune system not only prevents cancer development by eliminating transformed cells, it can also cause chronic inflammation driving tissue insult and carcinogenesis. Acute and chronic pancreatitis are strongly implicated in the development of pancreatic ductal adenocarcinoma (PDAC), the most frequent form of pancreatic cancer and one of the leading causes of cancer deaths worldwide.

Objectives: To investigate how Roquin function in T cells prevents the development of autoimmune pancreatitis and the mechanisms by which it promotes the development of PanIN lesions.

Material & methods: Pancreas pathology was assessed in mice with and without combined conditional ablation of Roquin-1 and Roquin-2 in T cells at the age of 5, 10 or 20 weeks. Spleens and pancreas draining lymph nodes were analysed for changes in lymphocytes that may lead to the observed pathologies.

Results: We observed that Roquin deficiency in T cells caused chronic T cell activation and can lead to autoimmune pancreatitis, acinar–to–duct metaplasia (ADM) and formation of PanIN1A (pancreatic intraepithelial neoplasia) lesions in mice. Pancreatic damage increased with age, with half of the mice aged 10 – 20 weeks showing pathologic changes. Pancreata were infiltrated with inflammatory cells and pSTAT3 levels were strongly increased in immune as well as acinar cells, pointing towards a potential role of IL-6 mRNA deregulation in T cells as a driver of Roquin mediated pancreas pathology. Roquin-1/2 DKO mice produced autoantibodies against pancreatic antigens, suggesting an involvement of Tfh cells. Consistently, we detected strongly increased frequencies of Th17 and T follicular helper (Tfh) cells in draining lymph nodes, and increased Th17 and Tfh associated mRNAs in pancreas tissue. Importantly, combined genetic inactivation of the Roquin encoding alleles as well as of its target Ox40 lead to a partial rescue and an improved phenotype.

Conclusions: We propose that mice with conditional Roquin deletion in T cells are a useful model to study how chronic inflammation and autoimmune pancreatitis trigger the development of neoplasia and pancreatic cancer. Currently, we are investigating whether chronic autoimmune pancreatitis is driven by Th17 or Tfh cells and whether Roquin deficiency works synergistically with the Kras mutation, a major driver of PDAC development.

DOI: 10.1002/eji.201970300

Dendritic and myeloid cells II (O99-O102)

O99

Effect of lipid metabolism on monocyte-derived DC maturation and functional interaction with effector cell

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Introduction. Dendritic cells (DC) are professional antigen presenting cells playing a central role in the development of immune responses and have been therefore implemented as vaccine either as monotherapy or in combination with other treatments. Recently it has become evident that the cellular metabolism is important for fate decision and consequent functional capabilities of immune cells, including DC. **Objective.** Aim of this study was to evaluate the influence of metabolic changes on the functionality of *in vitro* produced vaccine DC and possible ways to improve their anti-tumor efficacy.

Materials and methods. Monocytes purified from healthy donors were differentiated *in vitro* toward DC and then stimulated with various maturation cocktails. Resulting mature DC were characterized by flow cytometry for expression of costimulatory molecules as well as for metabolic properties, whereas their functionality was evaluated upon co-culture with autologous effector cells.

Results. Preliminary characterization of differently matured DC highlighted that DC stimulated with MPLA and IFNγ (Mγ-DC), that are potent stimulators of both Natural Killer (NK) as well as antigen specific $CD8^+$ T cells, have the highest lipid content and lowest glucose uptake. Addition of the anti-obesity drug Orlistat during maturation had limited consequences on their phenotype and functionality, whereas its presence throughout the differentiation process resulted in a highly reduced expression of costimulatory molecules. However, the DC were not functionally impaired, since they were still able to induce NK cells to degranulate to different tumor targets as well as to secrete IFNγ in equal of even higher amounts than untreated Mγ-DC. Evaluation of the secreting capabilities of the DC highlighted that the IL12p70 production was either unaffected or reduced upon differentiation in the presence of Orlistat whereas under these conditions IL10 secretion was highly reduced in all donors. Preliminary evaluation of the variability in Orlistat influence on DC functionality highlighted a possible role of the body mass index (BMI) of the donor with stronger activation of NK cells in those with a BMI>30.

Conclusions. Manipulation of the lipid metabolism in the protocol of vaccine DC production can improve their functional capabilities and should be further evaluated using other available inhibitors to dissect the mechanism.

O100

Mast cell and Dendritic cell communication and mutual arming to ensure acute host defense

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Mast cells (MCs) and dendritic cells (DCs) are essential innate sentinels at host-environment interfaces. While MCs remain stationary upon skin inflammation, DCs migrate to lymph nodes (LN) to prime effector T cells. Using longitudinal intravital multiphoton microscopy of DC/MC double reporter mice, we found that, before leaving the inflamed skin, DCs executed targeted and long-lasting interactions with MCs. These innate synapse-like contacts ultimately culminated in DC-to-MC protein transfer including MHCII complexes. Importantly, the extent of MHCII cross-dressing of MCs by DCs correlated with the subsequent *ex vivo* efficiency of MCs to prime T cells. Moreover, impeding MC cross-dressing by prior depletion of DCs diminished T cell-mediated skin inflammation.

On the other hand, we established a method to stain the intracellular secretory granules in intact MCs *in vivo* and found that upon skin inflammation dermal DCs ingest intact MC granules exocytosed by MC degranulation. DCs bearing MC granules showed a highly enhanced maturation and migration to draining LNs and a boosted T cell priming capacity. Most importantly, the engulfed MC granules were actively shuttled to skin draining LNs and finally degraded inside DCs within the lymphoid tissue. We consequently highlight a unique feature of peripherally released granule-embedded MC mediators to impact on LN borne adaptive immunity over distance by modifying DC functionality.

DOI: 10.1002/eji.201970300

Consequently, we identify an intercellular communication and mutual arming between motile DCs and resident MCs that might contribute to adaptive immunity priming on one hand and the acute defense potential at the skin barrier during critical periods of migration-based DC absence on the other hand.

O101

Repeated exposure to house dust mite drives persistent activation of inflammatorymacrophages

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Background: Airway macrophages are critical to pulmonary immunity and maintenance of lung function as they produce a wide array of pro-inflammatory cytokines and eicosanoids. Thus, aberrant or chronic macrophage activation can contribute to inflammatory diseases. Repeated exposure to microbes evokes an "innate memory", resulting in stronger responses or tolerance upon secondary exposure. Whether repeated contact to allergens persistently alters macrophage cytokine and eicosanoid profiles and how this impacts allergic inflammation remains unknown.

Method: Alveolar-like monocyte-derived macrophages (aMDM) were differentiated from CD14+ monocytes of healthy human volunteers. Cells were "trained" with house dust mite (HDM) for 24h before washout and re-stimulation a week later. Culture supernatants after initial, and before and after secondary exposure were analyzed by LC-MS/MS and multiplex cytokine assays. Metabolic profiles of trained aMDM were assessed using Seahorse technology.

Results: Training of aMDM with HDM resulted in an activated cell morphology, increased spare respiratory capacity and secretion of pro-inflammatory cytokines (IL-1β, IL-6, IL-10, TNFα) 24h after training, which mostly returned to baseline levels after 6 days. In contrast, CCL17 was initially induced by HDM and further increased with time (until day 13). Similarly, CXCL10 and microsomal prostaglandin E2 synthase (mPGES1) remained elevated in HDM-trained aMDM for 7 days. Treatment with a histone desacetylase inhibitor during training decreased PGE2 production of trained aMDM after 7 days. After re-exposure to HDM, trained aMDM produced more eicosanoids (PGE2, TXB2, LTB4 and 12-HETE) than acutely exposed aMDM.

Conclusion: HDM training of differentiated macrophages profoundly and persistently changed cytokine production. Increased metabolic capacity could enhance macrophage responsivity to inflammatory stimuli. The increased capacity to produce CCL17 could increase influx of TH2 cells into the lung leading to increased allergic inflammation. Augmented inflammatory cytokine responses and epigenetic modifications causing an altered PGE2/LTB4 ratio upon HDM re-exposure could perpetuate ongoing type 2 inflammation. Thus, targeting allergen-driven macrophage reprogramming could lead to new therapeutic options for allergic diseases.

O102

Genome-wide analysis of H3K27Ac and PU.1 binding patterns in human neutrophils activated *via* TLR8

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Question. The signalling pathways that regulate inducible genes by inflammatory stimuli have been extensively studied in macrophages or dendritic cells, but not in neutrophils. Herein, we aimed at extending our knowledge, at genome-wide level, of the molecular and epigenetic mechanisms controlling gene expression in human neutrophils activated *via* TLR8.

Methods. Human neutrophils, isolated from buffy coats by negative selection (99.7 ± 0.2 % pure), were stimulated *via* TLR8 for up to 20 h. Whole genome deposition of both histone H3 lysine 27 acetylation (H3K27Ac) and the transcription factor (TF) PU.1 were investigated by chromatin immunoprecipitation assays followed by high-throughput sequencing (ChIP-seq), while transcriptomes were obtained by RNA-seq.

Results. We have previously shown that the R848 imidazoquinoline represents a potent activator of human neutrophils *via* the exclusive binding to TLR8. In particular, we reported that, through chromatin remodelling events, R848 triggers the expression of IL-6, a cytokine otherwise poorly induced by other inflammatory stimuli in human

DOI: 10.1002/eji.201970300

neutrophils. Analysis at a whole genome level of the distribution of H3K27Ac, which marks active genomic regulatory regions, and the recruitment of PU.1, a lineage-determining TF, revealed that neutrophils profoundly reprogram their chromatin status in response to R848. Moreover, we were able to identify and characterize thousands genomic regions, defined as latent, that upon stimulation with R848 acquire H3K27Ac and PU.1 binding to promote gene transcription. By motif discovery analysis, we found that these regulatory regions are enriched in NF-κB and AP-1 motifs, usually observed in TLR stimulated cells, but also in PU.1 and CEBP motifs, TFs specific of the myeloid compartment.

Conclusions. In this study, by a whole genome level approach, we demonstrate that human neutrophils, even if considered terminally differentiated cells with poor transcriptional capacity, can reorganize their chromatin upon TLR8 stimulation, in turn modifying a vast repertoire of target genes.

Regulatory and helper T cells II (O103-O108)

O103
Comparison of co-inhibitor expressing CD4$^+$ T cell subsets from patients with severe and uncomplicated malaria and healthy donors

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Introduction: During the life cycle of *Plasmodium falciparum* (*Pf*), the parasite that causes malaria, two different stages occur in the human host: the liver and the blood stage. During the asymptomatic liver stage CD8$^+$ T cells are activated and are able to recognize plasmodium specific antigens presented by infected hepatocytes. The blood stage is characterized by a massive activation of CD4$^+$ T cells which produce proinflammatory cytokines like IFN-γ and TNF-α which are associated with severe malaria. Other subsets of these malaria-induced CD4+ T cells express various co-inhibitory molecules like CTLA-4, PD-1, TIGIT, TIM-3 and LAG-3 and show regulatory properties. One subset co-expresses LAG3 and Cd49b, recently identified markers of Type 1-regulatory T cells (Tr1 cells).

Objectives: We hypothesize that the induction of regulatory T cells during the blood stage of malaria might prevent an overwhelming inflammatory response but might also restrict the development of protective immunity against the liver-stage of the disease.

Patients & Methods: To compare the different CD4$^+$ T cell subsets of patients with severe and uncomplicated malaria and healthy donors in an endemic region, a study in Ghana was conducted. Whole blood samples of *Pf*-infected children and healthy donors were analyzed via flow cytometry and Hierarchical Stochastic Neighbour Embedding (HSNE).

Results: The HSNE Analysis revealed an induction of CD4$^+$CD49$^+$LAG-3$^+$ T cells (Tr1-cells) only in patients with uncomplicated malaria. These cells also show high expression of the co-inhibitory molecules PD-1, TIGIT and TIM-3 and of the chemokine receptor CCR5, indicating that these are additional markers for human Tr1-cells. The CD8$^+$ effector-T cells of patients with uncomplicated malaria were not reduced in number, but showed a higher expression of LAG-3 compared to the cells of patients with severe malaria. LAG-3 was described to be a marker of exhaustion on CD8$^+$ T cells indicating an impaired effector-T cell function in patients with uncomplicated malaria.

Conclusion: The induction of regulatory Tr1 cells in malaria patients might not only lead to a prevention of severe symptoms but also to an impaired effector-T cell function which could prevent the development of a long-term immunity, making these cells a putative target for different strategies of immune therapies.

O104
Antigen-specific Helios$^-$, Neuropilin-1$^-$ Tregs induce apoptosis of autoreactive B cells via PD-L1.

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DOI: 10.1002/eji.201970300

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Regulatory T cells (Tregs) maintain self-tolerance and prevent autoimmunity by controlling autoreactive T cells. We recently demonstrated in vivo that Tregs can directly suppress auto-reactive B cells via programmed death ligand 1 (PD-L1) that ligated PD-1 on B cells and caused them to undergo apoptosis. Here, we asked whether this mechanism is utilized by thymus-derived natural Tregs and/or by peripheral lymphoid tissue-induced Tregs. We first demonstrated that antigen-specific PD-L1-expressing Tregs were induced in the draining lymph node of autoantigen-expressing tissue and characterized them by their lack of the transcription factor Helios and of the surface marker Neuropilin-1 (Nrp-1). Next, we established an in vitro co-culture system to study the interaction between B cells and Treg subsets under controlled conditions. We found that Nrp^- Treg, but not Nrp^+ Treg suppressed autoreactive B cells, whereas both were able to suppress T-helper cells. Such suppression was antigen-specific and was facilitated by PD-L1/PD-1-induced apoptosis. Furthermore, it required physical cell contact and was MHC II-restricted, providing an explanation for the antigen-specificity of peripherally-induced Tregs. These findings identify a role for peripherally induced $Helios^-$ $Nrp\text{-}1^-$ inducible Treg in controlling peripheral B-cell tolerance against tissue auto-antigens.

O105

Regulatory T cells oscillate between a TNF-producing and a TNFR2-expressing status, and TNF-TNFR2 positive loop sustains Treg proliferation.

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Question: Tumor necrosis factor alfa (TNFα) is a pleiotropic cytokine traditionally considered a major actor in inflammation, but some studies highlighted the TNFα capacity to exert also anti-inflammatory and immunomodulatory effects. It has been described that TNFα promotes survival, proliferation and effector function of regulatory T cells (Treg), a CD4 T cell subset with immune suppressive function that constitutively express TNF receptor 2 (TNFR2), and that can upregulate it at high levels in condition of activation and especially in tumor tissue, where Treg are expanded. Moreover, there are some pieces of evidence that Treg themselves are able to produce TNFα and this may support their proliferation.

Here, we want to dissect the possible role and the effects of TNFα production by Treg on their expansion and function, especially in tumor context in which Treg are potentially more responsive to this cytokine.

Methods: A multiparametric flow cytometry approach and the isolation of TNFR2+Treg from Foxp3YFPcre mice, allowed to characterize TNFα-TNFRs axis in Treg subsets from human blood, different organs of wild type mice and murine tumor models ex vivo, but also in vitro, in proliferation assays and in culture experiments in presence of a TNFα blocking antibody.

Results: Our data confirmed TNFα production by human and murine Treg after short restimulation, and showed that TNFα neutralization in vitro suppressed Treg proliferation and reduced TNFR2 expression, suggesting the existence of a positive feedback mechanism. Interestingly, we found a mutually exclusive expression of TNFα and TNFR2 in Treg, and the culture in vitro of YFP+TNFR2+ and YFP+TNFR2- sorted Treg revealed significant differences between these populations: TNFR2+Treg were more proliferative and expressed TNFα at lower level than the counterpart, while TNFR2-Treg upregulated TNFR2 in culture while decreasing their TNF production. In tumors, Treg showed a general TNFα and TNFR2 increased expression, but also in this context we recognized different TNFα+ and TNFR2+Treg subsets, the latter with higher expression of activation markers.

Conclusion: Our data indicate the existence of different functional states of Treg with different ability to produce and sense TNFα, that may promote a circuit of cell maintenance that may be essential in condition of Treg expansion, like in tumor microenvironment. This mechanism may endorse the pro-tumoral effects of TNFα-TNFRs axis described in literature.

O107

Blimp1 prevents methylation of *Foxp3* and loss of regulatory T cell identity at sites of inflammation

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DOI: 10.1002/eji.201970300

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Introduction: Foxp3+ regulatory T (Treg) cells restrict immune pathology in inflamed tissues; however, an inflammatory environment presents a threat to Treg cell identity and function.

Objectives: To identify regulatory pathways in Treg cells that preserve their cellular identity in the face of inflammation in vivo.

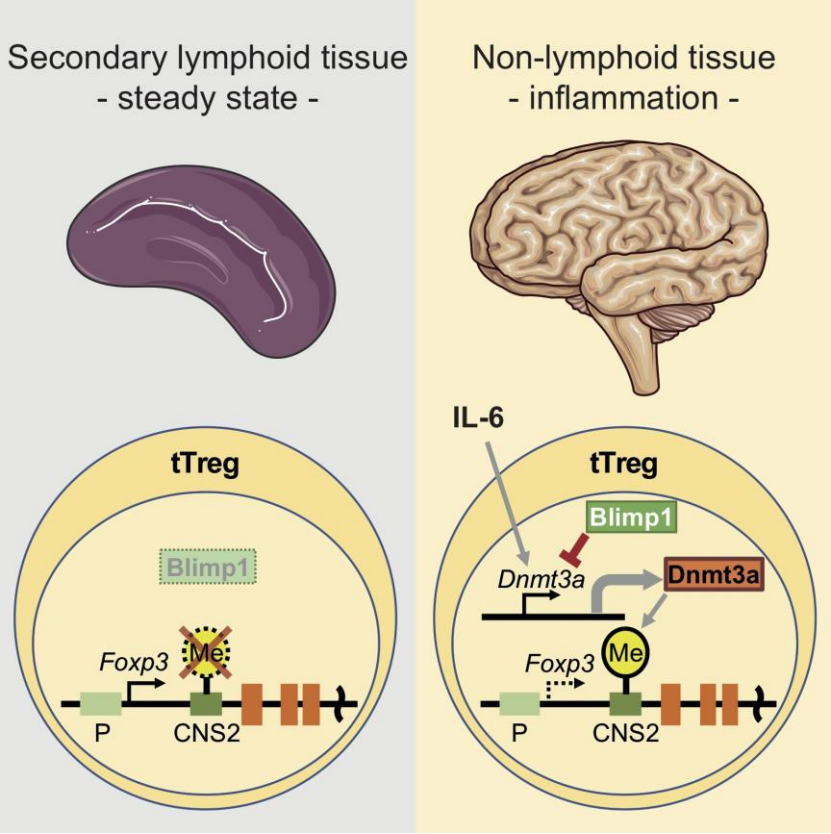
Methods: We combined genome-wide transcriptional and epigenetic profiling of Treg cells in the central nervous system (CNS) and in the secondary lymphoid tissue during experimental autoimmune encephalomyelitis to identify transcriptional modules selectively upregulated in the CNS.

Results: We identified the transcriptional modulator Blimp1 (encoded by *Prdm1*) to be expressed in the majority of Treg cells in the CNS while only a minority of Treg cells in the secondary lymphoid tissue was Blimp1+. In the CNS, Blimp1 was induced in Treg cells in part in a STAT1 dependent manner. Expression of Blimp1 in CNS Treg cells was indispensable for the maintenance of high Foxp3 levels in CNS Treg cells since CNS Treg cells with a conditional ablation of *Prdm1* downregulated Foxp3 in mixed bone marrow chimeric mice. However, *Foxp3* was not a direct transcriptional target of Blimp1. Rather, Blimp1 suppressed the expression of the methyl transferase Dnmt3a, which contributed in *de novo* methylating the non conserved sequence 2 in the first intron of the *Foxp3* locus, which is also known as "Treg cell specific demethylated region (TSDR)" since it needs to be demethylated in stable Treg cells. Thus, Blimp1 negatively regulated the IL-6- and STAT3-dependent Dnmt3a expression and function restraining methylation of TSDR in the *Foxp3* locus. In the absence of Blimp1, TSDR was methylated, Foxp3 expression was lost. Blimp1-deficient Treg cells abandoned their identity and gained toxic functions by expressing (among others) IL-17.

Conclusion: Blimp1 in Treg cells is instrumental in preserving Treg cell stability in inflamed non-lymphoid tissues.

DOI: 10.1002/eji.201970300

Figure 1



O108

m6A deficiency on mRNAs by T cell-specific depletion of Wtap causes spontaneous colitis.

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Introduction: Post-transcriptional gene regulation involves a number of molecular mechanisms. m6A (N6-methyladenosine) is the most prevalent modification in mRNAs that affects mRNA splicing, translation and stability, and has recently been involved as a new mechanism to regulate a number of cellular functions.

Objectives: The molecular details and the physiological significance of m6A modification on mRNAs in T cells remain unclear. Our analysis aims to discriminate the role of Wtap and m6A modification in T cells and to unravel the molecular mechanisms underlying T cell-driven pathology.

"Materials & Methods": We investigate a conditional knock-out mouse model and determine the function of Wtap in a T cell-specific manner.

Results: We show that genetic inactivation of Wtap, an accessory protein of the methyltransferase complex, causes a loss of m6A (N6-methyladenosine) modification. Importantly, young mice lacking Wtap in peripheral T cells spontaneously develop a colitis phenotype and display shortening of the gut. Their CD4+ T cells show reduced abundance in the periphery but produce proinflammatory cytokines in the colon. These data indicate a specific role of Wtap in Treg cells and/or conventional T cells. We also investigated Rorgt+Helios–Foxp3+ Treg cells, a specialized subpopulation, that are induced from conventional T cells in response to commensal antigens in the colon. Interestingly, these induced Treg cells are less frequent in the mutant mice, providing one possible explanation for the observed intestinal inflammation. We are currently analyzing the cell-intrinsic effects of Wtap deficiency on

DOI: 10.1002/eji.201970300

conventional T cells and Treg cells by utilizing inducible deletion mediated by a CD4CreERT2 or subset-specific deletion by a Foxp3-Cre transgene.

Conclusion: Our data involve a new layer of post-transcriptional gene regulation by the epi-transcriptome in the control of T cell homeostasis and Treg cell differentiation.

Immunometabolism and immunosignaling (O111-O113)

O111
Spermidine is a novel modulator of Src Kinase-mediated signaling pathway in dendritic cells

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Introduction. Polyamines (i.e. putrescine, spermidine and spermine) are highly bioactive polycations capable of modulating several signaling pathways. Although polyamines functions have been mainly studied in tumors, it has been recently shown that spermidine can be produced by, and exert effect on, dendritic cells (DCs; professional antigen-presenting cells of the immune system). Specifically, spermidine promotes the non-enzymatic functions of indoleamine 2,3-dioxygenase 1 (IDO1), an immunoregulatory molecule endowed of both catalytic activity and signal transducing properties due to the presence of phosphorylable ITIMs (immunoreceptor tyrosine-based inhibitory motifs).

Objective. To dissect the specific mode of action of spermidine in DCs.

Methods. Molecular modeling approaches, mutagenesis and co-immunoprecipitation studies were used to evaluate the key residues on Src kinase involved in the interaction with the activator (namely, spermidine) and with the substrate (i.e., IDO1).

Results. We found that spermidine directly activates Src kinase that, in turn, phosphorylates IDO1 and thus confers immunoregulatory properties on DCs. In particular, spermidine-treated DCs foster the differentiation of T cells into LAP^+Foxp3^+ regulatory T lymphocytes in IDO1-dependent fashion. At molecular level, we found that Src kinase directly interacts with the substrate IDO1. Moreover, we identified a stretch of negative surface on the SH2 domain of Src kinase involved in the binding with spermidine. Specifically, when the key amino acidic residues are mutated, the interaction between Src and spermidine is lost as well as the substrate binding and catalytic activation of the enzyme is blunted.

Conclusion. Our data suggest that spermidine may act as positive allosteric modulator of Src kinase. Moreover, although it has been previously shown that IDO1 undergoes tyrosine phosphorylation, we demonstrated, for the first time, that this is a direct effect through physical interaction between Src and IDO1. Overall, this study may pave the way toward the design of novel allosteric modulators able to switch on/off the Src-mediated pathways, including those involving the immunoregulatory protein IDO1.

O112
Type-I interferon regulates ghrelin-levels leading to anorexia and weight loss while promoting inflammatory immune responses

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DOI: 10.1002/eji.201970300

The acute phase of immune responses is often associated with a loss of appetite (anorexia) and body weight. Although anorexia is one important facet of sickness behavior, its link to immunity is only poorly understood. Here we demonstrate that cells of the innate immune system such as plasmacytoid dendritic cells release type-I-interferon (IFN-1) to communicate with the endocrine system. Interestingly, even the ablation of pDCs via the DTR system increased IFN-1 and IL-6 levels, indicating that the application of DT (Diphtheria Toxin) in BDCA-2-DTR mice is inducing inflammation. Here we identify the mechanism how IFN-1 initiates a decrease of the hunger hormone ghrelin - leading to anorexia, reduction in body weight and hypoglycemia.

In further studies we analyzed the relevance of this regulation during immune responses. We found that the efficiency of CD8 T cell priming is critically influenced by this hormonal axis. These results unravel the tight crosstalk between early IFN-1 driven innate immunity and the endocrine system and the feedback pathway of hormones to the immune system.

We think that besides cytokines, hormones act in more distant action to steer body responses and adjust behavioral action to energetic immune challenges.

O113

Mast Cells Trap Neutrophils to Recover from Degranulation and to Overcome Nutrient Limitations

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Introduction: Anaphylaxis is characterized by massive mast cell degranulation and accompanied tissue influx of neutrophils. During degranulation mast cells release the majority of their cellular protein content. However they survive degranulation and start to restore their granules in nutrient-deprived inflammatory tissues.

Objectives: The recovery of exhausted mast cells after degranulation and the dynamic behavior of neutrophils in anaphylactic tissues are poorly characterized. Here we investigated direct interactions of exhausted mast cells with neutrophils during anaphylaxis. Finally we analyzed whether and how neutrophils modify the recovery of mast cells.

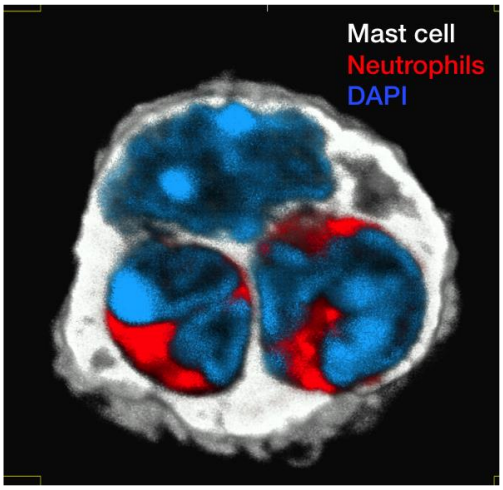
Material & Methods: We performed state-of-the-art intravital and extensive live cell imaging to characterize cell-cell interactions during and after degranulation of primary mouse mast cells. Further we used Ribotag precipitation and RNAseq analysis with co-cultivated cells to identify the impact of neutrophils on recovering mast cells. After we confirmed candidate hits with various tests on mast cell functionality and survival.

Results: Heavily degranulating mast cells attract neutrophils by releasing LTB4, which can lead to the trapping of neutrophils inside mast cells. The fast migrating neutrophils interact with mast cell degranulation channels, actively push through plasma membrane and actin cortex, and become ultimately trapped in large intracellular mast cell granules (Fig.1). We call this novel form of cell-in-cell structure "Mast Cell Intracellular Trap" (MIT), in which mast cells and trapped neutrophil are both viable in the first 12 hours after MIT formation. However, mast cells start to kill and degrade trapped neutrophils by ATPase-dependent acidification, which helps them to gain lipids from neutrophils. Therefore, mast cells with trapped neutrophils recover faster and are less dependent from environmental nutrient availability.

Conclusions: The invasion of a living cell into the cytoplasm of another cell (entosis) has gained much attention in cancer cell biology. By studying degranulating mast cells, we here show for the first time that immune cells employ an entosis-like process to promote its regeneration and regain functionality. In addition we define a new function of neutrophils during anaphylaxis, which is the supply of nutrients to recovering mast cells. Our studies may have potential implications for chronically activated mast cells in mast-cell related immune disorders.

DOI: 10.1002/eji.201970300

Figure 1



Immunotherapy (O115-O120)

O115
Reprogramming Macrophages using Toll Like Receptor Ligands-loaded Polymeric Nanoparticles for the treatment of Lung Cancer

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Introduction: Tumor-associated macrophages (TAMs) play a key role in tumor progression, metastasis, and recurrence after treatment. In the tumor microenvironment of many solid tumors, such as lung cancer, they acquire an immunosuppressive phenotype, preventing the immune system to fight against cancer cells. Toll like receptor (TLR) ligands are well-known immunostimulants, however their ability to reprogram TAMs from their M2-tumor-promoting phenotype towards an M1-antitumor one has still not been demonstrated *in vivo*.

Objectives: Here, we studied the ability of TLR3 and TLR7/8 ligands (Poly I:C and Resiquimod [R848]), both as free drugs or loaded in TAM-targeting polymeric nanoparticles (NP), to re-educate macrophages into cytotoxic effectors.

Methods: Polarization of macrophages has been investigated *in vitro* in primary human and murine M-CSF-differentiated macrophages. Their production of cytokines and nitric oxide (NO) was quantified and an *in vitro* growth inhibition assay was performed to measure the cytotoxic activity of "re-educated-macrophages" on cancer cells (PANC-1 and CMT 167). Finally, immunocompetent murine models of lung cancer have been developed and optimized for preclinical evaluation of both free drugs and TAM-targeting-NPs

Results: Primary human and murine macrophages exposed to Poly I:C and R848 produced significant levels of NO and pro-inflammatory cytokines, such as CXCL10 and CCL5. Both drugs increased the cytotoxic activity of macrophages toward cancer cells. *In vivo*, they were able to slow the growth of a subcutaneous lung cancer model. Interestingly, Poly I:C and R848 were shown to synergize both *in vitro* and *in vivo* in murine models. Polymeric NPs loaded with Poly I:C and R848, presented spherical shape, size around 100 nm, were free of LPS and have a satisfactory toxicological profile. Compared to the free drugs, the NPs showed a superior capacity to induce an antitumor M1 macrophage phenotype *in vitro*. Further studies are required to evaluate their effect on tumor growth *in vivo*.

Conclusions: Poly I:C and R848 are effective inducers of an antitumor M1 macrophage phenotype and can be further improved by encapsulation into TAM-targeting-nanoparticles. Furthermore, combination treatments with TLR agonists may offer new opportunities for macrophage-based cancer immunotherapy.

DOI: 10.1002/eji.201970300

O116

Polymer-mediated tumour immunotherapy by *in situ* activation of antigen presenting cells

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Introduction: Immunotherapy has become a promising tool to treat cancer as shown by the use of checkpoint inhibitors like anti-PD-1 or anti-CTLA-4. However, dependent on the tumor entity, there are still high recurrence rates and many patients suffer from immune related adverse events. Consequently, there is still a high medical need for the development of tumor-specific immunotherapies.

Objectives: Nanoparticles (NP) play an important role as carrier systems when developing new strategies for targeted immunotherapies. Here, we want to establish new nanogel-formulations covered with immunostimulatory drugs and tumor antigens as a tool for an antigen-specific and targeted immunotherapy.

Materials and methods: We designed pH-sensitive nanogel formulations which are functionalized by covalent binding of the TLR7-agonist IMDQ and the model antigen OVA, both fluorescently labeled. For all analyses, differentially functionalized NP were injected i.v.. For biodistribution analyses, splenocytes were analyzed by flow cytometry 24h after injection. Effects of NP on BMDCs were assessed by flow cytometry and [3H]-Thymidine proliferation assay. Antigen-specific antibody production and induction of CTLs were determined by ELISA and IFNg-ELISpot analyses. Finally, we performed prophylactic and therapeutic tumor experiments by inoculation of OVA expressing MC38 colon adenocarcinoma cells s.c. into the flank of mice.

Results: I.v. application of NP resulted in their accumulation in the spleen with OVA-positive macrophages and B cells. Additionally, we found an uptake of NP in BMDCs as well as an enhanced BMDC maturation status. Interestingly, we observed a higher CD8+ T cell response after treatment with NP which were functionalized through covalent binding of IMDQ and OVA (NP(IMDQ+OVA)) compared to a higher CD4+ T cell response after treatment with NP to which IMDQ was covalently bound but OVA added in a soluble form (NP(IMDQ)+sOVA). In line with these results, we could show an increased antibody production in mice which were immunized with NP(IMDQ)+sOVA and an increased CD8+ T cell response in mice treated with NP(IMDQ+OVA). Finally, we observed a significantly reduced tumor growth in mice immunized with NP compared to PBS treated mice.

Conclusion: Taken together, these results show that functionalized NP were able to induce strong antibody-production, CD4+ and CD8+ T cell responses and therapeutic effects in mice carrying OVA-expressing tumors.

O117

Targeting of CXCR3 improves anti-tumor efficacy of activated natural killer cells *in vivo*

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Introduction: The peculiar multiple myeloma microenvironment, characterized by up-regulated levels of several inflammatory chemokines, including the CXCR3 receptor ligands CXCL9 and CXCL10, limits NK cell positioning into the bone marrow by interfering with CXCR4 function. It is still unclear if the consequent reduced influx of transferred cells into the tumor represents a potential limiting factor for the success of NK cell-based adoptive therapy.

Objectives: We hypothesize that inhibition of CXCR3 function on NK cells will result in increased tumor clearance, due to higher NK cell bone marrow infiltration.

Material and methods: Since different activation protocols differently affect expression and function of homing receptors, we analyzed the bone marrow homing properties and anti-tumor efficacy of NK cells stimulated *in vitro* with two independent protocols. NK cells were purified from wild-type or *Cxcr3-/-* mice and incubated with IL-15 alone or with a combination of IL-12, IL-15, IL-18 (IL-12/15/18). Alternatively, CXCR3 function was neutralized *in vivo* using a specific blocking antibody. NK cell functional behavior and tumor growth were analyzed in bone marrow samples by FACS analysis.

DOI: 10.1002/eji.201970300

Results: Both activation protocols promoted degranulation and IFN-γ production by donor NK cells infiltrating the bone marrow of tumor-bearing mice, although IL-15 promoted a faster but more transient acquisition of functional capacities. In addition, IL-15-activated cells accumulated more in the bone marrow in a short time but showed lower persistence *in vivo*. Targeting of CXCR3 increased the bone marrow homing capacity of IL-15 but not IL12/15/18 activated NK cells. This effect correlated with a superior and durable myeloma clearance capacity of transferred cells *in vivo*.

Conclusions: Our results demonstrate that *in vitro* activation affects NK cell anti-myeloma activity *in vivo* by regulating their BM infiltration. Furthermore, we provided direct evidence that CXCR3 restrains NK cell anti-tumor capacity *in vivo* according to the activation protocol used, and that the effects of NK cell-based adoptive immunotherapy for multiple myeloma can be improved by increasing their bone marrow homing through CXCR3 inhibition.

O118

Emerging Immuno-Oncology Targets for Glioma and Multiple Sclerosis

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Introduction: Dysregulation of immune checkpoint molecule (ICM) signaling is one of the immune evasion mechanisms exerted by many tumors such as glioma. There are recent ongoing immunotherapy clinical trials for gliomas, but when targeting the CNS tumors the main challenge is to balance the immune response to prevent autoimmune reactions. The severest autoimmune disease of the CNS is Multiple Sclerosis (MS). Like there are tumor reactive t cells in glioma, myelin-specific cytotoxic CD8+ T cells were found in the brains of MS patients, which were indicated to play an important role in the immunopathology of MS.

Objectives: We hypothesize that dysregulated ICM signaling on oligodendrocytes can contribute to glioma immune evasion and may modulate autoreactive CD8+ T cell activity in MS patients. Our aim is to identify novel therapeutic targets on oligodendrocytes that could potentially play a role in the immunopathology of glioma and MS.

Methods & Results: To identify novel MS-glioma associated ICMs, we performed a high-throughput screen by co-culturing Flu-specific CD8+ T cells with human oligodendrocyte cell line MO3.13-A2-Luc cells that are transfected with a siRNA library consisting 4160 genes. The impact of gene knockdown on T-cell cytotoxicity was measured using a luciferase readout system. Out of top 150 HITs we selected 56 surface HITs and performed a secondary screen both using MO3.13-A2-Luc and primary glioma cells. We validated 7 overlapping HITs that increase T cell mediated killing upon knockdown in target cells. One of our HITs ANM1 (masked name) is a single-pass membrane protein and it is a ligand for a RTK, which is also identified as a HIT in the screen. ANM1 is significantly up-regulated in different GBM types according to TCGA dataset and its higher expression correlates with worse survival in glioma patients. Our cytokine assays revealed that IFNγ and TNFα secretion by T cells strongly decreased when they are co-cultured with ANM1 downregulated oligodendrocytes. We also showed that overexpression of ANM1 on oligodendrocytes decreased FluT mediated killing. Right now we are working further on the impact of ANM1 and its receptor on T cell functionality.

Conclusions: We established an *in-vitro* co-culture model for MS and glioma to identify novel ICM molecules that play a role in the antigen-specific CD8+ T cell mediated glial cell killing. ANM1 is one of the candidate ICMs that can be used as a target in the immunotherapy of glioma and MS.

O119

Re-activation of anti-viral immune cells and antigen reduction enables clearance of chronic HBV in mice

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DOI: 10.1002/eji.201970300

Chronic infection with the hepatitis B virus (HBV) is caused by weak and dysfunctional T-cell responses. The mechanisms responsible for failure of the immune response to clear HBV from infected hepatocytes remains largely unknown. Persistent high viral antigen load is believed to impair T-cell function. To study the link between viral antigen load and T-cell immunity, we developed a new HBV infection model in mice using recombinant adenoviral transfer of an expression cassette with 1.3 overlength HBV genome linked to luciferase gene via a P2Asite (Ad-HBV-Luc) that allows for daily, sensitive measurement of viral antigen levels in hepatocytes by bioluminescence imaging. In this model, we found that immune-mediated clearance of HBV-infected hepatocytes occurred depending on the level of presented antigen. A high infectious dose results in chronic infection associated with low numbers of HBV-specific CD8 T cells with a dysfunctional phenotype. These T-cells are characterized by strong and stable upregulation of inhibitory surface receptors (PD1, TIGIT, Lag3) and the transcription factor TOX and a weak *ex vivo* killing capacity. However, animals infected with a lower infectious dose mount a strong and functional HBV-specific immune response and eliminate HBV infection within 3 weeks. The reduction of antigen load in chronically infected animals by hepatocyte-specific expression of IFNβ induces a stable and robust HBV-specific CD8 T cell response. Immediately after IFNβ-expression HBV antigen levels are reduced leading to a CD8 T cell mediated elimination of infected hepatocytes starting at day 2 after IFNβ-expression. In those mice numbers of HBV-specific CD8 T cells are strongly increased, expression of inhibitory receptors is reduced and activation marker (KLRG1, CD69) are induced compared to mice with sustained high levels of HBV-antigen. In addition, the killing capacity *ex vivo* is restored in mice with hepatocyte-specific expression of IFNβ. Our data now show, that both, antigen reduction and boosting the immune response, is necessary to overcome chronic HBV infection.

O120

IL-9 is an immunotherapeutic target of Collagen Induced Arthritis

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Question: IL-9 was demonstrated to be a mediator of tissue damage in psoriatic and rheumatoid arthritis. In this study we aimed to evaluate the possible immunomodulatory role of antibodies neutralizing IL-9 in mice developing Collagen Induced Arthritis (CIA).

Methods: DBA/1 mice were immunized with collagen and Freund's complete adjuvant (CFA) at the base of tail to induce chronic arthritis. Anti-IL-9 was injected at the base of the tail after arthritis" onset (Group A) or in the same day of sensitization and in the day of challenge (Group B) of CIA mice. An histological analysis was performed in joints of diseased mice. Paired splenocytes were also analysed by cytofluorimetric analysis in the different experimental groups.

Results: IL-9 was over-expressed in murine swollen joints of CIA mice. Anti-IL-9 treatment efficiently down-modulated joint inflammation in mice treated after arthritis onset (Group A) conversely, it delayed the appearance of arthritis signs when it was administrated in the same day of sensitization and in the day of challenge. A decrease of CD4+ TNF-a+ and an increase of CD4+FOXP3+IL10+ among splenocytes, was detected in Group A CIA mice after treatment (week 8). Additionally, Pu.1 deficient mice were protected by CIA.

Conclusions: In CIA neutralization of IL-9 reduces joint swelling.Furthermore, the decrease of tissue damage was associated to increase of FOXP3+IL10+Tregs indicating IL-9 as a possible immunotherapeutic target for arthritis.

B-cell biology (P1-P28, P407)

P1

The role of Krüppel-Like-Factor 2 (KLF2) in plasma cell homeostasis

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Introduction: Krüppel-Like-Factor 2 (KLF2), as a key regulator of cell differentiation and organ development, regulates quiescence, migration and homing of B cells, plasmablasts (PB) and plasma cells (PC). Upon boost

DOI: 10.1002/eji.201970300

immunization, KLF2-deficient mice show a striking reduction in the number of antigen-specific IgG plasma cells in the bone marrow.

Objectives and Methods: To elucidate the role of KLF2 on plasma cell homeostasis in more detail, we analyzed CD138+/TACI+ PB/PC subpopulations in various lymphatic organs such as spleen, bone marrow (BM) and gut associated lymphoid tissues (GALT) for their Immunoglobulin (Ig)-isotype as antibody secretion by flow cytometry and Elispot.

Results and Conclusion: We found that frequencies of IgM-, IgA- and IgG-producing CD138+/TACI+ PBs and PCs are clearly altered in BM and spleen of KLF2-deficient mice. The vast majority of PBs and PCs in spleen and BM of KLF2-deficient mice were IgM positive whereas IgA-producing PBs/PCs were virtually absent. In addition, lowered serum IgA as well as fecal (s)IgA levels observed in KLF2-deficient mice indicate a crucial role of KLF2 for migration and/or survival of class switched plasma cells or in IgA processing/secretion.

Outlook: To address the systemic consequences of the dysregulated PB/PC distribution in KLF2-deficient mice, we are currently investigating GALT-dependent immune responses by immunization with recombinant Flagellin (sFliC), an antigen known to trigger mucosal IgA and systemic IgG responses. Furthermore, we are performing transfer experiments with KLF2-deficient PB/PC to analyze their migration and homing behavior. Moreover, we are analyzing gene expression profiles of PB and PC subsets from KLF2-deficient mice compared to their wildtype-cre controls, to reveal the regulatory networks and signaling pathways controlled by KLF2.

This work was supported, in part, by the Deutsche Forschungsgemeinschaft (DFG) through research grant TRR130 to Hans-Martin Jäck and Wolfgang Schuh and GRK1660 to H.M. Jäck

P2

SLy2-dependent Modulation of B-Cell Immune Responses towards Pneumococcal Antigens

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The immunoinhibitory adapter protein SLy2 (Src homology domain 3 lymphocyte protein 2) is located on human chromosome 21 and belongs into a group of additionally amplified genes in Down"s syndrome. Among others, invasive pneumococcal infection is one of the leading causes of death concerning Down"s syndrome patients. SLy2 has been reported to be a crucial regulator of B-1 cell function and activity, thereby influencing immune responses towards pneumococcal antigens. To further elucidate SLy2-mediated modulation of B cell immunity, we have created SLy2-transgenic (tg) and SLy2-knockout (ko) mice. If compared to wild type mice, SLy2-tg mice display significantly reduced levels of natural serum-IgM as well as decreased numbers of B-1 cells.

In this study, SLy2-tg and SLy2-ko mice will be immunized with pneumococcal vaccine (Pneumovax23 and Prevenar13) and a phenotypic profile of consequent B cell responses will be assessed using flow cytometry. Complementary, serum-antibody levels will be examined and compared. In addition, we aim to perform survival rate analysis of SLy2-tg and SLy2-ko mice during invasive pneumococcal infection to investigate their resistance towards S*treptococcus pneumoniae* with or without preceding immunization.

In summary, our studies aim to enhance the understanding of the role of the adaptor protein SLy2 during pneumococcal infection and give indications about its possible contribution to the susceptibility towards S*treptococcus pneumoniae* that is given in Down"s Syndromes patients.

P3

The role of BH3-only proteins in lymphocyte development and function

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Lymphocyte survival, function and development depend on the balance of anti-apoptotic and pro-apoptotic Bcl-2-family proteins (such as pro-apoptotic Bim and anti-apoptotic Bcl-2, A1 and Mcl-1) that control the mitochondrial apoptosis pathway. Leukemia, lymphoma or immunodeficiencies can develop from abnormally surviving immune cells already at early developmental stages, and dysregulation of mitochondrial apoptosis may play an important role here. BH3-mimetic drugs such as the Bcl-2/Bcl-XL/Bcl-w-inhibitor ABT-737 or the Bcl-2-inhibitor ABT-199/ Venetoclax can

DOI: 10.1002/eji.201970300

block individual anti-apoptotic Bcl-2-family proteins. Here we investigated the role and importance of anti-apoptotic Bcl-2 family proteins and of BH3-only proteins for life and death regulation of lymphocyte progenitors and early differentiating B cells. We used a conditionally active Hoxb8-oncogene to establish mouse lymphoid-primed multipotent progenitor (LMPP) like cells that can be differentiated towards myeloid or lymphoid cells in vitro. We have generated cell lines that are single and double deficient for the major pro-apoptotic BH3-only proteins. We treated the progenitor cells with selected Bcl-2-family inhibitors, alone or in combination, and tested for the induction of apoptosis. Specific inhibition of individual anti-apoptotic proteins revealed striking differences in apoptosis induction between distinct progenitor cell types and differentiation stages. We show that ABT-737 (inhibiting Bc-2 and Bcl-XL) is able to kill progenitor cells already at low doses and that combined treatment with an Mcl-1 inhibitor leads to elevated cell death rates. ABT-199 (inhibiting Bcl-2) but not A-1155463 (inhibiting Bcl-XL) had similar pro-apoptotic activity. Deletion of the BH3-only protein Bim protected progenitor cells against ABT-737, ABT-199 and Mcl-1 inhibitor treatment at low doses. It is known that neutrophils require Mcl-1 for their development. Conversely, our data suggest that early lymphocyte progenitors depend on Bcl-2. These data are relevant for understanding lymphoid cell development and may be a basis of therapeutic decision when Bcl-2-family antagonists are used in tumour treatment.

P4

Polymorphic variants change activity and function of human BAFF-receptor.

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B-cell-activating factor of tumor necrosis factor family (BAFF) is a cytokine which has indispensable pro-survival activity for the development of B cells. It binds to three surface receptors expressed by B cells. One of these receptors termed BAFFR is expressed from the transitional B cell stage on. BAFF binding to BAFFR triggers activation of the non-canonical NF-kB pathway, the phosphorylation of c-Jun activating kinases (JNKs), and induces an increase in glycolysis, protein synthesis and metabolic fitness through the PI3K-AKT-mTORC1 axis. While the deletion of the BAFFR-gene in humans causes severe B-lymphopenia and agammaglobulinemia, the impacts of many single nucleotide polymorphisms (SNPs) found in the human population causing missense mutations in BAFFR remains unclear. To elucidate the causal relation between the BAFFR variants and their impact on BAFFR function, we established an experimental model system based on their expression in the Burkitt's lymphoma cells. Analyzing the recruitment of TRAF2/3/6 as well as the activation of NF-κB2, PI3K and JNK signaling pathways we were able to correlate different BAFFR missense mutations with changes in BAFFR signaling and the pro-survival activity of BAFFR. Based on these results we can now predict if the different BAFFR variants would promote the development of immunodeficiency or autoimmunity.

P5

Investigation of the Association between the Chemokine Receptor CXCR4 and the B Cell Receptor (BCR) Isotype IgM or IgD in Healthy and CLL Patient Samples

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Introduction: Chronic lymphocytic leukemia (CLL) is the most frequent type of leukemia in western countries. It is an age-associated disease in which signaling trough the B cell receptor (BCR) is deregulated. For predicting clinical outcome of CLL patients besides BCR, the expression of chemokine receptors such as CXCR4 is having a prognostic value.

Objectives: It is already known, that CXCR4 is expressed at different stages of B cell development. In contrast, the interaction between the BCR isotypes IgD or IgM and CXCR4 is not clearly understood in B cell malignancies, so far.

Methods: Addressing this point, we investigated the interaction of the two BCR isotypes, IgD and IgM, and the chemokine receptor CXCR4 in both healthy and CLL patient B cells. First, we checked by the help of flow cytometry for the expression of our targets of interest (IgD, IgM, CXCR4) by performing a cell surface staining. Afterwards, intracellular calcium was stained for analyzing the CXCL12-responsiveness of the cells. In addition, proximity of BCRs and CXCR4 was detected by proximity ligation assay (PLA), where the detectable distance of both targets to each other should be between 10 to 40 nm.

DOI: 10.1002/eji.201970300

Results: Our results show, that CXCR4-proximity and signaling is correlated to IgD-BCR expression in healthy human B cells, while no correlation between IgM and CXCR4 is detectable. In contrast, in CLL samples an IgM:CXCR4 association was seen in 5 out of 14 tested samples. Within those samples, the interaction is more frequent in unmutated than mutated CLL. In addition to those results, proximity of IgD:CXCR4 is observed in 10 out of 15 samples, suggesting that IgD:CXCR4 association is crucial for CXCR4 signaling in healthy donor and CLL B cells.

Conclusions: Summarizing the data, the interaction of IgD and CXCR4 drives CXCL12-induced signaling in healthy and malignant mature B cells. In contrast, proximity of IgM:CXCR4 is observed in some CLL samples with being more frequent in unmutated than mutated cases.

P6

Circulating myelin oligodendrocyte glycoprotein (MOG)-specific B cells stratify patients with serum MOG-antibodies

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Autoantibodies to myelin oligodendrocyte glycoprotein (MOG) are found in a proportion of patients with inflammatory demyelination of the CNS, but the source of these antibodies (Abs) is unknown. Recent evidence indicates that only part of the patients with MOG-Abs benefits from B cell depletion.

In this study, we identified circulating MOG-specific B cells in peripheral blood of patients by differentiating B cells *in vitro* into Ab-producing cells via engagement of toll-like receptors (TLR) 7/8. We compared 21 patients with MOG-Abs and 26 healthy controls. MOG-Ab positive patients had significantly more MOG-specific B cells in blood than controls, but MOG-specific B cells were only detected in about 60 % of the patients. MOG-specific B cells in peripheral blood showed no correlation to anti-MOG Ab levels in serum, neither in the whole group of patients nor in the untreated patients, indicating an additional source of MOG-Abs apart from circulating B cells. Epitope analysis revealed an intraindividual heterogeneity of MOG-Abs secreted by MOG-specific B cells and partially also differences compared to MOG-Abs in serum.

Together, our study shows that patients with MOG-Abs can be stratified based on the abundance of circulating MOG-specific B cells which does not match with serum titer levels. This could be of therapeutic relevance for selecting patients with MOG-Abs for B cell directed therapy.

P7

Impaired B cell-specific LPS-signaling culminates in infection-induced preterm birth via decreased IL-10 expression

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Introduction: Despite enormous progress on the field of neonatal medicine, complications related to preterm birth (PTB) are still the leading cause of death among children under five years of age. A significant number of PTB are associated with maternal infections. B cells might be involved in LPS-induced PTB via different signaling pathways, among them TLR4/MyD88-dependent and CD19/RP105-dependent.

Objectives: In order to understand whether B cells are involved in the protection against preterm delivery, we used B cell-deficient mice and animals with altered B cell-specific MyD88- and CD19-signaling in a murine model of LPS-induced PTB.

Materials & methods: The following females were included in our study: C57Bl/6 WT mice, µMT mice (B cell deficient), mice with MyD88 deficiency in B cells (BMyD88-/-; generated by mating CD19cre/wt/ MyD88flox/flox), mice

DOI: 10.1002/eji.201970300

and CD19-deficient mice. 0.4 mg LPS/kg body weight (BW) was injected intraperitoneally (i.p.) at gestational day (gd) 16. Induction of preterm delivery was monitored with a camera within the following 24h.

Results: µMT, BMyD88-/- and CD19-/- mice were all more sensitive towards LPS compared to WT mice.

Since B cells are the major immune cell population in the peritoneal cavity, impaired B cell response towards LPS resulted in altered secretion of cytokines in peritoneal lavage, maternal and fetal serum. B cells from WT mice, fully competent to respond to LPS, secreted more IL-10 upon LPS challenge when compared to B cells from µMT, BMyD88-/- and CD19-/- mice.

Conclusion: B cells play an important role in LPS-induced murine PTB, probably by interfering in the balance between pro-and anti-inflammatory immune response.

Our data implicate that B cells might be a potential new target for therapeutic approaches in PTB.

P8

Synergism between IL7R and CXCR4 drives BCR-ABL induced transformation in Philadelphia chromosome positive acute lymphoblastic leukemia.

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Background: The Philadelphia chromosome-positive (Ph+) ALL is identified as high-risk group of ALL. IL7/IL7R signals regulate survival and proliferation of B cells and hence serve as obvious targets for oncogenes. BCR-ABL was reported to mimic signals from constitutively active IL7R in B-ALL cells. CXCR4 mobilizes malignant cells toward the bone marrow, which provides protection against chemotherapy.

Objectives: The functional correlation between CXCR4 and IL7R raises the question of a potential physical association of CXCR4 and IL7R. Thus, studying the synergism between CXCR4 and IL7R elucidates the molecular regulations of B-ALL, identifies unexpected resistance mechanisms and may provide alternative targets for novel treatment.

Methods: We investigated the surface expression of CXCR4 and IL7Raon Ph+ALL cells by the flow cytometry. Afterwards, the direct physical interaction of CXCR4 and IL7Rawas detected by proximity ligation assay (PLA).

Results: Our results revealed that the interaction between CXCR4 and IL7R is increased on the surface of BCR-ABL-transformed cells when compared with non-transformed cells. This suggests that the "crosstalk" between these receptors is important for survival of the leukemic cells. In fact, inducible deletion of *Il7ra*or *Cxcr4*in BCR-ABL transformed cells severely compromised the survival of BCR-ABL-transformed cells.

Conclusions: Our data suggest that co-localization of IL7R with CXCR4 on the same protein island on the cell surface physically connects homing with the availability of growth factors. High risk of relapse may represent one consequence of this concerted action of CXCR4 and IL7R signaling. In addition, our results revealed that disruption of *Il7ra*or*Cxcr4*gene expression resulted in cell death of the transformed cells. This suggests that CXCR4 may drive ALL cells into specific microenvironment containing IL7 and thus provides additional selective advantage in the presence of ABL-kinase inhibitors.

DOI: 10.1002/eji.201970300

P9

Master and servant - The complex interplay of a long non-coding RNA and its transcription factor STAT3 in human Multiple Myeloma

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Multiple Myeloma, also known as plasma cell myeloma, is a cancer of antibody-producing B-cells. To better understand the molecular mechanisms underlying the disease, we focused our interest on non-protein-coding RNAs (ncRNAs). Using the human Multiple Myeloma cell line INA-6, which strictly relies on STAT3 activation by IL-6, we identified STAiR18 as a novel STAT3-dependent ncRNA. Capture-RNA-sequencing revealed a complex splice pattern, each responsible for distinct cellular functions. Knockdown of the most abundant STAiR18 isoforms led to a dramatic decrease in INA-6 vitality, reduced STAT3 levels and changes in the histone methylation of the STAT3 locus. Moreover, STAiR18 and STAT3 knockdowns yielded overlapping changes of transcription patterns. Further, STAiR18 was found to associate with STAT3 mRNA. Taken together our data clearly indicate that STAiR18 and STAT3 are tightly involved in the dysregulation of human B plasma cells.

P10

Functionality of the BAFF/APRIL system in B-cell acute lymphoblastic leukemia

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Introduction: BAFF, APRIL and their receptors (BAFFR, TACI, BCMA) regulate the survival, maturation and homeostasis of mature B-cells. Despite the lack of a functional role of BAFF/APRIL system during normal early B-cell development, previous studies indicated a contribution of these molecules in the pathogenesis of B-lineage acute lymphoblastic leukemia (B-ALL).

Objectives: We evaluated the expression of this system in B-ALL and its involvement in spontaneous and drug-induced apoptosis of B-lymphoblasts, taking into consideration the distinct disease subtypes.

Patients and methods: Seventy-one patients with ALL (59 children, 12 adults) were enrolled in the study. The mRNA expression of ligands and receptors was examined by RT-PCR and the surface expression of the receptors was evaluated by flow cytometry. Chromatin immunoprecipitation was performed in the 697 pre-B-ALL cell line (E2A-PBX1$^+$ BAFFR$^+$) to investigate a possible transcriptional upregulation of *BAFFR* gene by the E2A-PBX1. Primary BAFFR$^+$/BAFFR$^-$ B-lymphoblasts and 697 cells were treated with BAFF and were analyzed with western blot for the NF-κB2 activation and with flow cytometry for BAFF-dependent survival. 697 cells and the Jurkat cell line (T-ALL, BAFFR-) were treated with BAFF and hypotoxic doses of chemotherapeutic drugs and were analyzed using an annexin V apoptosis kit.

Results: BAFFR is the most predominant aberrantly expressed receptor of the BAFF system in B-ALL and its expression, along with BCMA and APRIL, positively correlates with the maturation stage of B-lymphoblasts. Moreover, high *BAFFR* mRNA levels were found in B-ALL patients carrying the t(1;19)/E2A-PBX1 translocation. Chromatin immunoprecipitation showed binding of the E2A-PBX1 chimeric protein to *BAFFR* promoter suggesting that the transcriptional activator could contribute to the premature BAFFR expression in B-lymphoblasts. BAFF binding to BAFFR leads to the activation of the NF-κB2 pathway in pre-B-lymphoblasts suggesting that despite its premature expression, the receptor has the potential to activate downstream pathways. Surprisingly, we found that BAFF treatment enhances the cell death of primary BCR-ABL$^+$BAFFR$^+$ pre-B-lymphoblasts. It also enhances the glucocorticoid-induced apoptosis in the E2A-PBX1$^+$ pre-B-ALL cell line 697.

Conclusion: These data suggest a disease-subtype role of BAFF/BAFFR signaling in B-ALL that differs from normal mature B-cells and might affect the pathogenesis and prognosis of the disease.

DOI: 10.1002/eji.201970300

P12

Effects of Macrophage Migration Inhibitory Factor (MIF) Deficiency on B Cell Phenotypes and Population Diversity in Atherosclerosis

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Introduction: Macrophage migration-inhibitory factor (MIF) is an inflammatory cytokine that is upregulated in chronic inflammatory diseases including atherosclerosis. Although MIF does not structurally belong to the class of CXC chemokines, it "mimics" chemokine activities and has been defined as the prototypical member of the emerging class of atypical chemokines (ACKs). Accordingly, the pro-atherogenic and inflammatory effects of MIF are prominently driven by interactions with the classical chemokine receptors CXCR2 and CXCR4, mediating monocyte/neutrophil and T cell recruitment, respectively. More recently, B cells have been identified as important players in atherogenesis but their effects are subtype-dependent.

Objectives: We recently examined *Mif* gene deletion in an *Apoe-/-* background and revealed a link between MIF and B cell autoreactivity featuring vascular site-specific atheroprotection in *Mif*-KO mice and a strong reduction in peripheral B cell counts due to a maturation defect in the bone marrow (BM) associated with an atheroprotective auto-antibody profile. We now aim at an in-depth understanding of this phenotype focusing on the impact of *Mif*-deficiency on B cell development and population diversity.

Materials and methods: *Mif*-deficient *Apoe-/-* mice were fed a high cholesterol diet for different time periods. We performed FACS-based hematopoietic profiling of blood, spleen and bone marrow as well as phenotypical lesion analysis applying different histological and immunofluorescence staining methods. Moreover, we have employed single cell (sc)-RNAseq for the transcriptomic analysis of bone marrow and splenic B cells in different developmental stages (Fig. 1).

Results: Our results indicate that *Mif*-deficiency leads to a striking B cell subset-dependent enrichment of several gene clusters for pathways related to mitochondrial metabolism, cell cycle and migration. Moreover, *Mif* gene deletion alters the B cell localization and favors their redistribution in peri-adventitial clusters showing hallmarks of ATLOs stage I-II.

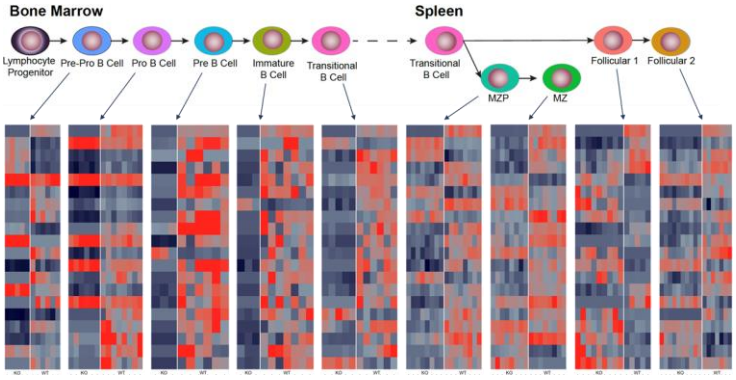
Conclusion: We revealed a novel role of MIF in B cell development. The observed B cell-related phenotypes and the scRNAseq-based transcriptomic data will be discussed against the background of MIF"s complex role in immune cell recruitment and advanced atherogenesis.

Figures:

Figure 1: Heatmap of differentially expressed genes between *Mif* KO and WT B cell developmental subsets.

DOI: 10.1002/eji.201970300

Figure 1



P13

Dysregulated miR-155 and miR-125b are related to impaired B-cell responses in Down Syndrome

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Down Syndrome (DS) is the most frequent chromosomal disorder in humans caused by an extra copy of chromosome 21 (HSA21). Life expectancy has increased in the last decades but the infections remain among one of the major causes of death in DS. Immune deficiency is an integral feature of DS. Moreover, DS respond poorly to primary immunization and have a significant reduction of switched memory B cells (MBCs) in the peripheral blood. The germinal centre (GC) is a secondary structure in peripheral lymphoid organs where activated B cells refine their antigen receptors. If it is dysfunctional it may lead to antibody deficiency and to recurrent infections and autoimmune diseases. HSA21 encodes two microRNAs (miRs), miR-125b and miR-155, that regulate B-cell responses. We studied B- and T- cell subpopulations in tonsils of DS and age-matched healthy donors (HD) and we found that the GC reaction was impaired in DS. GC size, numbers of GC B cells and Follicular Helper T cells (TFH) expressing BCL6 cells were severely reduced. The expression of miR-155 and miR-125b was increased in tonsillar MBCs and miR-125b was also higher than expected in plasma cells (PCs). AID (Activation Induced Cytidine Deaminase) protein, a miR-155 target, was significantly reduced in MBCs of DS patients and was significantly overexpressed in PBMCs activated with CpG, whereas miR-125b was constitutively higher than normal. The upregulation of both miRs is mostly evident in MBCs, activated B cells, and plasma blasts (PBs)/plasma cells (PCs) of DS, highlighting the crucial role of HSA21-derived miRs in the regulation of antigen experienced B cells. The increase of miR-155 and its functional consequences were blocked by antagomiRs in vitro, partially reverse the abnormalities observed in MBCs and PBs of DS. Because of HSA21-encoded miRs play a role also in DS-associated dementia and leukemia, our study suggests that antagomiRs may represent pharmacological tools for the treatment of DS.

P14

Memory B cell responses to malaria vaccines are potently inhibited by sub-protective antibody titres

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Introduction: The development of vaccines which induce high titres of protective antibodies has proved challenging for complex diseases such as malaria and HIV. In the case of malaria the circumsporozoite protein (CSP) antigen of *Plasmodium* encases the parasite and is a major vaccine candidate; however, protective antibody responses to this antigen are short-lived in vaccinated humans.

Objective: This study aimed to clarify the cellular mechanisms responsible for the sub-protective B cell response to malaria vaccines.

Materials & methods: Participants in a clinical trial (NCT02015091) for a radiation attenuated *Plasmodium* vaccine regime had regular blood collections, from which we examined the anti-CSP B cell response via ELISA, flow cytometry

DOI: 10.1002/eji.201970300

and BCR sequencing. To investigate the cell dynamics in the memory and plasma cell compartments which were not accessible in humans, we developed an anti-CSP BCR-knockin mouse and utilised a mouse malaria model analogous to the human vaccine trial.

Results: In trial participants we observed an expansion of CSP-specific plasmablasts after the first and second vaccinations, which correlate with increased titres of protective antibodies. However, additional vaccinations failed to further enhance titres. Hypothesising that malaria vaccination induces poor B cell memory, we investigated the cellular dynamics further in our BCR-knockin mice. Contrary to our hypothesis, we found that memory B cells from vaccinated mice adoptively transferred into naïve recipients were able to differentiate after a recall-vaccination. However, if the immune environment in the donor mice was matched in the naïve recipient mice, by transfer of an anti-CSP antibody, then memory cells failed to respond. Importantly, we determined that the amount of antibody required for protection against infection was ~3-fold higher than the amount required to inhibit recall responses by memory B cells.

Conclusions: These data suggest that simple prime-boost strategies will not be sufficient to generate durable immunity to complex pathogens because of antibody mediated feedback. Instead, targeted approaches to generate potently protective antibody lineages may be required.

P16

Insights into the functional role of the IgD B cell receptor

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Question: Development and responses of B cells are mainly controlled by the action of the B cell antigen receptor (BCR) expressed as IgM- and IgD-isotype on mature B cells surface. IgM- and IgD-BCRs follow the same intracellular signaling pathways upon engagement, leading to the hypothesis of their redundant function. However, the strict regulation of the two BCR isotypes in developing B cells and the important structural differences suggest different functional roles for the two isotypes. By mean of *in vivo* experiments, we aim at defining a set of isotype-specific functions in the immune response and the overall B cell function.

Methods: To study the function of the two BCR isotypes, we employed IgD- and IgM-deficient mice and compared their immune reaction to the wild type (WT) counterpart. To this aim we utilized flow cytometry analysis of lymphoid cells, serum analysis via ELISA and fluorescence microscopy to analyze spleen sections. We also generated and analyzed bone marrow chimeric mice to study IgM- and IgD-only B cells in direct competition with WT cells.

Results: We found that the generation of germinal center (GC) B cells and consequent antibody production in response to 2,4,6-trinitrophenol (TNP) coupled to ovalbumin (OVA) is impaired in IgD-deficient mice, which explains previous reports of a delayed immune response in these mice. Of note, we assessed that the immune response against a large polyclonal antigen, such as sheep red blood cells (sRBC), does not differ between IgM-, IgD-KO and WT mice. Moreover, we observed a reduced presence of IgD deficient B cells in secondary lymphoid organs of BM chimeric mice.

Conclusions: Taken together, our data indicate that, while being unresponsive to monovalent (auto-)antigens, the IgD-BCR is required for immune reactions against foreign antigens, especially during the early phases of the response. Moreover, we found that cells lacking IgD expression display impaired homing to secondary lymphoid organs and/or homeostasis compared to the WT counterpart. These findings are in agreement with previous reports and point to an important evolutionary advantage, rather than a redundant function, provided by the IgD-BCR.

P17

Stromal cell contact-induced PI3K signaling prevents caspase-3 induced cell death of memory plasma cells

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In the bone marrow, long-lived memory plasma cells (mPC) can survive for years, in niches organized by mesenchymal stromal cells. In order to identify the critical signals for survival of the plasma cells, we have shown earlier that ST2 stromal cells and the cytokine APRIL, under hypoxic cell culture conditions, together suffice to

DOI: 10.1002/eji.201970300

maintain mPC alive *ex vivo* for up to 6 days. We could further show that direct cell contact between stromal cells and mPC is required for mPC to survive. Apparently cell contact induces PI3K signaling, while APRIL is known to induce NF-kB signaling.

We now show that PI3K signaling downregulates the transcription factors FoxO1 and FoxO3, and that this is critical for survival of the mPC. SiRNA mediated knock-down of FoxO1/3 allows mPC survival in the absence of ST2-contact-mediated survival signaling. ST2-mediated downregulation of FoxO1/3, but not APRIL, protects mPC from caspase 3-mediated cell death, a consequence of mitochondrial stress. APRIL rather protects from caspase 12-mediated death, a consequence of endoplasmatic reticulum stress, i.e. protein synthesis.

P18

Siglec-G controls the severity of Chronic Lymphocytic B-cell Leukemia

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Siglec-G is a negative regulator of B-cell receptor (BCR) signalling. Siglec-G deficient mice show an expansion of the population of CD5+ B1a cells and increased Ca2+ signalling in these cells. Human chronic lymphocytic B-cell leukemia (B-CLL) is characterized by B cell leukemic cells developing from CD5+ B cells. BCR signalling is crucial for the maintenance of the B cell leukemic populations, as BCR signaling inhibitors such as Ibrutimib are successful new treatments of B-CLL. We used the mouse B-CLL model of the Tcl1 transgenic mice to determine how the inhibitor of BCR signalling, Siglec-G, influences the severity of B-CLL. Siglec-G-deficient x Tcl1 transgenic mice showed a significantly faster and higher expansion of the pre-leukemic CD5+ B cell population in the blood than Tcl1 tansgenic mice. Bone marrow, spleen, lymph nodes and liver were affected by an earlier infiltration of the leukemic-like cells. The Ig repertoire of Siglec-G-deficient x Tcl1 transgenic mice was changed in comparison to Tcl1 transgenic controls. Furthermore, we crossed Siglec-G knockin mice that overexpress Siglec-G about 5-fold on B cells, compared to the normal Siglec-G expression level, to Tcl1 transgenic mice. These mice develop a delayed onset of the disease, compared to Tcl1 transgenic mice with wild type levels of Siglec-G. We will also analyse the expression level of human Siglec-10 (the homolog to Siglec-G) in human B-CLL patients. In conclusion the inhibitory receptor Siglec-G has a crucial role in the pathogenesis of B-CLL, as revealed by the Tcl1 transgenic mouse model.

P19

Abatacept modulates CD80 and CD86 expression and memory formation inhuman B-cells

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Background: Cytotoxic T lymphocyte antigen-4 (CTLA-4) limits T-cell activation and is expressed on T-regulatory cells. Human CTLA-4 deficiency results in severe immune dysregulation. Abatacept (CTLA-4 Ig) is approved for the treatment of rheumatoid arthritis (RA) and its mechanism of action is attributed to effects on T-cells. It is known that CTLA-4 modulates the expression of its ligands CD80 and CD86 on antigen presenting cells (APC) by transendocytosis. As B-cells express CD80/CD86 and function as APC, we hypothesized that B-cells are a direct target of abatacept.

Methods: *In vitro*, kinetic of CD80 and CD86 expression was measured by flow cytometry on isolated B cells after stimulation via CD40 and IL-21R in presence and absence of abatacept. *In vivo*, nine abatacept-treated RA patients were followed up to 12 months, and seven up to 24 months and treatment response, immunoglobulins, ACPA, RF concentrations, B-cell phenotype and ACPA-specific switched memory B-cell frequency were assessed.

Results: Abatacept treatment *in vitro* resulted in a dose-dependent decrease of CD80/CD86 expression on B-cells by dynamin-dependent internalization, without influencing B-cell development. *In vivo*, RA patients treated with abatacept showed a progressive decrease in plasmablasts and serum IgG. While ACPA-titers only moderately declined, the frequency of ACPA-specific switched memory B-cells significantly decreased in response to treatment.

Conclusion: Abatacept directly targets B-cells by reducing of CD80/CD86 expression. The consequent impairment of antigen presentation and T-cell activation may result in altered B-cell selection and control of the generation of ACPA-

DOI: 10.1002/eji.201970300

specific memory B-cells providing a new therapeutic mechanism and a base for abatacept use in B-cell mediated autoimmunity.

P20

Are Chronic lymphocytic leukemia subset #4 B cell receptors derived from Rheumatoid factor-expressing B cells?

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Question: Chronic lymphocytic leukemia (CLL) is the most prevalent adult B-cell neoplasia in western countries. The B cell receptor (BCR) is central to the disease pathogenesis. Most CLL cases present a remarkably skewed repertoire of immunoglobulin (Ig) genes which allow their classification in different subsets. Among all CLL subsets, subset #4 is peculiar for the specific expression of IgG-switched BCRs encoded by the IGHV4-34 and IGKV2-30 gene pair. Crystal structure of subset #4 CLL BCR revealed clear evidence of homotypic interactions which are relevant to the cell-autonomous signalling, detected by Ca^{2+} influx, that extensively characterize the disease. In this study, we investigated the interactions that define CLL subset #4 BCRs focusing on the importance of IgG-isotype switching and on the affinity of the self-association complexes.

Methods: For the purpose of this study, conventional CLL subset #4 γ1 and μ-reverted BCRs were cloned and expressed in our well-established Triple Knock Out (TKO) pre-B cell line system. Enrichment of transduced cells was monitored and a proliferation assay was performed. Soluble CLL subset #4 antibodies encoding wild type or association-deficient mutant sequences were purified from HEK 293T cells. Binding assays and Indo-1 AM staining were performed to analyse Ca^{2+} influx: FACS measurement was performed in both cases.

Results: In our study, TKO cells expressing CLL subset #4 BCRs showed an increase in the number of transduced cells over time upon isotype switching to IgG. The latter is also required for cell-autonomous signalling, as CLL subset #4 BCRs expressed as IgM showed no autonomous signalling upon Ca^{2+} induction. Furthermore, our data pointed out that the affinity of the homotypic interaction in IgG molecules is sufficiently high to make IgG self-assembled complexes impermeable to soluble self or total IgG. On the other hand, CLL subset #4 BCR expressed as IgM acquired the ability to interact with association-deficient soluble CLL subset #4 antibodies.

Conclusions: Altogether, our findings shade light on the intramolecular interactions of subset #4 CLL BCR confirming their fundamental role for the cell-autonomous signalling. Furthermore, the ability of subset #4 CLL BCR expressed as IgM to bind soluble IgG prompts the idea that CLL cells might be derived from Rheumatoid factor-expressing B cells, suggesting that deeper investigation is required to speculate on the origin of CLL cells.

P24

The role of IgD BCR during late B cell development and humoral immunity

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The expression of IgM B cell antigen receptor (BCR) is crucial for B cell development and activation, as it regulates survival, proliferation and selection. During early developmental stages, B cells express exclusively IgM BCRs, while at later stages, IgD becomes the predominant isotype. Interestingly, IgD expression has been conserved throughout evolution. As IgD is rarely secreted and expressed mainly as a membrane-bound receptor, indicates a specialized role of the IgD BCR in B cell signalling. However, to date the function of IgD still remains elusive.

Recent findings by our group indicated that IgD is selectively responsive to stimulation by polyvalent antigen, while signalling can be inhibited by increased concentrations of monovalent antigen. Moreover, our results suggest that IgD expression is regulated by the PTEN/FoxO1 pathway, as inhibition of PI3K signalling by its catalytic antagonist PTEN, leads to upregulation of the splicing factor ZFP318, which promotes alternative splicing of Cμ-Cd pre-mRNA. In addition, we found that IgD-deficient mice show a delayed and abnormal germinal centre reaction upon immunization with TNP-Ova. Together, these data indicate a crucial regulatory role of IgD BCR in tolerance induction and the initiation of high-affinity and long-lived humoral immunity. Fundamental understanding might lead to improved vaccination protocols.

DOI: 10.1002/eji.201970300

P25

B-Raf and Raf-1 are dispensable for activation of the MAPK/Erk signaling pathway in murine B cells but contribute to plasma cell differentiation

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Introduction: Raf is a family of serine/threonine-kinases, consisting of Raf-1 (C-Raf), B-Raf and A-Raf in mammals. Raf-kinases were identified as intermediates in the MAPK/Erk-signaling pathway. The role of Raf-kinases in B cell development and activation is not completely understood.

Objectives: In this study, we want to investigate the role of Raf-kinases in B cell development and activation.

Materials & Methods: We studied the B cell development and activation in mice with B cell specific deletion of Raf-1 and B-Raf (DKO) by flow cytometry and westernblot. Additional ELISPOT and ELISA analysis were performed to investigate the capability of inducing a functional immune response in Raf-deficient mice.

Results: B cell specific Raf-1//B-Raf deficiency resulted in an incomplete block of B cell development from the late-pro- to early-pre-B cell stage and in decreased B cell numbers in the spleen. The diminished numbers of splenic B cells in DKO mice were caused by a reduced B cell efflux from the bone marrow and an impaired differentiation of transitional B cells. In contrast, the survival and persistence of mature B cells was comparable to wild type B cells. Furthermore, the differentiation of mature DKO B cells to plasma cells (PC) was impaired *in vivo* and *in vitro*. Flow cytometric analysis revealed a very early block of PC differentiation in the transition from activated B cells to pre-plasmablasts.

Surprisingly, *in vitro* stimulation of DKO B cells with anti-IgM, anti-CD40 and LPS led to a pronounced Erk-activation. In the absence as well as in the presence of stimulation Erk-phosphorylation was even higher in DKO than in control B cells. Since in DKO and control B cells pan-Raf-inhibitors were not able to inhibit Mek/Erk-phosphorylation, we suppose that A-Raf is not the mediator of Mek/Erk phosphorylation in DKO B cells. However, treatment with the PI3K-inhibitor LY294002 resulted in reduced Mek/Erk-phosphorylation upon BCR- and CD40-stimulation in both genotypes.

Conclusions: These data suggest an important role of Raf kinases during B cell development and plasma cell differentiation. The defects in B cell activation of mature DKO B cells could be due to a deregulated Erk-phosphorylation upon chemical inhibition or genetic knockout of the Raf kinases. The mechanisms leading to an impaired PC differentiation in DKO mice is still under investigation.

P26

CXCL13 elevation in early kidney allograft rejection in mice and men

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Background: Early recognition of allograft rejection after kidney transplantation (ktx) is important to initiate timely treatment strategies. In delayed graft function (DGF) indicators of renal function impairment such as serum creatinine are missing. Therefore, biomarkers are required to identify ongoing rejection and activation of the immune system as early as possible. CXCL13, a potent chemoattractant for B-cells, is a candidate biomarker, which is known as an indicator of autoimmune disease activity. Here, we determined CXCL13 levels in patient blood samples longitudinally after ktx. Furthermore, we characterized CXCL13 expression in blood and tissue samples of mice with mixed T-cell and antibody mediated allograft rejection (TCMR-AMR).

Methods: Patients scheduled for ktx were enrolled in a prospective clinical study and had blood sampling prior to ktx, at day 1, 3 and 7 thereafter. Serum-creatinine elevation and histology were analysed. CXCL13 expression was

DOI: 10.1002/eji.201970300

measured by ELISA. For allogenic ktx in mice Balb/C (H-2d) donor kidneys were transplanted into a completely mismatched C57Bl/6 (H-2b) male recipient and control isogenic ktx was performed with C57Bl/6 donors and recipients. Blood samples were drawn weekly and flow-cross match was performed with BalbC splenocytes. At the designated endpoints at 2, 3 and 6 weeks after ktx renal tissue was analysed for CXCL13 mRNA and pathological changes. Flow cytometry was performed to characterize infiltrating leukocyte subsets of the graft 10 days after ktx.

Results: Patients with initial function had stable CXCL13 levels. In contrast, two patients with early TCMR had >5 fold CXCL13 elevation within the first week after ktx. Biopsies revealed TCMR with B-cell infiltrates in both patients.

In the mouse model CXCL13 levels increased over time and correlated with enhanced CXCL13 mRNA levels in the allograft and with acute rejection. Severe interstitial inflammation with CD3 positive cellular infiltrates and perivascular B-cell rich clusters were observed. By flow cytometry enhanced plasma cell infiltrates in the allograft were measured.

Conclusion: Determination of increased levels of systemic CXCL13 in patients after ktx is a promising candidate biomarker for B-cell activation and needs further clinical evaluation in a larger patient cohort.

P27

Monoclonal antibodies against porcine IgE: characterization and analytical application

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Introduction: IgE is one of the key molecules involved in allergic diseases. But the lack of specific antibodies against porcine IgE (pIgE) has limited the usage of pigs as large animal models for allergic diseases in the past.

Objectives: The aim of this attempt was the generation of monoclonal antibodies (mAb) directed against pIgE as well as their characterization and testing their eligibility in diverse immunological assays.

Material and Method: BALB/c mice were immunized with HEK 293T cell derived 6xHIS-tagged recombinant porcine IgE heavy chain (CH2-CH4; HIS-IgE) of about 50 kDa. Splenic lymphocytes of immunized mice were fused with SP2/0 cells. Clones producing mAb were screened on HEK 293T cells expressing intracellular Flag-tagged pIgE heavy chain (CH2-CH4; Flag-IgE) by flow cytometry (FCM) for specificity. For epitope characterization 32 15-mere peptides, overlapping in five amino acids created of the respective pIgE sequence were tested by means of a direct ELISA and in blocking assays on Flag-IgE expressing HEK 293T cells by FCM. Cross-reactivity with other porcine immunoglobulins was also tested by ELISA and FCM blocking assays. Furthermore their applicability in immunoblots, immunoprecipitation and allergen specific ELISA were tested. The IgG-class mAb were purified by affinity chromatography on Protein G and partly conjugated to biotin for further usage.

Results: We got three specific mAb producing clones of IgG1 isotype and one of IgM class. Each of the clones recognized a different peptide in ELISA. These results could be confirmed in the FCM blocking test. In Immuno-blots all mAb bound to HIS-IgE and none to the HIS-protein. Cross-reactivity tested to pIgG or pIgM was not detected. The IgG1 isotypes were also applicable for immunoprecipitation.

Conclusion: These newly generated mAb provide good tools for using pigs as large animal model for allergies.

P28

Fas non-apoptotic signaling contributes to positive B cell selection by modulating the mTOR signaling pathway

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DOI: 10.1002/eji.201970300

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Activation of B cells in the germinal center (GC) is a central mechanism for the formation of memory cells and plasma cells. Intact positive and negative B cell selection in the GC ensure potent memory responses and maintenance of humoral tolerance. Defective Fas/CD95 signaling is linked to autoimmune lymphoproliferative syndrome (ALPS), a human disorder characterized by autoantibody-mediated cytopenia and lymphoproliferation. So far, development of disease is attributed to impaired negative selection due to deficient Fas mediated apoptosis which translates into disturbed removal of autoreactive B lymphocytes. However, the impressive improvement of autoimmune cytopenias under mTOR inhibition can not be sufficiently explained by this concept. Since Fas has both apoptotic and non-apoptotic functions we sought to investigate a potential non-apoptotic role of Fas as a modulator of human B cell activation and selection. The major signal inducing mTOR in GC B cells is CD40 ligation and the dynamic modulation of mTOR is a key mechanism driving positive selection and shuttling between the GC dark and light zone. Mass cytometry studies showed that transient ligation of Fas in CD40L stimulated B cells induced a steady decrease of pAkt and pS6 that was independent of apoptosis. This signal modulation was absent in Fas-mutant ALPS patients. Accordingly, mTOR signaling was enhanced in GC B cells and plasmablasts of secondary lymphoid organs of ALPS patients. Proteome analysis revealed a possible link between Fas and mTOR signaling via regulation of a DAXX-USP7-PTEN axis. Finally, transient Fas triggering promoted expression of transcripts that regulate the shuttling and the permanence of B cells in the GC, uncovering a non-apoptotic role of Fas in the process of positive selection of activated human B cells. This study contributes to the understanding of B cell selection, the pathogenesis of ALPS and sheds light into the physiological role of Fas signaling in a non-apoptotic context.

P407

Phenotypically and functionally distinct plasma cell subsets reside in human bone marrow

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In humans, antibody-secreting plasma cells (PC) lacking the expression of CD19 preferentially reside in bone marrow (BM). While they candidate as long-lived plasma cells, the specific lifestyle conferring their stability and exceptional maturity in comparison to their CD19 counterparts also found in BM remains elusive.

Here we systematically compared the transcriptomes and the expression of cell-surface receptors of human CD19 and CD19- BMPC, revealing differential abundance of several receptors and molecules implicated in cell adhesion/migration and intercellular communication. Consistent with subset-specific PC behavior, we observed a survival advantage of isolated CD19- vs. CD19 BMPC after 5 and 13 days in in vitro co-cultures with human MSC grown from primary human BM.

49-parameter mass cytometry was employed to further delineate the phenotypical setup of human BMPC. This analysis identified distinct, non-redundant subsets of PC expressing or lacking CD56 or CD45, whose identity as antibody-secreting cells was confirmed by Elispot. Notably, CD56 expression by PC was associated with the expression of other receptors commonly absent in B cell lineage cells, such as CCR2 and CD28. A re-analysis of a public dataset (Bendall et al., Science 2011) indicates that CD45-negative PC exhibit enhanced phosphorylation of STAT3 and 5 proteins after treatment with PVO4, suggesting that the known function of CD45 as a negative regulator of Jak-dependent cytokine signaling is active in PC and may limit the processing of STAT-dependent cytokines signals in CD45 but not CD45- BMPC.

We here define new subsets of human BMPC that express distinct sets of cell adhesion and communication molecules which have the potential to confer differential survival in different BM microenvironments. At the same time, variable CD45 expression by PC has the capacity to mount differential responses of PC in an identical environment. We show that the normal human PC compartment in the BM is more complex than previously appreciated.

DOI: 10.1002/eji.201970300

Understanding the complexity and interrelatedness of mature PC subsets will aid the development of PC-directed therapies and vaccines.

Cytotoxic T cells (P29-P86, P398)

P29
CpG reinvigorates exhausted T cells and potentiates proliferation burst of stem-like CXCR5+ T cells

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Chronic hepatotropic infections are characterized by impaired T cell function and understanding T cell exhaustion as well as strategies aimed at restoring exhausted T cell function is of immense clinical interest. Here we exploit an experimental mouse model characterized by constitutive, hepatocyte-specific intracellular OVA-antigen expression. Upon intramuscular vaccination of these chronic antigen expressing mice with adenoviral OVA vectors, we observe diminished cytotoxic T cell responses. This mimics the situation in chronically infected patients. Interestingly, modulating liver microenvironment with CpG 28 days post vaccination rejuvenates exhausted T cells, facilitating efficient OVA-antigen clearance. In concordance, antigen specific T cells are dramatically increased in the liver. However, it is unclear which subset of exhausted T cell expedites extensive proliferative burst upon CpG administration. In this regard, we identified a prominent subset (~20-30%) of Ova-specific CXCR5+ T cells in the liver, which undergo enhanced proliferation upon CpG application, compared to CXCR5- cells. These observations prompted us to evaluate the functional fate of Ova-specific CXCR5+ T cells in the liver 21 days post vaccination. CXCR5+ cells show improved expression of CD69, CD127 and Ki67, which are markers associated with activation/tissue residency, memory and proliferation, respectively. Similarly, effector molecules such as GZMB, TNFa, and T cell cytotoxic marker CD107a are dramatically increased in CXCR5+ cells. Moreover, the presence of CXCR5+ cells in the liver is accompanied by increased serum CXCL13, the ligand for CXCR5, suggesting that CXCL13 expression coincides with antigen recognition. Further, CXCR5+ T cells express lower exhaustion marker TIM-3 and Lag-3. In addition, CXCR5+ cells show efficient mitochondria, elevated nutrient acquisition activity and high expression of the purinergic receptor P2X7R as well as reduced levels of ROS. These data suggest distinct mitochondria functions in CXCR5+ and CXCR5- T cells. Consequently, CXCR5+ cells are better able to self-maintain. Together, these findings suggest that OVA-specific CXCR5+ T cells in the liver possess stem-like cells features. Given that CXCR5+ cells possess features of "potent" memory and expand upon immunomodulation, they might represent a crucial population for efficient elimination of the viral burden in liver.

P30
Analysis of EV-decorated CD8+ T cells during viral infection

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The exposure of phosphatidylserine(PS) on the outer leaflet of the plasma membrane is a classical feature of apoptosis but also common on extracellular vesicles (EVs). EVs have been shown to play important roles in cellular communication and are involved in the regulation of CD8+ T cell responses during infection and cancer. However, the investigation of EV-attached CD8+ T cells in complex and dynamic *in vivo*settings has not been reported yet. Here, we have generated a fusion protein of the PS-binding protein Mfge8 and the reporter molecule eGFP (MFGe8-eGFP) to analyze naturally occurring EV-T cells-interactions *in situ*. PS+ cells were detected by intravenous injection of Mfge8-eGFP followed by imaging flow cytometry. In order to reliably discriminate apoptotic from live EV-decorated cells, we developed a deep learning algorithm. By using this novel approach, only low frequencies of EV-bound T cells were detected in the absence of infection. In contrast, upon LCMV-infection we found an approximately 10-fold increase in the frequency of EV-decorated CD8+ T cells. EV-binding was mostly confined to activated effector CD8+ T cells, but almost absent on naïve CD8+ T cells. Furthermore, bound EVs were enriched for proteins normally found on antigen presenting cells (APCs), suggesting an APC-origin. Our data demonstrate the propensity of activated CD8+ T cells to bind EVs. Moreover, we here introduce a new method to study EVs and their CD8+ T cell targets *in vivo*, which will give new insights into the complex immune modulating role of EVs in infection and antitumor immunity.

DOI: 10.1002/eji.201970300

P31

Identification of the unique phenotype of liver-resident memory CD8⁺ T cells in chronic viral infection

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Introduction: Tissue-resident memory CD8⁺ T (T_{RM}) cells play a pivotal role for the combat of several viral infections, which was mostly demonstrated for viruses replicating in mucosal tissues or secondary lymphoid organs, but not for hepatotropic viruses. Due to its unique anatomic structure and physiologic function, the liver represents a unique location for tissue resident cells and favors a chronic outcome of infections.

Objectives: We asked whether T_{RM} cells develop both in resolving and chronic hepatic viral infection and aimed at identifying and characterizing T_{RM} cells in chronic infection as possible target for therapeutic intervention.

Materials & methods: C57BL/6 mice received 100 naïve CD45.1⁺ OT-I T cells and were subsequently infected with liver-targeting adenoviruses encoding ovalbumin under the CMV- or the hepatocyte-specific TTR promoter causing resolving or persistent infection, respectively. 6 weeks later, hepatic OT-I T cells were isolated by fluorescence-activated cell sorting and subjected to RNA sequencing, or analyzed *ex vivo* by flow cytometry.

Results: RNA sequencing revealed a preserved tissue-residency core signature present in virus-specific CD8 T cells from both resolved as well as chronic infection. In accordance with those findings, OT-I T cells in both settings expressed the T_{RM} markers CXCR6 and CD69. However, T_{RM}-like cells from chronic infection exhibited a unique transcriptional signature implying functional impairment, which was verified *ex vivo* by showing reduced functionality, high expression of inhibitory receptors and a higher turnover rate. Bioinformatics analysis further suggested increased cAMP signaling as one mechanism inducing loss of functionality, which could be confirmed on protein level in preliminary experiments.

Conclusion: Adapted T_{RM} cells are formed during chronic infection of the liver. They share the chemokine receptor CXCR6 and the residency marker CD69 with their functional counterparts from resolved infection. However, these cells are functionally adapted to the persisting infection. Bioinformatics analysis suggests a transcription factor beyond TOX being responsible for liver-specific functional impairment of CD8 T cells. Still, the preserved tissue-residency signature of adapted liver T_{RM} cells during chronic infection suggests that these cells can be targeted for re-activation.

P32

The deubiquitinase OTUB1 regulates homeostatic proliferation of CD8⁺ T cells.

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Introduction: Ubiquitination is a crucial post translational modification regulating signal transduction and immune responses. The process of ubiquitination is counter-regulated by deubiquitinating enzymes (DUB's) by removing ubiquitin chains from the target protein. OTUB1, a DUB preferentially targeting K48-linked ubiquitin chains, thereby preventing proteasomal degradation of the target protein. It has been shown that OTUB1 regulates CD4⁺ T cells anergy in vitro by degradation of GRAIL (gene related to anergy in lymphocytes). However, the role of OTUB1 in T cells *in vivo* is unknown.

Objectives: We aim to investigate how OTUB1 regulates T cell homeostasis and response to bacterial infection.

Materials & methods: We generated CD4-Cre OTUB1$^{fl/fl}$ mice to deplete OTUB1 in both, CD4⁺ and CD8⁺ T cells and analyzed T cell development and homeostasis in naïve mice. In addition, we intravenously infected mice with *Listeria monocytogenes* and investigated the T cell response by flow cytometry.

Results: Based on flow cytometric analysis we found no disturbance in T cell development. However, in secondary lymphoid organs an accumulation of CD44^{high}CD62L^{high} CD8⁺ T cells was observed, indicating an important role of OTUB1 in homeostatic proliferation. Interestingly, OTUB1-deficient CD8⁺ T cells were hyperactivated and proliferated stronger in Rag1$^{-/-}$ mice, as well as *in vitro* upon IL-7 and IL-15 stimulation. Both cytokines are major regulators of homeostatic proliferation. Even though accumulation of CCD44^{high}CD62L^{high} CD8⁺ T cells increased over time, CD4-

DOI: 10.1002/eji.201970300

Cre OTUB1$^{fl/fl}$ mice survived for at least 1 year without clinical signs of autoimmune disease. Despite the pre-activated state of OTUB1-deficient CD8$^+$ T cells we observed no significant differences in colony forming units and T cell response when we infected mice with *L. monocytogenes*.

Conclusion: Our results indicate that OTUB1 plays a crucial role in CD8$^+$ T cell homeostasis by inhibiting cytokine signaling, but has no effect on the regulation of the T cell response upon *L. monocytogenes* infection.

P33

Identification of a miR-146b-FasL axis in the development of neutropenia in T-LGL leukemia

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T Large Granular Lymphocytes Leukemia (T-LGLL) is a rare disease characterized by LGLs proliferation. The main clinical manifestation of T-LGLL is a severe neutropenia that is also the most common indication for treatment in this disease. From the molecular point of view, the high levels of soluble Fas ligand (FasL) found in these patients are the main cause of neutropenia. We recently described that the increased FasL expression was induced by the high STAT3 activation that specifically characterizes T-LGLs of neutropenic patients. Although STAT3 is a transcriptional activator of a huge number of oncogenes, so far its relationship with microRNAs has not been evaluated in T-LGLL. Here, we investigated whether STAT3 could carry out its pathogenetic role in T-LGLL through an altered expression of miRNAs. Here, we investigated whether STAT3 could carry out its pathogenetic role in T-LGLL through an altered expression of miRNAs.

Hierarchical Clustering Analysis of miRNA expression profile of T-LGLs identified two groups of samples: in the first cluster, patients are characterised by CD8+ T-LGLs, neutropenia and high levels of activated STAT3, while CD4+ T-LGLs, normal neutrophil counts and low levels of STAT3 activation distinguish remaining patients. Remarkably, we demonstrate a CD8-specific and STAT3 activation-dependent inhibition of miR-146b expression, this latter being related to miR-146b promoter methylation. We also provide experimental evidence that the STAT3-dependent reduction of miR-146b expression allows increased HuR-mediated stabilization of FasL mRNA, leading to increased FasL production, which in turn has been shown to play a role in neutropenia. Restoring miR-146b expression in CD8 T-LGLs leads to a reduction of HuR protein and, in turn, of FasL mRNA expression. Finally, we demonstrated that treatment with Bortezomib, a proteasome inhibitor, lead to a 70% reduction of STAT3 activation and 67% reduction of DNMT1 expression in T-LGL. Finally, in line with our previous results, reduction of HuR protein expression (-44%) and FasL mRNA (-43%) was observed in Bortezomib-treated T-LGL, consistent with the restoration of miR-146b expression.

All together these data provide mechanistic insights for the link between STAT3 activation, miR-146b expression and neutropenia in CD8 T-LGLs and indicate a possible use of Bortezomib in the treatment of neutropenia in T-LGLL.

P34

Poor responsiveness of naïve CD8+ T-cells from elderly individuals is associated to their altered basal metabolism

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Quantitative and qualitative alterations of naïve CD8+T-cells are primary hallmarks of immune aging. The naïve T-cell compartment represents the source of de novo cellular responses, and its impairment an important obstacle to respond to and clear emerging pathogens and tumours in old individuals. Notably, elderly subjects also show severe defects in responding to vaccinations, and this has been associated to both the decline in the absolute numbers of Naïve CD8+T-lymphocytes as well as to the disruption of their priming and differentiation capacity upon TCR mediated activation.

DOI: 10.1002/eji.201970300

However, the intrinsic defects associated with their altered functionality remain unclear, which is a critical obstacle for the development of vaccines tailored for the elderly population. Considering the important role of intracellular metabolism in lymphocyte functionality and the systemic bioenergetics dysfunctions characterizing elderly subjects, we aimed here at studying the metabolic features of naïve CD8+T-cells in elderly humans. Our data indicate that naïve CD8+T-cells from elderly donors present an active basal state, which is supported by alterations at the level of mitochondrial metabolism and increased fatty acid uptake and storage. IL-7, known to drive T-cell homeostatic proliferation and present at elevated levels with aging, induces lipid uptake and storage, similarly to observations in naïve CD8+T-cells from elderly donors. This particular bioenergetics profile is associated with increased apoptotic levels and decreased proliferative response of old naïve CD8+T-cells upon TCR-triggering. Notably, lipid lowering strategies are able to restore normal TCR-induced caspase-3 expression and proliferation. The present work highlights that alterations of the lipid metabolism in naïve CD8+T-cells with old age may alter their responsiveness. Approaches favoring lipid catabolism may represent interesting therapeutic strategies to improve T-cell immunity and vaccine efficacy in the elderly.

P35

The transcription factor TOX controls differentiation and maintenance of CD8+ T-cells in chronic infections.

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During chronic infections, virus-specific CD8+ T-cells acquire a differentiation program that is distinct from the one acquired in acute infections. The persisting antigen stimulation causes T-cells to become dysfunctional which means that they show impaired effector functions and up-regulated inhibitory receptor expression. Antigen amount is a critical factor for the generation of the chronic phenotype, which is retained in persistent infections even after antigen removal. To investigate key determinants in the generation of the chronic phenotype, we use the LCMV mouse model with a newly developed experimental system that allowed us to compare T-cells with acute-functional and chronic-dysfunctional phenotype, and identify core genes responsible for the generation and maintenance of the chronic phenotype. The transcriptional profile analysis revealed the transcription factor TOX as one of the most upregulated genes in chronically stimulated T-cells. TOX expression correlates with the chronic phenotype and it is maintained after antigen withdrawal in chronically stimulated mice. TOX is induced by high antigen levels and its absence strongly affects dysfunctional T-cells, which are forced to acquire an effector phenotype, with decreased PD-1 but increased KLRG-1 expression and cytokines production. The induction of an acute phenotype in chronically stimulated T-cells reveals a deep alteration of the transcriptional program, and eventually results in impaired survival of dysfunctional CD8+ T-cells, explained by a substantial loss of the critical Tcf-1+ progenitor population. Interestingly, the enhanced effector phenotype is coupled with stronger effector function, which leads to a better viral clearance, but also to an increase in immunopathology. TOX promotes the dysfunctional phenotype in CD8+ T-cells, nonetheless ensures their fitness for long-term maintenance during chronic infections. Importantly, TOX prevents the acquisition of the effector phenotype and triggers a response in T-cells that protects the host form overwhelming immunopathology. Therefore, TOX is a key regulator of the differentiation program that leads chronically stimulated T-cells to acquire a dysfunctional phenotype. This essential role in tuning T-cells function under persistent stimulation makes TOX a major target for future immunotherapeutic approaches.

P36

SLA class-I deficiency by targeting b2m in pigs – phenotypical and functional characterisation for xenotransplantation

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DOI: 10.1002/eji.201970300

Introduction: Foreign MHC molecules are the main inducers of anti-graft responses and targets of cytotoxic effector cells during rejection. The interaction between porcine MHC (SLA, swine leucocyte antigen) molecules and the human T cell receptor is functional across the species-barrier. The absence of SLA class-I molecules should, therefore, lead to a decreased immunogenicity of porcine cells and tissues. Therefore, SLA class-I deficient pigs were generated and the immune status of the animals and the stimulatory potential of their cells and tissues were characterised.

Methods: For cell surface expression of functional SLA class-I molecules, the non-polymorphic beta2-microglobulin (b2m) is regarded to be essential. Thus, SLA class-I deficient pigs were generated by targeting the b2m gene using CRISPR/Cas9 in porcine cells following somatic cell nuclear transfer (SCNT). Resulting b2m "knock-out" (b2m-ko) pigs were characterised phenotypically and cells from these animals were tested for their potential to stimulate proliferative responses and cytotoxic activity of human cells in comparison to wildtype (wt) porcine cells.

Results: Histological staining showed no b2m or SLA class-I expression in tissues from organs relevant for transplantation (heart, kidney, liver). Flow cytometry analyses revealed a strongly decreased b2m and SLA class-I expression on PBMC. SLA class-I deficiency was also reflected by the total absence of CD8+ T cells in peripheral blood. Additionally, piglets showed reduced cytokine and immunoglobulin levels. In xenogeneic MLR assays we observed significantly reduced proliferation of human CD8+ T cells to stimulation with PBMC from b2m-ko pigs. Despite this, cytotoxic effector cells were generated in 6d MLRs which lysed SLA class-I negative targets as effectively as targets from SLA class-positive wt pigs. Experiments are ongoing to clarify whether this lysis reflects the activity of NK cells or killing mediated by human CD4+ T cells via the CD95/CD95L pathway.

Conclusion: Targeting of b2m is an appropriate strategy to generate pigs expressing significantly reduced levels of SLA class-I molecules. The functional data indicate a low immunogenic status of cells from SLA class-I deficient pigs, predominantly by preventing the induction phase of human anti-pig T cell reactivity. Additional strategies should be introduced to protect porcine cells from cytotoxic effector cells.

Supported by the DFG (SFB-TRR 127 "Xenotransplantation")

P37

Adenine nucleotide-modulated T cell differentiation and effector functions

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Introduction: Activation of T cells via the TCR provokes a Ca^{2+} signal. Adenine nucleotide-derived 2nd messengers including adenosine diphosphoribose (ADPR), 2"-deoxy-ADPR (2dADPR), cyclic ADPR (cADPR) and nicotinic acid adenine dinucleotide phosphate (NAADP) trigger different Ca^{2+} channels and initial data suggest that these 2nd messengers could regulate Ca^{2+} signalling in T cells. CD38 is involved in interconversion of these 2nd messengers and could modulate the response.

Objectives: We investigate the role CD38 and of Ca^{2+} channels activated by adenine nucleotide 2nd messengers in CD8 T cell activation and differentiation.

Materials & methods: CD8 T cells from mice deficient in CD38 or the Ca^{2+} channel TRPM2 will be used. In addition, deficient mice transgenic for an ovalbumin-specific TCR (OT1) are available. CD8 T cells will be incubated with anti-CD3 mAb or ovalbumin peptide-loaded BMDCs and Ca^{2+} signalling and induction of early activation genes will be analysed. TCR signalling will also be investigated in the presence of an NAADP agonist. TCR transgenic CD8 T cells will be transferred into recipients infected with ovalbumin-recombinant listeria and their response will be determined.

Results: Preliminary results show that early CD8 T cell activation is regulated by the strength of stimulation, which applies for anti-CD3 mAb and for incubation with DCs loaded with fully and partially agonistic ovalbumin peptides. FACS analysis of activated CD8 T cells revealed an "on/off" type of response for the expression of early response genes with an increase in the frequency of activated cells after stimulation with increasing strength as opposed to differences in the activation level of cells. This effect was also found in the frequency of CD8 T cells that generated a Ca^{2+}signals in response to stimulation with increasing strengths. The NAADP antagonist decreased the responding T cell population when added during activation. So far, no effect on early CD8 T cell responses was detected in cells

DOI: 10.1002/eji.201970300

from CD38 and TRPM2-deficient mice. Currently, the responses of CD38 and TRPM2-deficient CD8 T cells in vivo and the formation of memory T cells are investigated.

Conclusion: Our data suggest a stimulus-threshold for TCR activation of naive CD8 T cells and that the strength of the TCR signal primarily determines the number of T cells that reach this threshold. First experiments indicate that CD38 and TRPM2 do not alter early T cell activation in CD8 T cells.

P38

Removal of H3K27me3 is key for initiation of transcriptional programs required for early virus-specific CD8+ T cell differentiation.

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Understanding the molecular events that underpin initiation of programmed virus-specific CD8+ T cell differentiation is needed to improve optimal T cell responses. Profiling genomic wide changes in H3K27me3 deposition, chromatin accessibility and gene transcription, we identified that prior to the first cell division, there is extensive epigenetic reprogramming that results in stepwise engagement of specific transcriptional modules. This early reprograming involves rapid H3K27me3 demethylation at genes involved in a broad range of cellular support processes intrinsic to readying activated T cells for proliferation. Inhibition of H3K27me3 demethylation within early-activated T cells constricted the magnitude of an effective primary virus-specific CD8+ T cell responses and the formation of memory CD8+ T cell populations. H3K27me3 methylation therefore acts as a molecular handbrake with H3K27me3 demethylation required at the very earliest stages of T cell activation, enabling lineage-specific reprogramming of effector and memory CD8+ T cells

P398

Memory CD8 T cell protection from viral reinfection depends on interleukin-33 alarmin signals

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Memory CD8 cytotoxic T lymphocytes (CTLs) can protect against viral reinfection. However, the signals driving rapid memory CTL reactivation have remained ill-defined. Viral infections can trigger the release of the alarmin interleukin-33 (IL-33) from non-hematopoietic cells. IL-33 signals through its unique receptor ST2 to promote primary effector expansion and activation of CTLs. Here, we show that the transcription factor STAT4 regulated the expression of ST2 on CTLs *in vitro* and *in vivo* in primary infections with lymphocytic choriomeningitis virus (LCMV). In the primary antiviral response, IL-33 enhanced effector differentiation and antiviral cytokine production in a CTL-intrinsic manner. Further, using sequential adoptive transfers of LCMV-specific CD8 T cells, we deciphered the IL-33 dependence of circulating memory CTLs at various stages of their development. IL-33 was found dispensable for the formation and maintenance of memory CTLs, and its absence during priming did not affect their recall response. However, in line with the CTL-boosting role of IL-33 in primary LCMV infections, circulating memory CTLs required IL-33 for efficient secondary expansion, enhanced effector functions, and virus control upon challenge infection. Thus, beyond their effector-promoting activity in primary immune reactions, innate alarmin signals also drive memory T cell recall responses, which has implications for immunity to recurrent diseases.

Infection (P39-P86)

P39

PD1-PDL1 interaction is required for the expansion of a population of CD4^{+}Ly6C^{hi}effector T cells during *Leishmania major* infection.

DOI: 10.1002/eji.201970300

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Introduction Programmed cell death (PD)-1 receptor and its ligand PD-L1 (B7-H1) are important inhibitory receptors, which coordinates both T cell activation by dendritic cells and effector function of experienced antigen-specific T cells. The role of this pathway during infection by intracellular protozoan *Leishmania* has not been well studied yet. **Objective** The aim of this study was to investigate the role of PD1-PD-L1 during the infection by the intracellular parasite *Leishmania (L.) major*.

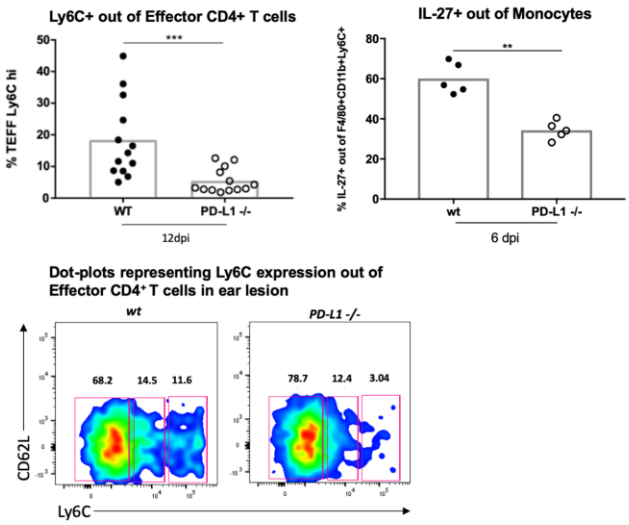
Materials & methods C57BL/6J and PD-L1$^{-/-}$ mice were bred and co-housed and subcutaneously infected in the left ear with 3 x 10^6 7-days stationary promastigotes of *L. major*. Uninfected mice were used as controls. Mice were followed over the infection course and euthanized in different time points and ear and draining lymph nodes (dLN) processed to obtain single cell suspensions. Ear lesions were measured with the aid of a digital calliper. Sample aliquots were used to quantify the amount of tissue parasites and for macrophages, monocytes, fibroblasts, effector ($CD44^+CD27^-CD62L^-$) and regulatory ($Foxp3^+$) $CD4^+$ T cells, and IL-27 staining with antibodies fluorochrome-conjugated for FACS. This research was approved by the authorities of the Hamburg-Germany state (Nr N 027/2018). Statistical calculations used two-tailed Mann Whitney test and assumptions were made at 95% confidence level.

Results This study has shown an important strong up-regulation of PD-L1 by different cell subsets at the infection site with *L. major,* and, at least in our model, mice lacking PD-L1 exhibited increased lesions, but with no impact on parasite burden. In the increased lesions analysis of monocyte/macrophage function revealed that the lack of PD-L1 may contribute to a less activation status and tissue remodeling function of these cells. Interestingly, the lack of PD-L1 contributes to an impaired expansion of a population of $CD4^+Ly6C^{hi}$effector T cells in the dLN and at the infected site, and the lack of PD-L1 contributes to a lower production of IL-27 by local infiltrating monocytes which comes along with the lower level of Ly6C on $CD4^+$T cells.

Conclusion This study suggests a role for PD1-PD-L1 interaction in inducing the expansion of a population of $CD4^+Ly6C^{hi}$effector T cells during the infection by *L. major* which might be dependent on IL-27.

Key-words: PD-1; PD-L1; $CD4^+Ly6C^{hi}$effector T cells; *Leishmania major*.

Figure 1



DOI: 10.1002/eji.201970300

P40

C. trachomatis serovar D interaction with TLR3 in epithelial cells of the human urogenital tract

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Introduction: *Chlamydia trachomatis* serovar D-K is one mayor cause of female genital tract infections leading untreated to chronic activation of the immune response resulting in tubal factor infertility. To enable an adequate immune reaction in case of pathogens' invasion epithelial cells of the human urogenital tract are provided with numerous pattern recognition receptors (PRRs) such as toll like receptors (TLRs). TLR3 in particular is localized in the endosome to recognize external dsRNA. When TLR3 binds such a structure the corresponding pathway, consisting amongst others of the intracellular adapter TRIF and the transcription factor IRF3, is activated and results in enhanced interferon beta (IFN-β) production.

Objectives: Our aim was to investigate the role of TLR3 in cells of the human urogenital tract in case of *C. trachomatis* serovar D infection.

Materials & methods: We detected TLR3 and TRIF mRNA by RT-PCR and the expressed proteins by immunofluorescence microscopy in various cervix and bladder cancer cell lines. Moreover IFN-β concentration in supernatants was measured by ELISA. To analyse the inclusion size *C. trachomatis* forms during infection in human bladder cancer cells we made use of immunofluorescence microscopy again. To receive an overview on the host cell mRNA, a RNA-Array was performed comparing host cell RNA-profiles under infection in a TLR3-dependent manner.

Results: We found that, as the basis for a functional pathway activation, TLR3 and TRIF are expressed and recruited to chlamydial inclusions in all tested cell lines. However, IFN-β production after infection with *C. trachomatis* did not take place in our experiments. When TLR3 was inhibited by a small molecule inhibitor, a significant diminishment of *C. trachomatis* inclusions was observed in the human bladder cancer cell line T24/83. Furthermore, these inclusions produced no reproductive progeny. Concerning the mRNA-levels a TLR3-dependent regulation of approximately 93 genes could be detected after infection, affecting various intracellular pathways.

Conclusion: This opens up a new perspective on the interaction between *C. trachomatis* and TLR3, assuming that TLR3 might play a crucial role in *C. trachomatis'* intracellular development among others probably through nutritional pathways which haven't been identified yet.

P41

Inhibition of type I interferon production by murine leukemia virus (MuLV)

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Introduction: The initial immune response to retroviruses is highly important to clear infection, since retroviruses integrate their reverse transcribed genome into the host genome which leads to persistent infection resulting in immunodeficiencies, cancers and other pathologies.

Several pattern recognition receptors (PRRs) like Toll-like receptors (TLRs) and cyclic GMP-AMP synthase (cGAS) have been reported to detect retroviral nucleic acids. Especially, plasmacytoid dendritic cells (pDC) are targets of retrovirus infection but murine leukemia virus (MuLV) infection does not lead to any type I interferon (IFN) response.

Modification of RNA influences its recognition negatively and for example 2'-O-methylation transforms RNA into an antagonist for TLR7. This leads to the hypothesis of modified RNA being present in MuLV.

Objectives:

- Investigating antagonistic components of MuLV.
- Analyzing modifications of RNA packaged within MuLV particles.

Materials & methods: MuLVs were generated from producer cell lines followed by ultracentrifugation. Murine pDCs were differentiated from bone marrow cells by induction with murine Fms-related tyrosine kinase 3 ligand (Flt3L) and

DOI: 10.1002/eji.201970300

human pDCs were isolated from buffy coats. Immune responses were analyzed by ELISA and RNA modifications were examined by HPLC.

Results: MuLV infection does not lead to type I IFN production within murine Flt3L-DCs as well as human pDCs. Furthermore, MuLV infection inhibits type I IFN secretion induced by TLR-ligands and this inhibitory effect does not depend on the envelope or glycogag polyprotein of MuLV and in addition the inhibitory SIGLEC-H/DAP12 pathway is not involved. Interestingly, heat inactivation destroyed the antagonistic function and rendered MuLV itself immunostimulatory. Immune stimulation with non-G18-methylated tRNA revealed an immunostimulatory effect, which however could not be confirmed with MuLV produced by methyltransferase knock-out cells. Genomic MuLV-RNA is sensed by murine Flt3L-DCs in a TLR7-dependent manner. RNA recognition by human pDCs is more complex, since RNA from wt- or ΔΨ-MuLV is equally recognized.

Conclusion: MuLV infection does not induce a type I IFN response within murine Flt3L-DCs or human pDCs. Furthermore, MuLV possess immune suppressive properties, which can be abrogated by heat treatment. Human pDCs are able to sense non-viral RNA in contrast to murine Flt3L-DCs by a receptor of unclear nature.

P42

Development of a two-step flow cytometry method for a field setting using whole blood for the description of phenotypic markers on peripheral blood cells

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Question: In resource poor settings, research activities are limited because local laboratories lack equipment or trained personnel. Polychromatic flow cytometry is a method commonly used to characterize different immune cell subsets but is often not available in tropical regions. Here infections, such as from helminths or HIV, frequently occur. Flow cytometry can be done using cryopreserved peripheral blood mononuclear cells and measurements can then be performed in specialized laboratories. However, this requires cryopreservation in liquid nitrogen and large blood draws, which are often difficult, particularly in small children.

Our aim was to characterize various cell populations in peripheral blood, including memory and regulatory T helper cells, along with a variety of activation markers and markers that may be relevant for HIV transmission within *Wuchereria bancrofti* (*W. bancrofti*) infected individuals. We have developed and optimized a method for measuring up to 9 phenotypic markers using 100 µl of blood, which can potentially be taken via finger prick.

Materials and Methods: Peripheral blood was collected in EDTA or heparin collection tubes. Extracellular antibodies (b7-PE, CD4-PerCpC5.5, HLADR-PeCy7, CD38-APC, CD27-APC-H7, CD45RA-Bv421, CCR5-APC, CD8-V500, CD25-Bv605) were added and after an incubation, cells were lysed, frozen and stored in liquid nitrogen for transport and further processing. After a period of up to 6 months, cells were thawed, permeabilized, and stained with intracellular antibodies (CD3-ECD, FoxP3-FITC, Tbet-PE, Eomes-PeCy7) before acquisition on a 13-parameter Flow Cytometer.

Results: Initial tests showed highly comparable results between freshly drawn and cryopreserved samples. This method has been performed in two studies in Ghana and Tanzania, which focus on the immunology of individuals infected with *W. bancrofti.* Over 200 participant samples have been analyzed with promising results. The participant samples that were preserved for 6 months before analysis are comparable to those that were freshly processed and analyzed.

Conclusions: We have developed a method which, to the best of our knowledge, is a new whole blood extracellular and intracellular assay that is easy to use. Due to the minimal amount of blood required and the robustness, we believe that the method is useful for both evaluating immunological parameters in children and field studies in remote settings.

P43

Membrane-bound proteases ADAM10 and ADAM17 regulate the T cell response against intracellular bacteria

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DOI: 10.1002/eji.201970300

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Introduction: The protease ADAM (*A Disintegrin And Metalloproteinase*) 17 is an important modulator of the immune system. Over 70 immunorelevant targets are currently known, including TNF-α and its receptors. However, we could previously show that the immune response against *Listeria monocytogenes* of mice with a T-cell specific ADAM17 knockout is comparable to that of control mice. ADAM10 is involved in the NOTCH signaling pathway during thymic T-cell development and differentiation of T cells in the periphery. ADAM10 also shares substrate specificity with ADAM17. A potential redundancy in ADAM protease function might explain the limited effect of ADAM17 in our infection model and serves as the focus of this research project.

Objectives: In the current study, we aim to elucidate the function of ADAM10, alone or combined with ADAM17, in the activation and differentiation of T cells during the primary immune response and in the generation of memory T cells.

Materials & Methods: We used mice with a T-cell restricted deficiency in either ADAM10 ($A10^{\Delta CD4}$), or ADAM10 and ADAM17 ($A10/A17^{\Delta CD4}$) combined and corresponding control mice. We infected mice with a recombinant Listeria monocytogenes strain expressing the ovalbumin antigen (LmOVA) and analysed the T-cell response after 8 days (primary response). Memory T-cell development was assessed in different tissues after 10 weeks. In addition, mice were rechallenged with a lethal LmOVA dose in order to elucidate the influence of ADAM10 and ADAM17 on the recall response.

Results: Under homeostatic conditions, distribution of peripheral T cells was comparable between $A10/A17^{\Delta CD4}$ and control mice. However, during primary infection the total number and the number of listeria-specific $CD4^+$ and $CD8^+$ T cells in spleen and liver from $A10/A17^{\Delta CD4}$ mice was significantly reduced when compared to numbers in control mice. Following secondary infection, deficient and control mice had similar total numbers of T cells, but tissues of $A10/A17^{\Delta CD4}$ still contained diminished numbers of specific T cells.

Conclusion: Combined deficiency of ADAM10 and ADAM17 in T cells does not significantly impact T-cell numbers and distribution. However, deficiency significantly impairs the T-cell response during primary and secondary L. monocytogenes infection. Currently, we aim at determining the underlying mechanisms.

P44

Role of receptor specificity and membrane fusion activity of influenza viruses in immune cell tropism

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Introduction Hemagglutinin protein (HA) of influenza A viruses (IAVs) mediates binding to cellular receptors and fusion of viral and endosomal membranes at low pH. Both properties of HA vary depending on viral host species and evolutionary lineage. Growing evidence suggests that this variation may affect cell tropism and pathogenicity of IAVs. For example, avian IAVs such as zoonotic H5N1 that predominantly bind to 3-linked sialic acids (Sia) and have high pH optimum of fusion are associated with increased fatal outcomes related to severe immune dysregulation.

Objective To characterize how receptor-binding specificity and pH optimum of membrane fusion of HA affects viral infection and induction of immunological responses in different types of human immune cells

Material & methods A panel of 2:6 recombinant IAVs was generated with 6 gene segments of A/PR8 virus and distinctive HA and NA from seasonal, pandemic or zoonotic IAVs. Point mutations in the HA were introduced which affected receptor-binding specificity or pH of fusion. The IAVs were used to infect either human PBMCs or isolated plasmacytoid DCs. Flow cytometry was used to determine susceptibility of different cells to IAVs and to analyse expression of Sia. The innate immune response was determined by measuring IFNα using ELISA.

Results Studies on interaction of IAVs with purified human pDCs revealed that levels of virus-induced secretion of IFNα correlate with viral receptor specificity rather than pH optimum of fusion. Thus, IAVs with avian-virus-like binding preference for 3-linked Sia showed eight times higher IFNα secretion than human-type IAVs with favoured binding to 6-linked Sia and HA fusion mutants. These findings agreed with abundant expression by pDCs of 3-linked Sia. The

DOI: 10.1002/eji.201970300

infection of PBMCs with single point HA mutants of recombinant pandemic IAV A/HK/68-PR8 (H3N2) revealed differences in susceptibility of different immune cells. Whereas $CD14^+$ monocytes and $CD123^+CD303^+$ pDCs were efficiently and equally infected, $CD20^+$B-cells and T cell subpopulations showed significantly lower susceptibility to the avian like IAV-mutant. This pattern correlated with the levels of expression by the cells of 6-linked Sia.

Conclusion Our results suggest that the receptor specificity of the HA has a more pronounced effect than pH optimum of fusion on IAV infectivity for pDCs and $CD3^+$ cells. Studies are in progress on susceptibility of PBMCs and isolated cell populations using an expanded panel of IAVs.

P45

The long pentraxin PTX3 has a non-redundant role in the control of *Streptococcus pneumoniae* invasive infections

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Pentraxin 3 (PTX3) is a fluid phase pattern recognition molecule which has served as a paradigm for linking the cellular and humoral arms of innate immunity. PTX3 is an important component of host resistance to pulmonary infections for selected pathogens. Our aim was to investigate the role of PTX3 in the control of pneumococcal infections caused by *Streptococcus pneumoniae*, the most common causative bacteria in community-acquired pneumonia and an important cause of mortality world-wide.

By using a model of invasive pneumococcal infection in young-adult mice, we observed a strong expression of PTX3 by non-hematopoietic cells. Comparing the pneumococcal load and survival of infected mice, we observed a higher sensitivity *of Ptx3$^{-/-}$* animals during the invasive phase of the infection which could be restored by a systemic administration of recombinant PTX3. Infected *Ptx3$^{-/-}$* mice also showed an increased inflammatory profile. Furthermore, the local exogenous instillation of PTX3 during the ongoing infection was able to reduce the expression of numerous inflammatory cytokines and the pulmonary pneumococcal load. We also observed that PTX3 specifically bind on *S. pneumoniae* but not in physiological conditions found during *in vivo* infection. The mechanism of the protective function of PTX3 remains to be fully elucidated but does not appear to be dependant of the opsonophagocytosis features of PTX3.

Our results suggest a non-redundant role of PTX3 in the control of *S. pneumoniae* infections by limiting the damaging inflammation during the invasive phase of infection. Given that PTX3 is characterized to bind P-selectin expressed by endothelial cells and damp the neutrophil recruitment at the local site of inflammation, we are currently investigating the involvement of the interaction P-selectin/PTX3 in the control of pneumococcal invasive infection.

P46

T cell-specific ablation of Neuropilin-1 attenuates experimental cerebral malaria

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Introduction: Malaria is induced by the parasite *Plasmodium* which, in 2017, affected 219 million people and led to 435,000 deaths worldwide. The course of infection is characterized by fever, anemia as well as respiratory distress and can culminate in severe neurological deficits, brain edema and coma leading to a mortality rate of 15-25%. Fundamental mechanisms of cerebral malaria can be investigated by infection of mice with *Plasmodium berghei ANKA* (PbA) parasites resulting in accumulation of peripheral immune cells in the brain.

Objectives: Here, we study whether Neuropilin-1, a semaphorin and VEGF co-receptor participating in immune cell migration during tumorigenesis, contributes to lymphocyte infiltration into the brain during experimental cerebral malaria.

DOI: 10.1002/eji.201970300

Material and methods: For this purpose, we infected T cell-specific Neuropilin-1-deficient mice with PbA parasites and monitored the development of cerebral malaria symptoms. The T cell response within the spleen, blood and brain was analysed by flow cytometry.

Results: Neuropilin-1-expressing T cells of PbA-infected mice showed a highly activated and migratory phenotype with increased effector function. T cell-specific ablation of Neuropilin-1 expression attenuated cerebral malaria pathology associated with significantly decreased numbers of peripheral immune cells in the brain compared to infected wildtype mice.

Conclusion: The results from our study suggest a role of Neuropilin-1 in immune cell migration into the brain during experimental cerebral malaria.

P47

Detrimental role of CD47 in protective immunity during severe influenza virus infection

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Introduction: Influenza viruses are among the most common causes of human respiratory infections. However vaccination is seasonally limited to date. Therefore, new strategies are needed to treat severe influenza virus (IV) infection. The integrin-associated surface protein CD47 has been implicated in various immune cell functions but its role for anti-viral immunity is still poorly understood.

Objectives: Here we determine the impact of CD47 on innate and adaptive immune responses during IV infection and define the therapeutic potential of CD47 blockade.

Materials and Methods: To this end C57BL/6 mice as well as mice deficient for CD47 ($CD47^{-/-}$) were infected with IV strain A/PR8/34. Moreover, IV infected C57BL/6 mice were treated 1 day post infection (dpi) with a monoclonal CD47 blocking antibody. The immune responses were examined by flow cytometric analysis and viral loads in the lungs of infected mice were determined by plaque assay as well as qRT-PCR.

Results: While the expression of CD47 and its ligands signal regulatory protein α (SIRPα) and thrombospondin 1 (TSP-1) is enhanced on various immune cells upon infection, our results depict CD47 to modulate the immune response against IV early during infection as frequencies of alveolar macrophages were increased in $CD47^{-/-}$ mice compared to the wildtype. Furthermore, we found that the generation as well as the activation of mature NKT cells was enhanced in absence of CD47. Beside their direct effects on the viral clearance both types of these innate immune cells may further shape the functionality of the subsequent adaptive immune response. Consequently, $CD47^{-/-}$ mice showed lower viral loads as well as less severe pathology. In line with this, our data also demonstrates that the blockade of CD47 by a monoclonal antibody likewise reduces the viral load in the lung of infected mice.

Conclusion: Thus, the data implies CD47 as a potential therapeutic target for the treatment of acute IV infection.

P48

The T cell epitope repertoire of polyomavirus BK

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Introduction: Polyomavirus BK (BKV) is a persistent virus, widespread in the population, and an important pathogen in immunocompromised persons. No specific treatment is currently available.

BKV-specific T cells probably protect against disease, for example after transplantation. T cell epitopes of BKV antigens have been identified, but limited to subsets of BKV antigens and HLAs. Antiviral protection appears associated with certain HLAs, but protective epitopes have not been identified.

Objectives: A more complete picture of the BKV-specific T cell and epitope repertoire would allow to improve immunomonitoring and immunotherapy. Therefore, we have studied the BKV-specific T cell response at the level of antigens, epitopes and HLA restriction in healthy donors.

DOI: 10.1002/eji.201970300

Materials & Methods: We used CD40-activated B cells loaded with peptide libraries to establish T cell clones from PBMC of healthy donors. Phenotype and function of T cell clones were analyzed using FACS, ELISA and ELISPOT.

Results: Our results indicate that individual BKV carriers recognize multiple epitopes per antigen. Analyses of ex vivo reactivity in ELISPOT (n=27) indicated that most donors recognize 2-5 antigens (out of 5). CD8 and CD4 T cell clones against regulatory proteins (t and T antigens) and structural proteins (VP1 and VP2/3) were established, and multiple target epitopes were identified.

Conclusion: We identified various new epitopes targeted by the BKV-specific T cell response in healthy donors. In the next step, the presence of such T cells in patients after stem cell transplantation in association with BKV infection will be analyzed. The results of these studies will improve monitoring and therapy of patients.

P49

Differential effects of HLA-A- and HLA-E-restricted CD8⁺ T lymphocytes on *Mycobacterium tuberculosis* and HIV-1 co-infection.

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Introduction: We have investigated the contribution of HLA class Ia- and HLA-E-restricted CD8⁺ T cells in patients with *Mycobacterium tuberculosis* and HIV-1 co-infection on the ground, that HIV-1 downregulates HLA-A, -B and -C molecules from the infected cell surface, and this in turn might influence infected cell recognition by HLA class Ia-restricted CD8⁺ T cells.

Objective.: We have then addressed whether HLA class Ia- and HLA-E-restricted CD8⁺ T cells were equally capable to recognize and kill macrophages co-infected with HIV-1 and *Mycobacterium tuberculosis* and to reduce the viability of both intracellular pathogens. In addition, we have analysed the frequency and functions of HLA class Ia- and HLA-E-restricted CD8⁺ T cells in patients with *Mycobacterium tuberculosis* and HIV-1 co-infection.

Materials and Methods: HLA-E- and HLA-A2-restricted CD8⁺ T cells were tested *in vitro* for cytotoxic and microbicidal activities toward *Mycobacterium tuberculosis*-infected macrophages and their frequencies, phenotypes and functions evaluated *ex vivo* in patients with active tuberculosis and concomitant HIV-1 infection.

Results: HIV-1 and *Mycobacterium tuberculosis* co-infection caused downmodulated of HLA-A2 expression in human monocyte-derived macrophages, which was associated with resistance to lysis by HLA-A2-restricted CD8⁺ T cells and failure to restrict the growth of intracellular *Mycobacterium tuberculosis*. Conversely, HLA-E surface expression and HLA-E-restricted cytolytic and microbicidal CD8⁺ T cell responses were not affected. HLA-E-restricted CD8⁺ T cells were expanded in the circulation of patients with *Mycobacterium tuberculosis*/HIV-1co-infection, as measured by tetramer staining, displayed a dominant terminally-differentiated phenotype but had an exhausted profile. Blocking of the PD-1/PD-L1 pathway with the anti-PD-1 mAb Nivolumab consistently improved proliferation of HLA-E-restricted CD8 T cells and decreased apoptosis rate.

Conclusions: HLA-E-restricted and *Mycobacterium tuberculosis*-specific CD8⁺ T cells in patients with *Mycobacterium tuberculosis*/HIV-1co-infection have a dominant terminally-differentiated profile, display an exhausted phenotype and fail to expand *in vitro* in response to antigen stimulation, which can partially restored by blocking PD-1 pathway.

P50

Role of cytosolic MRP8/14 in leukocyte recruitment *in vivo*

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DOI: 10.1002/eji.201970300

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Introduction: Extracellular Myeloid Related Protein 8/14 (MRP8/14) was shown to activate β2 integrins thereby mediating neutrophil adhesion during an inflammatory process both *in vitro* and *in vivo*. Despite increasing evidence of the extracellular functions of MRP8/14 as an alarmin, its intracellular function remained elusive.

Objectives: We wanted to investigate a putative role of intracellular MRP8/14 in post arrest modification steps enabling neutrophil spreading, adhesion strengthening and crawling under flow conditions.

Materials & methods: We investigated MRP8/14 dependent neutrophil adhesion, spreading, crawling and resistance to increasing shear rates in flow chambers coated with E-selectin, ICAM-1 and KC using WT and *Mrp14-/-*, which are functional MRP8/14 deficient, mice.

Results: *Mrp14-/-* neutrophils showed reduced adhesion to E-selectin, ICAM-1 and KC coated microflow chambers when compared to WT neutrophils. They were unable to spread properly, crawled more in the direction of flow and were more susceptible to detach at increasing shear stress levels.

Conclusion: Based on our findings we propose a role for intracellular MRP8/14 in leukocyte adhesion under flow, by affecting outside in signalling processes and adhesion strengthening of neutrophils.

P51

Synthetic oligosaccharide-based vaccines protect mice from Clostridium difficile infections

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Introduction: *Clostridium difficile* is the leading cause of antibiotic-associated diarrhea worldwide. Recent increases in incidence and case-fatality rates have been associated with an expansion of emerging hypervirulent strains with elevated production of the two disease-mediating toxins TcdA and TcdB, the main virulence factors of *Clostridium difficile*. As commonly used frontline antibiotics become less effective and frequently induce recurrent disease by disrupting the intestinal microbiota, the development of vaccines to prevent this devastating and emerging disease is highly desirable.

Objectives: The clinically most advanced vaccine approaches prevent symptoms once infection has established by inducing immunity to the secreted clostridial toxins. However, they do not inhibit bacterial colonization and thereby might favor asymptomatic carriage. Thus, to preclude colonization with *Clostridium difficile*, we have identified several bacterial surface glycans such as lipoteichoic acid (LTA) and the polysaccharides I and II (PS I and II) as promising vaccine candidates.

Materials & Methods: We conjugated PS-I, PS-II and LTA to CRM197, a carrier protein used in commercial vaccines. Mice were vaccinated with these glycoconjugate vaccines against PS I, PS II and LTA. The composition of the intestinal microbiota, *Clostridium difficile* counts and intestinal inflammation were assessed using plating assays, qPCR, 16S rRNA sequencing and histopathological analyses.

Results: PS I-, PS II- and LTA- glycoconjugate vaccines induced glycan-specific antibodies in mice and substantially limited colitis and colonization with *Clostridium difficile* after experimental infection. These anti-glycan antibodies selectively inhibited the colonization of the gut with *Clostridium difficile* without disrupting the intestinal microbiota. The glycoconjugates induced long-term protection and were even superior to a toxin-targeting vaccine candidate in preventing *Clostridium difficile* mediated disease.

Summary: Glycoconjugate vaccines against *Clostridium difficile* represent a complimentary approach to toxin-targeting strategies and are advancing through preclinical work. As anti-toxin immunoglobulins correlate with asymptomatic carriage, toxin-based vaccines may even expand the presence of *Clostridium difficile* in the population. Vaccines targeting bacterial surface structures, in contrast, could limit the human reservoir due to the inhibition of intestinal colonization.

DOI: 10.1002/eji.201970300

P52

Helminth-specific CD4⁺ T cell responses during *Ascaris* infection in the pig

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The pig represents the ideal human-relevant research model to study the immune response to *Ascaris* spp., the most common of the soil-transmitted helminths. The large roundworms infecting humans and pigs, *A. lumbricoides* and *A. suum,* are genetically almost identical and cross-transmission occurs, thus highlighting the role of pig studies to understand the host-parasite interaction.

There is an urgent need to be able to assess porcine, pathogen-specific CD4⁺ T cells - cells that are rare, but of upmost importance in orchestrating the host immune response to the worm.

In our study we used CD40L (CD154) expression as an early TCR activation marker of swine CD4⁺ T cells to investigate frequency, phenotype and specificity of *Ascaris*-reactive T cells in the pig as a natural host. A time course study demonstrates the onset of a robust, antigen-specific Th2 response early during larval tissue migration.

Ascaris-specific CD4⁺ T cells are directed against both excretory-secretory proteins and parasite lysates. To improve functional analysis of helminth-specific T cells we adapted the method of antigen-reactive T cell enrichment (ARTE) to porcine CD4⁺ T cells. Enrichment analysis revealed phenotypic differences in CD4⁺ T cells from peripheral *vs.* migration affected compartments such as the lung parenchyma with regard to Th2 and Th1 marker cytokines.

Our approach thereby offers a novel strategy to identify and characterize *Ascaris*-specific CD4⁺ T cells directly in the pig, and will be used to unravel mechanisms of protection, immune evasion and protective antigens.

P53

Infection with Orf virus (ORFV) in organotypic cultures (OTC) of skin: histological changes, viral gene transcription and modulation of human keratinocytes differentiation

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Parapoxviruses (PPV) are epitheliotropic viruses causing localized lesions. In contrast to systemic Orthopoxvirus infections, the human zoonotic infection with PPV, especially with Orf virus (ORFV), is a localized event resulting in a normally benign lesion commonly known as milker"s nodule that is completely resolved within a few weeks. Even though the development of lesions caused by ORFV was extensively studied in animals, only limited knowledge exists about the course of human skin infection. Organotypic cultures (OTC) of skin have been established for various purposes and can be a suitable equivalent, e.g. for studies of wound healing or infectious diseases manifesting in the skin. The aim of the present study was to evaluate a OTC as a human skin model for ORFV infection to study replication of the virus, viral gene transcription, histological changes and modulation of differentiation of human keratinocytes. Infection was performed at the setup and near termination of OTC using a human ORFV wild-type isolate. Primary normal human keratinocytes (KC) were isolated from epidermis of juvenile foreskin. The transcription of *keratin1*, *10*, *14, loricrin and* ORFV-specific early and late genes were analyzed. by reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Keratin 1, 10, 14, loricrin, and ORFV env at the protein level were detected by immunohistochemistry. Upon ORFV infection, OTCs exhibited histological cytopathic changes including hyperkeratosis and ballooning degeneration of the keratinocytes. Viable ORFV was able to persist for ten days and was located in keratinocytes of the outer epidermal layers. ORFV-specific early, intermediate and late genes were transcribed, but limited viral spread and restricted cell infection were noticed. ORFV infection resulted in downregulation of K1, K10, and loricrin at the transcriptional level without affecting proliferation as shown by PCNA or Ki-67 expression. In conclusion, OTC provides an excellent model to study the interaction of virus with human keratinocytes in a similar structural setting as human skin. Here, it revealed a new escape strategy of ORFV by downregulation of several differentiation markers in the epidermis of human skin.

P54

Bacterial-strain dependency in secondary pneumococcal pneumonia in the post-influenza lung – a role for alveolar type II epithelial cells?

DOI: 10.1002/eji.201970300

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Introduction: Influenza A virus (IAV) predisposes the host for subsequent secondary infections with *Streptococcus pneumoniae* (*S.pn.*). Alveolar type II epithelial cells (AECII) are not only primary targets of IAV but also important effectors of antipneumococcal defense *in vivo*. However, it is still unknown whether the immunological responsiveness of AECII to pneumococci is modulated by a preceding IAV infection.

Objectives: We aimed to assess and compare AECII-specific immune responses towards *S.pn.* in healthy *vs.* post-IAV lungs. The overall goal is to uncover the contribution of viral infection-imprinted AECII to impaired antipneumococcal immunity in the post-IAV lung.

Materials & methods: We established a murine model of IAV/*S.pn.* co-infection using mouse-adapted influenza A/PR/8/34 virus in combination with three clinical pneumococcal isolates [serotype 4 (highly invasive), 7F (invasive) & 19F (low invasiveness, colonizing strain)], which are administered at d14 post IAV (recovery phase). AECII from lung single-cell suspensions were purified by flow sorting and transcriptomic profiling was performed using microarray analyses.

Results: Infection at d14 post IAV was associated with increased pulmonary CFU (serotypes 4 & 7F) and increased incidence of bacteremia (serotype 4) in IAV/*S.pn.* superinfected mice compared to *S.pn.* only. Interestingly, bacterial CFU with *S.pn.* 19F was unaffected by previous IAV infection. In order to assess AECII responsiveness to *S.pn.* post IAV, infection with *S.pn.* 19F was performed at d14 post IAV and AECII from *S.pn.* and IAV/ *S.pn.* mice were purified for transcriptomic analyses. We detected a rapid induction of pro-inflammatory cytokines and chemokines at 4h post *S.pn.* that was however blunted in AECII from previously IAV-infected lungs. This effect was even more pronounced at advanced pneumococcal infection (18h).

Conclusions: Altogether, we found that susceptibility to *S.pn.* persists during the recovery phase of IAV infection in a bacterial strain-dependent manner. Our first characterization of AECII immune responses revealed pronounced alteration of antipneumococcal immune responses in the post-influenza lung. Our future comparative analyses with serotypes 7F & 19F will aim at answering the questions: a) Is reduced AECII responsiveness following IAV a pneumococcal serotype-specific effect? and b) Does altered AECII immunity contribute to susceptibility following IAV?

P55

Improving the diagnosis of tuberculosis: Using single-cell transcriptomic profiling of pathogen-specific T cells for the identification of novel biomarkers

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Tuberculosis (TB) caused by *Mycobacterium tuberculosis* (MTB) is one of the leading causes of death worldwide. One of the major obstacles in the fight against TB is the lack of rapid and accurate diagnostic tools for TB detection, especially for a clear differentiation between active and latent disease. The current reference standard relies on the detection of live *Mycobacteria* by culture, a time-consuming process that can only be performed in well-equipped laboratories. Novel non-invasive methods to quickly and accurately diagnose TB are therefore urgently sought.

A novel, flow cytometry based approach assessing T cell activation markers on MTB-specific T cells in peripheral blood has shown great potential in accurately differentiating between the two disease states and provides results within 24 hours (Ahmed, et al. 2018). In order to identify additional biomarkers on MTB-specific T cells that differentiate active and latent MTB infection, we applied single-cell transcriptional analysis to MTB-specific T cells from clinically well-characterised patients of the Munich ReFuScreen TB cohort. Live, MTB-specific CD4 memory T cells

DOI: 10.1002/eji.201970300

were sorted after autologous in vitro stimulation and submitted to low-input RNA sequencing. Bioinformatic analysis of RNA sequencing data, revealed a promising selection of genes in MTB-specific CD4 T cells significantly regulated between patients with active or latent stages of TB. We identified a set of genes, many of which are involved in immune regulation pathways, such as NFKappaB and cytokine signalling as well in regulation of T cell activity that can clearly distinguish between active and latent MTB infection

The biomarkers and pathways differentially expressed in active and latent TB infection, identified and characterised here, will not only contribute to the development of novel TB diagnostic tools, but might also deepen our understanding of the cellular pathways involved in the adaptive CD4 T-cell response against MTB.

P56

Profiling of epitope-specific antibody responses and biomarker discovery in viral and parasite infections utilizing high-density peptide arrays

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Introduction: Highly sensitive diagnostic tests and the identification of targets for the development of preventive measures are fundamental to fight against infections. The significance of humoral responses is multi-facetted. Antibodies do not only play an important role in combating a wide range of infectious diseases but can also be utilized for serology. Hence, a comprehensive analysis of humoral responses will ultimately result in the identification of novel target antigens of protective immune responses and disease-specific biomarkers for the development of innovative serological tests.

Objectives: The precise knowledge of antigenic properties of proteins and their underlying epitopes could be a rich source for comprehensive diagnostic markers and novel vaccines. Relating to HEV this could be the basis for an innovative, multiplex serological assay with a higher sensitivity and specificity. Antibody-mediated defense mechanisms play an important role in combating malaria infection, however, the entire picture of the antigenic targets is still elusive.

Materials & Methods: High-density peptide arrays can display large numbers of antigen proteins translated into overlapping peptides. Antibody responses to linear and conformational epitopes can be analyzed in a high-throughput mode yielding high and low immunogenic specific epitopes.

Result: As examples we chose HEV and malaria to conduct extensive epitope mappings for antigen characterization and biomarker discovery. In case of malaria, we analyzed the antibody responses of individuals from malaria-endemic areas and from malaria-naïve European individuals. The results show distinct antibody patterns according to immune status of infected individuals.

In our HEV screenings, we identified common immunogenic regions in patients that clearly discriminate between infected and non-infected individuals. The corresponding peptide epitopes can potentially serve as starting points for peptide based diagnostic tests.

Conclusion: In conclusion, high content peptide microarrays are an ideal tool for the comprehensive identification of epitope-based biomarkers for infectious diseases such as HEV or malaria. The discovery of novel linear or conformational epitopes can lead to the development of innovative multiplex serological assays and identify novel vaccine candidates.

P57

***Chlamydia trachomatis*-induced pyroptotic exit from epithelial cells enables silent infection of pro-inflammatory macrophages**

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DOI: 10.1002/eji.201970300

Chlamydia trachomatis causes one the most abundant sexually transmitted diseases in the western world and chronic infections with *Chlamydia* often lead to infertility in women. As an obligate intracellular pathogen, *Chlamydia* infects different host cells in which it can persist or replicate and then exit to infect the next host cell. Here, we focus on chlamydial exit from epithelial cells into human immune cells. Using Hep2 cells as model for epithelium, we found that, after finishing its replication, *Chlamydia* is released from these cells by pyroptotic cell death rather than apoptosis as measured through AnnexinV/PI staining and caspase-1 activity. This pro-inflammatory cell death can attract inflammatory macrophages (hMDM1) whereas apotosis recruits anti-inflammatory macrophages (hMDM2). These two phenotypes of hMDM were compared as potential secondary host cells. Our studies revealed that infection rates are higher in hMDM2 cells whereas the bacterial load per cell is higher in hMDM1. Interestingly, we could show that surface receptor expression and uptake of further pathogens by primary hMDM1 is significantly reduced by *Chlamydia* and that this silencing of immune functions is absent upon infection of hMDM2. Moreover, *Chlamydia* manipulates cytokine production of the infected hMDM1 to allow survival and replication of the bacteria without eliciting a T cell-mediated adaptive immune response.

Our data demonstrate that pyroptosis is a preferred exit mechanism for *Chlamydia* and that pro-inflammatory macrophages are more suitable host cells as compared to anti-inflammatory macrophages.

P59

Virus-encoded fluorescent caspase-1 reporters as a novel tool for the study of virus-induced inflammasomes

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Introduction: Inflammasome sensors are an important part of the innate immune system. Upon sensing infection or cell damage, they are activated and nucleate macromolecular structures defined as ASC specks. Downstream signaling from these inflammasome complexes is mediated by the adaptor molecule ASC and caspase-1, leading to cytokine secretion and pyroptotic cell death. Bacteria-triggered activation of inflammasomes is well established, but the activation and function of virus-dependent inflammasomes is still poorly understood.

Methods: Inflammasome assembly in human primary cells can not be easily studied due to several limiting factors such as; difficulty in generating primary cells expressing genetically engineered inflammasome components- or reporters and the evident pyroptotic cell death of cell populations assembling inflammasomes. To overcome these limitations, we have developed a novel, fluorescent caspase-1 reporter, which allows the visualization and quantification of inflammasome activation. The reporter, a fusion protein of the caspase-1 recruitment domain and fluorescent EGFP, is recruited to inflammasome specks upon treatment with inflammasome triggers, changing the diffusely distributed EGFP signal into a single EGFP spot, allowing the quantification of activated cells by flow cytometry.

Results: We have engineered recombinant strains of vaccinia virus, vesicular stomatitis virus and herpes simplex virus 1 encoding the caspase-1 reporter. For all viruses the reporter is expressed along with the virus proteins at early time points of infection and a reporter-dependent inflammasome speck is observed in settings where the host cell responds to virus infection by activating inflammasome pathways.

Conclusion: Inflammasome activation of infected primary cells and mixtures of different cell types can be easily visualized and quantified by the established inflammasome reporter. The reporter tool encompasses a range of opportunities, including the characterization of virus-activated inflammasome sensors by use of additional tools as mass spectrometry and proximity ligation assays.

P60

Tumor-derived G-CSF increases susceptibility for *Pseudomonas aeruginosa* acute lung infection in mice

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Objectives. Patients with head and neck cancer (HNC) are predisposed to bacterial complications even in the absence of immunosuppressive therapies. HNC patients have characteristic increase of granulocyte colony-stimulating factor (G-CSF) in a blood, which increases along tumor progression. As G-CSF is known to influence multiple neutrophils functions and neutrophils are known to be the first line of defense against bacteria, we aimed to

DOI: 10.1002/eji.201970300

study the role of tumor-derived factors (e.g. G-CSF) in the alterations of in antibacterial properties of these cells. As a readout system we have used the model of acute lung infection with *Pseudomonas aeruginosa* in tumor-bearing mice.

Materials and methods. Mouse tonsil epithelial carcinoma cell lines, control and transfected with G-CSF cDNA, were injected subcutaneously into mice. At day 14 tumor-bearing mice were infected with *P. aeruginosa* ($2x10^6$ CFU/mouse) intratracheally. Next 20 hours after infection the clinical status mice was monitored. Subsequently, mice were sacrificed, bacterial load in the lower respiratory tract assessed and the lung tissue damage evaluated. Neutrophils were isolated from the lung tissue, and their antibacterial properties analyzed: gene expression (RT-qPCR), ROS production, phagocytosis and NET release in response to *P. aeruginosa*.

Results. In spite of the prominent neutrophil infiltration in organs, mice bearing G-CSF-expressing tumors had increased bacterial load in the lower respiratory tract, compared to control tumor-bearing mice. This correlated with worse clinical outcome. Assessment of the functions of isolated neutrophils revealed decreased expression of antibacterial molecules, such as defensin (def1), diminished NET formation and phagocytosis in mice bearing GCSF-producing tumors, while the expression of molecules responsible for tissue damage (mpo, mmp9), as well as ROS production, were elevated.

Conclusion. Taken together, we could show that G-CSF released by tumor affects the maturation and antibacterial properties of neutrophils. Importantly, factors that are responsible for tissue damage are strongly expressed in these animals, which deteriorate their bacterial clearance. Further investigations are needed to reveal the significance of G-CSF expression in HNC patients as a biological marker for a high risk of bacterial complications.

P61

Proteomics and immunoproteomics provide insights into bacterial and host factors contributing to the outcome of *Staphylococcus aureus* infective endocarditis

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Infective endocarditis (IE) is a complex and heterogeneous disorder associated with high mortality. *Staphylococcus (S.) aureus* is the leading cause of IE, and the high rate of antibiotic-resistant strains aggravates therapy. IE is common in elderly patients, but also affects i.v. drug users. It is well known that i.v. drug users have a better outcome of IE than non-drug users, but the reasons for this are still enigmatic. Here, we used OMICs approaches to study whether bacterial factors (genomics, proteomics) or the host"s antibody response (immunoproteomics) impact on IE outcome by comparing 11 i.v. drug users with 14 non-drug users. Genomic studies revealed that clonal complex 30 (CC30), CC12 and CC59 are the most common lineages in i.v. drug users. Virulence gene patterns were too variable to identify IE-specific virulence genes as compared to matched colonizing *S. aureus* strains. On protein level, however, some immune evasion proteins were upregulated in the strains isolated from IE patients. Immunoproteomics, using a panel of 80 recombinant *S. aureus* antigens, revealed an increase in antibody titers against secreted toxins and immune evasion factors, adhesins, and iron uptake systems over the course of IE (28 days vs. disease onset). This antibody increase was more pronounced in i.v. drug users than non-drug users. Moreover, pre-existing antibody titers tended to be higher in i.v. drug users. In conclusion, the higher antibody titers at disease onset as well as the more pronounced increase of anti-*S. aureus* antibodies during infection in i.v. drug users could explain their better outcome in *S. aureus* IE.

P62

Differentiation and tissue-adaptation of type-2 innate lymphoid cells during helminth infection

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DOI: 10.1002/eji.201970300

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Type-2 innate lymphoid cells (ILC2) have been implicated in tissue immunity, repair and immune regulation, particularly at mucosal barrier sites. Our previous work revealed that ILCs are locally maintained as tissue-resident cells in lymphoid and non-lymphoid organs. In addition to resident cells being generated early during ontogeny, we found that ILC2s travel through peripheral blood and are recruited into tissues during late stages of helminth infection of adult mice, suggesting a "dual origin" of tissue ILC2s.

How tissue-resident versus recruited ILCs differentiate, whether they represent specialized subsets with unique functions and how each of these putative subsets contribute to tissue-immunity is currently unclear. To address these questions we tracked the differentiation and tissue-adaptation of migrating versus tissue-resident ILC2 during different phases of infection with the helminth N. brasiliensis (NB). We combined lineage-tracing, photoconversion and parabiosis and sequenced mRNA from thousands of individual ILC2s isolated from peripheral blood and different tissues. We employed a newly developed algorithm to visualize the population structure and differentiation trajectories of tissue ILC2s during homeostasis and NB infection and mapped these cells based on their local tissue versus hematogenous origin at single-cell resolution. Using advanced multiplex imaging we are currently visualizing tissue- and context-specific niches of ILC2 subsets. Based on these combined analyses and our experimental *in vivo* validation we will discuss a model for the ontogeny and local tissue-adaptation of ILC2s during infection.

P63

Assessment of the percentages of T-lymphocyte subsets in the peripheral blood of mediterranean spotted fever patients

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Introduction: Mediterranean spotted fever (MSF) is a rickettsial disease, caused by Rickettsia conorii conorii. This study aims at investigating the T-lymphocyte subsets in the peripheral blood of patients with MSF in order to clarify the immunopathogenesis of the disease.

Material and methods: The percentages of T-lymphocytes subsets were assessed in peripheral blood of 62 patients in the acute stage of MSF. Controls were 32 age and sex matched healthy individuals. MSF patients were assigned into three groups, based on the severity of disease – with mild, moderate or severe forms. The diagnosis of MSF was confirmed by immunofluorescence assay. Immunophenotyping was performed using Epics XL-MCL Coulter, USA flow-cytometer.

Results: The percent of the immune competent (CD3+ total) cells decreased, while that of the helper/inducer (CD3+CD4+) and suppressor/cytotoxic (CD3+CD8+) did not alter substantially compared to the controls. Unlike their absolute values, the percentage ratios of all three T-cell subsets did not correlate with the disease's severity. The naïve T-cells (CD4+CD45RA+) showed a reduce level, with a tendency to decrease at the severe forms of the disease, while the activated memory (CD4+CD45RO+) T-cell subsets did not change significantly. Taken as a whole, the levels of activated (CD3+HLA-DR+) cells increased with high significance $p<0.001$ compared to the controls, but without correlation with the MSF forms of severity. The stimulatory molecules (CD38+ total) increased in the acute stage, and their values grew parallel to the severity of the disease; so did CD8-CD38+ as well as CD8+CD38+ T-cell subsets. Values of the presenting accessory molecules CD28+ total, CD8-CD28+ and CD8+CD28+ did not show a statistically reliable difference to the controls, but corresponded to the disease severity as their levels increased significantly at mild forms with an evident downward trend at the severe forms of MSF.

DOI: 10.1002/eji.201970300

Conclusion: Reduced T-lymphocyte subset percents are likely related to trans-migration into perivascular inflammatory foci. The increased percent of T-lymphocytes bearing stimulatory molecules reflects the mobilization of the cell-mediated immune response in the healing process. An important issue of this study is the possible prognostic value of T-cell subset percentage, predicting the evolution of a clinical condition to clinical forms, according to the disease severity.

P65

Interleukin-33 administration increases host susceptibility to *Citrobacter rodentium* infection by dysregulating host mucosal immune responses

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Introduction: Interleukin-33 has recently emerged as novel cytokine with pleiotropic functions in both homeostasis and inflammation within the gut. Signalling via its receptor ST2, IL-33 modulates immune responses at barrier sites. The involvement of IL-33/ST2 axis in the development of autoimmune intestinal disorders and colon cancer is currently under investigation. However, the impact of IL-33 on bacteria-driven intestinal inflammation remains unrevealed. Interestingly, we identified enhanced colonic ST2 expression following infection with the gut-specific mouse pathogen *Citrobacter (C.) rodentium* suggesting an upregulation of the IL-33 signalling during intestinal infection.

Objectives: In the present study we carefully determined the immunological relevance of IL-33/ST2 axis in bacteria-induced colitis.

Materials & Methods: *C. rodentium*-infected mice were treated or not with recombinant IL-33. Colon tissues were analysed for inflammatory and immunological parameters by histological analysis and flow cytometry, respectively. In the same settings, DEREG transgenic mice were used to deplete regulatory T cells (Treg) by administering diphtheria toxin. Furthermore, intestinal epithelial integrity upon IL-33 challenge was evaluated both *in vivo* and *in vitro*.

Results: IL-33 administration during *C. rodentium* infection increased the intestinal bacterial burden, which was accompanied by exacerbated colitis and severe systemic bacterial distribution. Well in line, IL-33 application was found to impair the gut integrity and to negatively affect tight junction expression. Interestingly, the frequencies of Treg cells in the infected colons were boosted by IL-33 treatment, suggesting that IL-33-induced Treg expansion inhibits protective effector T cell responses. However, Treg depletion did not reverse the deleterious effect of IL-33. Finally, IL-33 administration impaired protective Th17 responses against infection.

Conclusion: We provide novel evidence that IL-33/ST2 axis has a detrimental impact on bacteria-induced colitis by altering intestinal epithelium integrity and by limiting protective Th17 immune responses that are essential for pathogen clearance. Thus, IL-33 could be a therapeutic target during infectious colitis, but further analyses are needed for better understanding the molecular mechanisms specifically involved.

P66

Essential role of $I\kappa B_{NS}$ for the induction of an inflammatory program in myeloid immune cell subsets during *Listeria monocytogenes* infection in mice

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Introduction: The activation of the ubiquitously expressed transcription factor NF-κB is tightly regulated by inhibitory proteins which retain NF-κB in the cytoplasm. The inducible inhibitory protein $I\kappa B_{NS}$ binds to NF-κB in the nucleus. Though $I\kappa B_{NS}$ deficiency is known to affect the expression of pro-inflammatory cytokines in T cells and macrophages, no data are available regarding the impact of $I\kappa B_{NS}$ on the course of a systemic bacterial infection such as *Listeria monocytogenes*.

DOI: 10.1002/eji.201970300

Methods: *Listeria monocytogenes* (LM) infections were performed in $I\kappa B_{NS}$-deficient ($I\kappa B_{NS}^{-/-}$) and wild type ($I\kappa B_{NS}^{+/+}$) mice followed by disease monitoring, determination of bacterial burden, liver enzymes, cytokine profiling, liver microarray analysis, histopathological and flow cytometric analyses.

Results: While $I\kappa B_{NS}^{+/+}$ mice succumb to high-dose LM infection within 4-6 days post infection, $I\kappa B_{NS}^{-/-}$ mice are fully protected. $I\kappa B_{NS}^{-/-}$ mice exhibit less pronounced pathology in spleen and liver, reduced serum alanine transaminase levels and an overall blunted inflammatory cytokine response compared to $I\kappa B_{NS}^{+/+}$ mice. Prompted by our observation that inflammatory monocytes and neutrophils exhibit a high promoter activity of the $I\kappa B_{NS}$ encoding gene, more detailed *ex vivo* analyses of these cellular subsets were performed which indeed revealed reduced expression of inflammatory mediators (e.g. *Nos2*, *Il1*, *Il6)* specifically in monocytes and neutrophils from $I\kappa B_{NS}$-deficient mice. Moreover, detailed flow cytometric analyses of LM-infected spleen and liver samples from both $I\kappa B_{NS}$ genotypes uncovered differences with respect to the effector function of neutrophils. Furthermore, data obtained in mice lacking $I\kappa B_{NS}$ expression specifically in neutrophils further support a contribution of $I\kappa B_{NS}$ in these cells to the striking different susceptibility to LM infection of $I\kappa B_{NS}^{-/-}$ and $I\kappa B_{NS}^{+/+}$ mice.

Conclusion: In summary, our data suggest that IkB_{NS}-dependent activation of an inflammatory program in myeloid cell subsets contributes to the detrimental course of the high-dose LM infection in mice.

P67

Phosphocholine-modified lipooligosaccharides of *Haemophilus influenzae* induce a cholinergic mechanism to evade innate immune responses

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Objective: The opportunistic pathogen of the airways *Haemophilus influenzae* can induce acute pneumonia in immunocompromised patients. To enable immune evasion and pathogen survival, *H. influenzae* decorates its cell surface with phosphocholine (PC)-modified lipooligosaccharide (PC-LOS). The underlying mechanisms are, however, poorly understood. We hypothesized that *H. influenzae* PC-LOS function as unconventional agonists of nicotinic acetylcholine receptors (nAChRs) and *H. influenzae* hijacks an endogenous cholinergic control mechanism of the lung to prevent ATP-induced release of the pro-inflammatory cytokine interleukin-1β (IL-1β).

Methods: *In vitro* experiments were performed on human pulmonary epithelial cell lines A549 and Calu-3. Cells were primed with lipopolysaccharide from *Escherichia coli* followed by stimulation with ATP in the presence or absence of wildtype *H. influenzae* PC-LOS or PC-free LOS that was isolated from *lic1*-mutant strains. ATP-induced IL-1β-release was measured in cell culture supernatants by ELISA. The involvement of nAChRs was tested by using a panel of specific antagonists. Furthermore, e*x vivo* experiments were performed on primed mouse precision-cut lung slices.

Results: Wildtype *H. influenzae* PC-LOS efficiently inhibited the ATP-mediated release of IL-1β by epithelial A549 and Calu-3 cells. In contrast, PC-free LOS had no impact on the ATP-mediated release of IL-1β. The inhibitory effect of PC-LOS was prevented by specific nAChR antagonists, suggesting that PC-LOS stimulates nAChRs containing subunits α7, α9, and/or α10 to exert the anti-inflammatory effect. The inhibitory effect of PC-LOS on the ATP-mediated IL-1β release was further confirmed in mouse precision-cut lung slices.

Conclusion: *H. influenzae* PC-LOS efficiently inhibit the ATP-induced release of IL-1β by pulmonary epithelial cells as well as by lung tissue. PC-LOS signaling involves the activation of nAChRs containing subunits α7, α9, and/or α10. Therefore, *H. influenzae* seems to hijack an endogenous cholinergic control mechanism of the lung to evade host innate immune responses. These findings may open the opportunity for the development of a novel host-centered anti-infective therapy beyond the classical antibiotic regimens.

DOI: 10.1002/eji.201970300

P68

Protective role of acid sphingomyelinase/ceramidase in bacterial-induced colitis

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Introduction: Complex sphingolipids are essential structural components of intestinal membranes. The role of sphingolipid signaling has been established in intestinal cell survival, growth, differentiation, and apoptosis. In the colon pathogenesis, inflammatory cytokines cause alterations of the lipid composition in the cell membrane by activating various phospholipases and sphingomyelinases. However, little is known about the role of the sphingolipid system in the context of bacterial-induced inflammation.

Objectives: In the present project, we analyze and compare the function of acid sphingomyelinase (Asm) which catalyzes the hydrolysis of sphingomyelin into ceramide and acid ceramidase (Ac) which catalyzes the degradation of ceramide into sphingosine during bacterial-induced colitis.

Materials & methods: Asm knockout (KO), Ac conditional knockout (cKO) mice and wildtype littermates were infected with the mouse specific, gram-negative bacterium *Citrobacter (C.) rodentium* to induce colonic inflammation. Colonic sphingolipid concentrations, colonic and systemic bacterial burden as well as the *C. rodentium* associated inflammatory scores were assessed. In addition, the impact of Asm and Ac on the gut architecture under homeostasis and infection was determined by organoid cultures.

Results: Sphingomyelin and ceramide concentrations were markedly altered in the colon of *C. rodentium* infected Asm KO and Ac cKO mice compared to infected wildtype littermates. Furthermore, Asm KO mice and Ac cKO mice were highly susceptible to *C. rodentium* infection with more severe colonic inflammation and enhanced crypt hyperplasia compared to *C. rodentium* infected wildtype mice. In contrast to infected wildtype mice, Asm KO as well as Ac cKO mice showed enhanced translocation of the non-invasive *C. rodentium* into the liver and the spleen.

Conclusion: The loss of Asm and Ac activity significantly enhances susceptibility to bacterial-induced colitis. Systemic distribution of the non-invasive *C. rodentium* indicated an impairment of intestinal integrity upon Asm/Ac deficiency. Therefore, the impact of the Asm/ceramide system on the intestinal stem/epithelial cells and the intestinal barrier function during bacterial-induced colitis will be investigated in detail.

P69

Characterization of the role of monocytes and neutrophils during the *Streptococcus suis* systemic and central nervous system infections

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Introduction: *Streptococcus suis* is an important porcine bacterial pathogen and a zoonotic agent causing sudden death, septic shock, and meningitis, amongst other pathologies. These diseases are the consequence of uncontrolled bacterial replication leading to an exacerbated inflammatory response, which is a hallmark of the *S. suis* systemic and central nervous system (CNS) infections. Though monocytes and neutrophils are important innate immune blood cells and are well-known to massively infiltrate the CNS during *S. suis*-induced meningitis, their role during infection caused by this pathogen remains unknown.

Objectives: To determine the role of inflammatory and patrolling monocytes and neutrophils during the *S. suis* systemic and CNS infections in the development of disease and clinical outcome.

Materials and methods: Well-established C57BL/6 mouse models of *S. suis* systemic and CNS infections were used, in which bacteria were inoculated via the intraperitoneal and intracisternal routes, respectively. To determine the role of $Ly6C^{hi}$ inflammatory and $Ly6C^{low}$ patrolling monocytes, $CCR2^{-/-}$ and $Nr4a1^{-/-}$ mice were used, respectively Meanwhile, neutrophil depletion was achieved using an anti-Ly6G neutralizing antibody. Host survival, development of clinical disease, blood, organ, and CNS bacterial burden, as well as systemic and CNS inflammation were evaluated.

DOI: 10.1002/eji.201970300

Results: Neutrophils, and to a lesser extent inflammatory monocytes, but not patrolling monocytes, participate in *S. suis*-induced systemic disease via their role in inflammation required for controlling bacterial burden. Moreover, inflammatory monocytes, but not patrolling monocytes, also partially contribute to the exacerbation of *S. suis*-induced CNS inflammation, while neutrophils participate in local CNS bacterial burden control. However, development of clinical CNS disease was independent of both cell types, suggesting that resident immune cells are most probably responsible for *S. suis*-induced CNS inflammation leading to clinical signs, with infiltrating monocytes and neutrophils amplifying the inflammatory cascade.

Conclusion: Inflammatory monocytes and neutrophils contribute to the development and exacerbation of the *S. suis* systemic infection but play differential roles during the CNS disease, while patrolling monocytes appear to play only a minor role. Moreover, this is the first study to demonstrate a differential role of monocytes and neutrophils during bacterial infection.

P70

T cell-specific overexpression of acid sphingomyelinase results in elevated T cell activation and reduced parasite burden during *Plasmodium yoelii* infection

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Introduction: Acid sphingomyelinase (Asm) is a hydrolyzing enzyme and part of the sphingolipid metabolism. After activation Asm converts sphingomyelin to ceramide at the outer leaflet of the plasma membrane. Self-accumulating properties of ceramide lead to the generation of ceramide-enriched platforms. These platforms are involved in regulating differentiation, proliferation and apoptosis in different cell types. However, the role of T cell-intrinsic Asm activity on T cell function remains elusive.

Objectives: We aimed to analyze the T cell-intrinsic role of Asm on T cell development and function by using t-Asm/CD4cre mice, which specifically overexpress Asm in T cells. Besides analyzing T cell differentiation and activation *in vitro* we wanted to elucidate whether T cell specific overexpression of Asm influences T cell activity during *Plasmodium yoelii (Py)* infection *in vivo*.

Material and methods: We performed flow cytometric analysis of T cells isolated from lymphoid organs of t-ASM/CD4cre mice. Furthermore, we stimulated naïve CD4+ T cells from t-Asm/CD4cre mice *in vitro* to determine their proliferative activity as well as their capacity to differentiate into distinct T cell subsets. For *Py* infection t-ASM/CD4cre mice were infected with 1 x 105 infected red blood cells (iRBC). The frequency of iRBCs was monitored by microscopic examination of Giemsa-stained blood smears.

Results: Flow cytometric analysis of splenocytes from t-Asm/CD4cre mice revealed decreased frequencies of regulatory T cells compared to wildtype (wt) littermates. Moreover, T cells from t-Asm/CD4cre mice showed higher proliferation rates and were more prone to differentiate into Th1 cells *in vitro* than T cells from wt mice. In response to CD3/CD28 stimulation CD4+ T cells from t-ASM/CD4cre mice showed stronger activation of T cell receptor signaling molecules. Strikingly, *Py*-infected t-Asm/CD4cre mice showed higher T cell activation accompanied by higher cytokine secretion and better parasite clearance than *Py*-infected controls.

Conclusion: Our results indicate that T cell-intrinsic Asm activity has an impact on T cell function *in vitro and in vivo*. In the context of *Py* infection T cell specific overexpression of Asm lead to enforced T cell activation and to reduced parasite burden.

P71

Impact of the ceramide pathway on immune responses during *Plasmodium* infection

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Introduction: Malaria, caused by *Plasmodium* parasites, is still one of the most common infectious diseases in the world. Sphingolipids are bioactive molecules with various functions. Beside their physical function as structural components in the plasma membrane, they are proposed to play a pivotal role in several cellular processes.

DOI: 10.1002/eji.201970300

Objectives: We aim to investigate how sphingolipids modulate the course of infection and the function of immune cells during *Plasmodium* infection.

Material and Methods: We assessed the role of the ceramide-hydrolysing enzyme acid ceramidase (AC) by using global AC knockout mice and by pharmacological inhibition of AC with carmofur. Mice were infected with non-lethal *Plasmodium yoelii* parasites and compared to wildtype and control littermates.

Results: Systemic AC deficiency resulted in delayed parasite burden and elevated serum levels of pro-inflammatory cytokines. Moreover, we examined T cell frequencies and phenotypes in spleen from infected AC-deficient mice and detected altered T cell responses compared to infected wildtype mice in the early phase of infection. In line with these findings, the pharmacological inhibition of AC with carmofur improved parasite clearance and impaired T cell responses in the initial phase of infection.

Conclusion: Thus, our study indicates the involvement of ceramide in modulating immune responses during *Plasmodium* infection. Future studies of infected mice with cell type specific AC-deficiency ($AC^{flox/flox}$ x CD4cre and $AC^{flox/flox}$ x LysMcre) should clarify the impact of AC and the ceramide pathway in T cells and the innate immune compartment.

P72

The antigen-specific T-cell receptor repertoire against human cytomegalovirus

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Introduction: Human cytomegalovirus (CMV) establishes a persistent infection in its host. Primary infection and reactivation frequently cause severe disease in immunodeficient persons who cannot control the virus. T cells are crucial for virus control and the presence of specific CD8+ T cells is associated with protection against CMV disease.

Objectives: To explore the CMV-specific CD8+ T-cell response in detail, we studied the T-cell receptor (TCR) repertoire that humans mobilise to fight CMV. We focused on donors expressing the haplotype HLA-B*07:02/HLA-C*07:02, which is common in Europeans. HLA-C–restricted epitopes deserve special attention because their presentation is not affected by CMV immunoevasins. We were particularly interested in the similarities and differences of TCRs with the same epitope specificity, in TCR sharing, and in the magnitude of the CMV-specific T-cell response.

Materials & methods: CMV-specific T cells were enriched from blood cells of healthy virus carriers by short-term *in vitro* stimulation with immunogenic viral peptides. High-throughput TCRβ sequencing was performed on bulk mRNA and CMV-specific TCRβ sequences were identified by comparing clonotype frequencies in the peptide-stimulated sample, the unstimulated sample, and a control peptide-stimulated sample, all derived from the same donor (three-sample comparison). CMV-specific TCRβ sequences were analysed with regard to their frequency, composition, and common features.

Results: We identified thousands of TCRβ sequences specific for various CMV epitopes. CMV-specific TCRβ clonotypes, especially those restricted by HLA-C*07:02, were highly frequent in the peripheral blood of virus carriers. Numerous TCRβ clonotypes with the same epitope specificity had identical or strikingly similar amino acid sequences and were shared by multiple donors. The cumulative *ex vivo* frequency of shared TCRβ sequences was considerably higher in CMV-positive persons and allowed for precise discrimination of CMV-positive and CMV-negative donors in our cohort and a large independent cohort (Emerson et al. 2017).

Conclusion: Shared virus-specific TCRβ sequences will be valuable in disease monitoring, for instance as markers for the presence of a CMV-specific T-cell response. In addition, such TCRs are promising for use in adoptive T-cell transfer, since they are tolerant to a wide range of HLA-self peptide complexes and are therefore less likely to cause autoimmunity in the recipient.

DOI: 10.1002/eji.201970300

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Changes in expression of ligands for NK cells on human gut epithelial cells infected with adenovirus

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Introduction: Human adenoviruses (HAdV) are a significant cause of enteric illness, leading to high morbidity and mortality in the immunocompromised host. HAdVs encode several immunosubversive functions in their E3 transcription unit that promote evasion from immune cell recognition. Previous HAdV studies were performed using immortalized cell lines and confirmative studies in primary epithelial cells are required. Human intestinal organoids (HIO) now enable studies of enteric HAdV infection in a primary cell setting.

Objectives: To assess modulation of the expression of ligands for immune cell receptors by replication-competent HAdV in human epithelial cells, with focus on ligands for NK cells.

Materials & methods: Stem cells were isolated from non-inflamed parts of the adult small intestine and cultured following standard procedures to promote formation of organoids. HIO lines were prepared for infection by switching to suspension culture conditions in differentiation medium, allowing access of the apical site of the organoids to viral particles and promoting differentiation into epithelial cell types. HIOs and human embryonic kidney (HEK293) cells were infected with HAdV5C (type 5, species-C) wildtype and E3 mutant strains to analyze the impact of infection on the expression of immunoligands.

Results: HIOs were successfully infected using a reporter strain (HAdV5C-mCherry). Infection of HEK293 cells with HAdV5C wildtype and an E3/19K-deficient virus showed a two-fold reduction in surface expression of HLA class I molecules and UL16 binding proteins (ULBP). Furthermore, cells infected with E3/19K- expressed higher levels of MIC A/B than wildtype infected cells, while HAdV5C resulted in increased expression of intercellular adhesion molecules (ICAM) 1 and 2 compared to uninfected controls.

Conclusion: We have established HAdV infection in a primary epithelial cell system. HAdV5C wildtype and E3/19K knockout strains differentially modulated immunoligand expression and we are currently investigating the consequences for interaction with NK cell receptors. This research could lead to new insights into viral disease interactions in the human gut epithelium and their implications for immune recognition.

P74

***Mycobacterium tuberculosis* drives expansion of low-density neutrophils equipped with regulatory activities.**

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Introduction: In human tuberculosis (TB) neutrophils represent the most commonly infected phagocytes but their role in protection and pathology is highly contradictory. Moreover, a subset of low-density neutrophils (LDNs) has been identified in TB, even if their functions and clinical significance remain unclear.

Objectives: We have studied neutrophil cell compartment in patients with active TB disease, analyzing total neutrophils and their LDN subset in terms of frequencies, phenotype, functional features and genes signature.

Patients & methods: Full blood counts from Healthy Donors (HD), Latent TB infected (LTBI), active TB patients and cured TB patients were performed. LDNs that co-purify with PBMCs in density gradient centrifugation were isolated by

DOI: 10.1002/eji.201970300

immunomagnetic sorting. Frequency, phenotype, burst activity and suppressor T cell activity of LDNs and NDNs from active TB patients were assessed by flow cytometry. NETosis and phagocytosis of the two different subsets were evaluated by confocal microscopy. The transcriptomic analysis of NDNs and LDNs from active TB patients was performed by the Nanostring technology.

Results: Elevated numbers of total neutrophils and a high neutrophil/lymphocyte (N/L) ratio distinguish patients with active TB from all the other groups. PBMCs of patients with active TB disease contained elevated percentages of LDNs compared with those of HD, and which exhibited an increased expression of CD66b, CD33, CD15 and CD16 compared to NDNs.

Differently than NDNs, LDNs failed to phagocyte the *Mycobacterium tuberculosis* bacilli, to make oxidative burst and NETosis, but caused significant suppression of antigen-specific and polyclonal T cell proliferation.

Transcriptomic analysis of LDNs and NDNs purified from the peripheral blood of TB patients identified 12 genes differentially expressed: *CCL5*, *CCR5*, *CD4*, *IL10*, *LYZ* and *STAT4* were upregulated while *CXCL8*, *IFNAR1*, *NFKB1A*, *STAT1*, *TICAM1* and *TNF* were downregulated in LDN, as compared to NDNs.

Conclusion: Our study identifies two populations of neutrophils with distinct biological activities during TB: NDNs mediate bacterial killing, through oxidative burst and NETosis, while LDNs to inhibit antigen-specific T cell responses *via* IL10 upregulation.

P75

Characterisation of γδ T cells in murine Cytomegalovirus infection

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Introduction: Upon Cytomegalovirus (CMV) infection immunocompromised patients are at substantial risk for developing severe organ disease eventually leading death through to multi-organ failure. Existing virus escape mechanisms to anti-viral drugs plus missing approved vaccination currently designate CMV as major health issue. Previous findings showed that γδ T cells can effectively control murine CMV (mCMV), remarkably when conventional immune mechanisms (like αβ T, B or Natural Killer cells) are insufficient or absent, corresponding to the immune-suppressed state in transplant recipients and neonates. Previous studies in mice and men suggest adaptive features of γδ T cells in context of microbial infections as CMV and malaria.

Objectives: Recognition mechanisms, antigen specificity and the formation of the immune response of γδ T cells are still not completely understood. The data presented aimed to examine, to what extent the γδT cell receptor (TCR) participates in sensing and controlling mCMV infection in the mouse model to better comprehend the development and immune responses of γδ T cells.

Material & Methods: We monitored γδ TCR repertoires in TCRα$^{-/-}$ mice for clonal diversity. In different organs RNA-based immune-profiling of V(D)J rearranged complementary-determining regions (CDR) from selected receptor chains (TRGV1, TRGV4, TRDV5, TRAV15/DV6) was performed in a time kinetic manner. Taking advantage of a fluorescent reporter system for TCR activation and hybridoma technology we identified γδ T cell clones which recognize mCMV infected target cells *in vitro*. Based on these data we created a new mouse line with pre-arranged VDJ cassette knocked into the TCRD locus via CRISPR/Cas.

Results: Alterations in clonality and focusing of the CDR3 length distribution after virus exposure implicate an antigen-driven response with TCR involvement. Additionally, *in vitro* experiments strengthened this hypothesis, which will be further addressed by analysing the newly generated mouse model.

Conclusions: The results of the presented study show new insights in the elusive biology of γδ T cells. Cellular therapies based on these unconventional T cells are promising approaches to combat cancer and infectious diseases. To this extent, it is crucial to fully understand their developmental pathes and immune capacity.

DOI: 10.1002/eji.201970300

P76

Deciphering the mechanism of action of the viral chemokine binding protein of varicella zoster virus

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Chemokines are small, basic cytokines that direct leukocyte migration during development, homeostasis, inflammation, tissue damage and infection. Dysregulation of chemokine activity is linked to autoimmune disorders, cancer and pain, among other pathologies. To function in vivo, chemokines are presented by glycosaminoglycans (GAGs) to chemokine receptors, which are members of the G protein-coupled receptor (GPCR) superfamily. Most chemokines bind to more than one receptor and each receptor normally interacts with more than one chemokine. Moreover, some chemokines interact with others modulating their activity, increasing the functional complexity of the network.

Some large DNA viruses express viral chemokine binding proteins (vCKBP) that interact with chemokines with high affinity. Most vCKBP inhibit chemokine activity by blocking the interaction of the chemokine with GAGs or the GPCR. Varicella zoster virus glycoprotein C (VZV gC) is a vCKBP that enhances chemokine activity, increasing leukocyte migration through an unknown mechanism. VZV gC is a type I transmembrane protein containing an N-terminal repeated region and three predicted beta-strand rich domains at the C-terminus of the ectodomain. To discover VZV gC mechanism of action we generated recombinant gC constructs lacking specific protein domains. We performed surface plasmon resonance and chemotaxis experiments to identify gC binding and functional domains. Our results show that VZV gC beta-strand rich domains bind chemokines with nanomolar affinity. Binding to the chemokine and signalling through the chemokine receptor are required for gC activity, i.e., to increase chemokine activity. VZV also interacts with GAGs and the functional relevance of this interaction is unknown. We are currently determining the relevance of gC-GAG interaction in the context of migration and whether gC modulates chemokine binding to GPCR and subsequent signalling and internalization. Understanding how VZV gC increases chemokine activity may provide novel insights on how the GAG-chemokine-GPCR axis is regulated.

P77

Phenotypic characterization of primary porcine macrophages and establishment of a PRRSV-specific serum neutralization test

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Introduction: Infections with the porcine reproductive and respiratory syndrome virus (PRRSV) are responsible for reproductive failures in sows and respiratory symptoms in piglets worldwide. The main target cells of PRRSV are CD163+alveolar macrophages. These macrophages are susceptible to PRRSV type-1 and -2. In vitro growth of PRRSV in cell lines like MARC-145 is possible but not consistently achieved. The antibody response against PRRSV can be determined by enzyme-linked immunosorbent assays (ELISA), usually measuring antibodies against the viral nucleocapsid protein (N). These antibodies are typically produced 2 weeks post infection, without neutralizing properties.

Objectives: The aim of this project was to phenotypically characterize porcine macrophage subsets, to investigate the individual cell subsets for PRRSV susceptibility and to establish a serum neutralization test (SNT).

Materials & methods: Monocyte derived macrophages (MoMph), Alveolar macrophages (AMph) and bone marrow derived macrophages (BMMph) were analyzed by FACS for their expression of CD172a and CD163. PRRSV infection in these cell populations was verified by RT-PCR and immunofluorescence assay (IFA). MARC-145 cells were used for further passaging of PRRSV field strains. For the establishment of a PRRSV-specific SNT, 2-fold serial dilutions of field sera from PRRSV-infected or vaccinated pigs were prepared and incubated with a selected PRRSV type-1

DOI: 10.1002/eji.201970300

vaccine strain. After cultivation on MARC-145 cells, cells were fixed, stained and analyzed for the presence of PRRSV using IFA.

Results: Flow cytometric analysis revealed cell-type specific expression of CD172a and CD163. Almost 100% of the AMph were CD172a+, in contrast to BMMph and MoMph with only reached 50-66% and 4-8% CD172a+ cells, respectively after cultivation with M-CSF. CD163 expression was observed on almost 100% of the AMph as well as MoMph. In BMMph, CD163 expression was detected on 40-60% of the cells. Growth of PRRSV type-1 and -2 has been detected in MoMph, AMph and MARC-145 cells. A SNT for the detection of PRRSV-specific antibodies was established.

Conclusion: Phenotypic analysis of BMMph and MoMph revealed that only a minor fraction of the cell populations differentiated to CD172a+CD163+ macrophages. Therefore further infection experiments focus on CD172a+CD163+ AMph. The established SNT will be used to trace the presence and progression of neutralising antibodies in PRRSV-infected and vaccinated herds.

P78

XIAP controls RIPK2 signaling by preventing its deposition in speck-like structures

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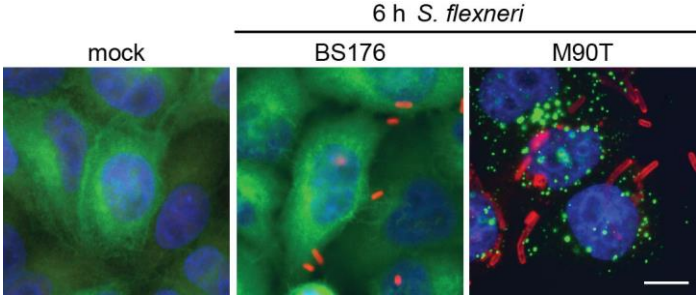
The receptor interacting serine/threonine kinase 2 (RIPK2) is essential for linking activation of the pattern recognition receptors NOD1 and NOD2 to cellular signaling events. Recently, it was shown that RIPK2 forms higher order molecular structures in vitro, which were proposed to activate signaling. Here, we demonstrate that RIPK2 forms detergent insoluble complexes in the cytosol of host cells upon infection with invasive enteropathogenic bacteria. Formation of these structures occurred after NF-κB activation and depends on the CARD of NOD1 or NOD2. Complex formation upon activation was dependent on RIPK2 autophosphorylation at Y474 and influenced by phosphorylation at S176. Inhibition of activityof the cIAP protein XIAP induced spontaneous complex formation of RIPK2 but blocked NOD1-dependet NF-κB activation. Using immunoprecipitation, we identified 14-3-3 proteins as novel binding partners of non-activated RIPK2, whereas complexed RIPK2 was bound by the prohibitin proteins Erlin-1 and Erlin-2. Taken together, our work reveals novel roles of XIAP, 14-3-3 and Erlin proteins in the regulation of RIPK2 and expands our knowledge on the function of RIPK2 posttranslational modifications in NOD1/2 signaling.

Full Manuscript available at bioRxiv:

bioRxiv 545400; doi: https://doi.org/10.1101/545400

Figure 1



P80

Identification and localization of bacteria from infected native and prosthetic valves

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DOI: 10.1002/eji.201970300

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Infective endocarditis (IE) of native and prosthetic heart valves is a severe inflammatory disease of the endocardium leading to death if left untreated. We based our studies on the premise that 1.) identifying the causative microorganisms is crucial for tailoring the type and duration of antibiosis and does for the patient survival and that 2.) Knowledge of the presence of immune cells and the localization bacteria within these cells will add to a better understanding of the cause of the disease. Using Next-Generation Sequencing (NGS) of 16S-rDNA we investigated the biodiversity of infectious species from valves of eight IE patients and immune cells were identified by ion beam scanning electron microscopy (FIB-SEM). Our data reveal that A) bacterial specimen detected by NGS of 16S-rDNA far exceed those identified by conventional culture-based diagnosis, B) the antibiotic treatment did not cover many of the newly detected species and that C) although phagocytosed, bacteria very likely survive by escape from entering phagocytic vacuoles. Thus, the here presented results shed new light on the bacteria-mediated endocarditis.

P81

Identification of bystander activated CD8⁺ T cells in the bone marrow during polymicrobial sepsis

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Introduction: Polymicrobial sepsis, a systemic bacterial infection, induces a state of immunosuppression which enhances the susceptibility to secondary infections, but has been described as less severe in TLR2 knockout (ko) mice. Immunosuppression is associated with reprogramming of myeloid cells which differentiate in the bone marrow depending on the cytokine microenvironment. We hypothesize that changes in the bone marrow are responsible for the development of post-septic immunosuppression.

Objectives: Previous work showed an altered differentiation of dendritic cells which is paralleled by an accumulation of activated T cells (TCs) in the bone in contrast to sepsis-induced apoptosis in the spleen. TCs, especially CD8+ virtual memory TCs, can get antigen-independently "bystander" activated by exposure to innate cytokines. In this study, the composition and functional properties of CD8⁺ TCs in the bone marrow 24 hours after sepsis induction were examined.

Materials & methods: Polymicrobial sepsis was induced by cecal ligation and puncture in wildtype BALB/c and TLR2 ko mice. Twenty-four hours after sepsis induction, bone marrow cells were isolated and analyzed via flow cytometry regarding TC subset composition as well as their activation level via CD69 expression and production of Interferon gamma (IFN-g) and Interleukin (IL-)10.

Results: The number of naïve, central and virtual memory CD8⁺ TCs but not of effector/effector memory CD8⁺ TCs significantly increased in the bone marrow 24 hours after sepsis induction. Additionally, central memory and virtual memory CD8⁺ TCs displayed increased activation. No difference in accumulation and activation level of TCs could be identified between wildtype and TLR2 ko mice. However, effector/effector memory CD8⁺ TCs showed an impaired ability to produce IFN-g in wildtype but not in TLR2 ko mice and instead produced IL-10. When adoptively transferred into wildtype mice, CD8⁺ TCs from TLR2 ko mice maintained their superior capacity for IFN-g production.

Conclusion: Polymicrobial sepsis induces an accumulation of antigen-independently activated CD8⁺ TCs in the bone marrow. So far unknown TLR2 ligands might modulate the cytokine secretion pattern of these cells. Since the cytokine environment plays a crucial role in the regulation of differentiation processes in the bone marrow, the alteration of CD8⁺ TCs might contribute to the reprogramming of myeloid cells during sepsis.

DOI: 10.1002/eji.201970300

P82

The increased protective granulomatous response in *Mycobacterium tuberculosis*-infected IL-27Rα-deficient mice is dependent on IL-17A and is associated with the IL-17A-induced expansion of multifunctional T cells

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Introduction: During Mycobacterium tuberculosis (Mtb) infection, mice lacking the IL-27Rα exhibit lower bacterial burdens in comparison to wild type mice accompanied by the formation of highly stratified protective granulomas with a center of Mtb-containing macrophages surrounded by a rim of lymphocytes**.**

Objectives: Because IL-27 has been shown to control the development of IL-17A-producing Th17 cells, we were wondering whether IL-17A might support the increased protection and improved granulomatous response in the absence of IL-27Rα-mediated signaling.

Materials & methods: C57BL/6, IL-27Rα-deficient ($^{-/-}$) and IL-27Rα/IL-17A-double deficient mice were infected with approximately 100 CFU Mtb H37Rv via the aerosol route and the outcome of experimental tuberculosis was compared at different time points post infection.

Results: IL-27Rα$^{-/-}$ mice exhibited a significant increase of IL-17A-producing CD4+ T cells during Mtb infection. Importantly, the augmented level of IL-17A was responsible for the increased protection and the formation extremely well-organized granulomas in IL-27Rα$^{-/-}$ mice. Whereas IL-17A did neither impact the development of IL-10-producing regulatory Tr1 cells nor the expression of programmed cell death protein-1 (PD1) and killer-cell lectin like receptor-G1 (KLRG1) on T cells in IL-27Rα$^{-/-}$ mice during infection, it regulated the presence of IFN-γ^{+}IL-2^{+}TNF^{+} multifunctional T-cells in the lung. Eventually, IL-17A supported *Cxcl9*, *Cxcl10* and *Cxcl13* expression in IL-27Rα$^{-/-}$ mice.

Conclusion: IL-17A contributes to protection in Mtb-infected IL-27Rα$^{-/-}$ mice probably through a chemokine-mediated recruitment and strategic positioning of multifunctional T cells in protective lung granulomas. As IL-27 restricts optimal anti-mycobacterial protection by inhibiting IL-17A production, blocking of IL-27Rα-mediated signaling may represent a strategy for improving vaccination and host-directed therapy in tuberculosis. However, because IL-27 also prevents IL-17A-mediated immunopathology such intervention has to be tightly controlled.

P83

BRONCHIAL MALFORMATION AND PROGRESSIVE B and CD4+ T CELL LYMPHOPENIA IN IMMUNODEFICIENCY, CENTROMERIC INSTABILITY AND FACIAL ANOMALIES SYNDROME 2

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Immunodeficiency, centromeric instability and facial anomalies syndrome 2 (ICF2) is a rare autosomal recessive primary immunodeficiency disorder. So far, 27 patients with ICF2 have been reported in the literature. Here we present 3 siblings with ICF2 due to a homozygous ZBTB24 gene mutation (c.1222 T>G), including two twin sisters. One patient had mycobacterial infection and bronchial malformation, which are for the first time associated with ICF2. All patients displayed a gradual reduction in immunoglobulin levels, B-cell and CD4+ T cell counts, suggesting a progressive course of immunodeficiency in ICF2. Despite their common genetic background, including the same causative mutation in ZBTB24, clinical heterogeneity (recurrent infections Vs. no significant infection record) and evident differences in immunological profiles (e.g. normal IgG levels Vs. severe hypogammaglobulinemia) among these patients suggest the pathogenic relevance of epigenetic modification in this monogenic immunodeficiency disorder. Considering the high mortality rate of ICF2, previous reports on severe complications, such as opportunistic infections, lymphomas and EBV-induced hemophagocytic lymphohystiosis as well as the here presented evidence on a progressive impairment of the immune system, we suggest early consideration of HSCT in all ICF2 patients.

P84

Aryl hydrocarbon Receptor (AhR)- and AhR Repressor (AhRR)-dependent immunoregulation in defense against *Salmonella Typhimurium* infection

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DOI: 10.1002/eji.201970300

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The Aryl hydrocarbon Receptor (AhR) is a ligand activated transcription factor, which binds exogenous ligands such as environmental toxins and natural compounds as well as endogenous ligands including tryptophan-derivatives. Recent studies have identified AhR as a potent immune regulator. AhR induces the expression of AhR Repressor (AhRR) which regulates AhR activation in a negative feedback loop. Using AhRR-eGFP reporter mice (AhRRE/+) our group showed that the AhRR is mainly expressed in immune cells of barrier organs. AhRRE/E mice showed an increased susceptibility to DSS-colitis as well as *Toxoplasma gondii* induced ileitis. These findings highlight the importance of the AhR/AhRR system in regulating inflammation and defense against pathogens.

In this project, we are investigating the role of AhR and AhRR in immunoregulation using a *Salmonella Typhimurium* infection model. AhRRE/E mice as well as WT control mice were infected with an attenuated *S. Typhimurium* strain, TAS2010. Splenomegaly was observed in both genotypes after infection with TAS2010 compared to uninfected controls although the spleens of AhRRE/E mice were slightly larger. While the total number of spleen cells was significantly increased in AhRRE/E mice compared to WT mice, no increase in myeloid cell infiltration was observed after infection. In addition, bacterial burden in organs was not different between genotypes. Since IFNg production is known to have a protective role in *S.Typhimurium* infection, we measured IFNg levels in serum and small intestine cultures. While there was no significant difference in IFNg serum levels, a slight decrease in IFNg levels in ileum cultures of AhRRE/E mice compared to WT mice was observed. FACS analysis of IFNg producing immune cells in the ileum of AhRRE/E mice showed a significant reduction of the IFNg+ CD4+ T cell population compared to WT mice. Initial analysis using AhR-/- mice revealed a strong increase in spleen weight compared to WT mice after TAS2010 infection. Also, the bacterial burden in spleen and liver of AhR-/- mice was higher compared to WT mice. TAS2010 infected AhR-/- mice will be analyzed further for production of IFNg in serum and small intestine cultures as well as changes in IFNg producing immune cell populations.

P85

The Long Pentraxin PTX3 Is an Endogenous Inhibitor of Hyperoxaluria-Related Nephrocalcinosis and Chronic Kidney Disease

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Patients suffering from primary hyperoxaluria type 1 display an elevated endogenous oxalate production, that leads to calcium oxalate crystals deposition in the kidneys (nephrocalcinosis) and to end-stage renal disease Herein lies an enormous unmet medical need. The acute phase protein Pentraxin 3 (PTX3) exerts a variety of functions in pathophysiological conditions associated with cell death and inflammation. Amongst them, PTX3 acts as a very efficient opsonin for a variety of pathogen and damage associated molecular patterns. We hypothesized, that PTX3 exhibits opsonin like functions on calcium oxalate crystals, inhibiting their further growth.

A simple in-chemico assay revealed a dose-dependent calcium oxalate crystal growth inhibition by PTX3, that was absent in the isomolar albumin control. Next, we studied PTX3 modulation in a model of nephrocalcinosis-induced CKD. PTX3 protein was detectable in the urine and upregulated in immunohistochemically stained kidney sections of wildtype mice fed for 3 weeks with a high oxalate diet whereas absent in control animals. To confirm the hypothesis, that PTX3 plays a pivotal role under hyperoxaluric conditions, we exposed B6;129-Ptx3^{tm1Mant} mice as well as their wildtype littermates to a high oxalate diet. Prior studies from our group led us to believe that the mixed BL6-129SV background found in these animals is not susceptible to hyperoxaluria induced nephrocalcinosis, enabling us to test, if the absence of functional PTX3 leads to the development of the respective pathology. The results showed, that nephrocalcinosis, as well as subsequent tissue damage, leukocyte infiltration and a decline in excretory kidney function are only to be observed in PTX3 deficient mice, but totally absent in their PTX3 competent littermates.

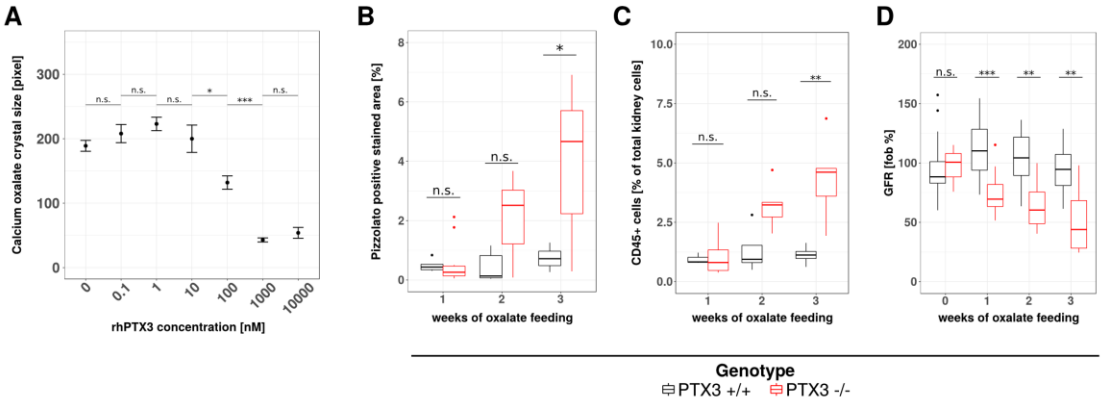
We were able to confirm, that PTX3 acts as a calcium oxalate crystallization inhibitor and exerts a non-redundant role in kidneys dealing with hyperoxaluric conditions. In future, PTX3 might serve as a valuable biomarker for assessing a patients susceptibility for nephrocalcinoses or even as a potential target molecule to be modulated in primary

DOI: 10.1002/eji.201970300

hypoeroxaluria treatment. Currently we investigate the precise molecular requirements that enable PTX3 to act as an crystallization inhibitor.

Fig 1: (A) PTX3 dose-dependent reduction of CaOx crystal size in chemico. Lack of (B) nephrocalcinosis, (C) leucocyte infiltration and (D) reduction in GFR in PTX3 competent vs PTX3 deficient mice.

Figure 1



T cell development (P88-P97)

P88
A new splice variant of the human transcription factor ONECUT2

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The transcription factor ONECUT2 (OC2) plays an important role in T cell development. Like other members of the ONECUT family of transcription factors, OC2 is characterized by the simultaneous presence of one CUT domain and one HOX domain. While establishing a cDNA library from the chemo-resistant Hodgkin lymphoma (HL) cell line L-1236, we identified a new splice variant of OC2 (OC2s). In contrast to OC2, OC2s uses an alternative second exon and contains only a CUT domain and no HOX domain. Expression analysis by quantitative Real Time PCR (qRT-PCR) indicated high expression of OC2 and OC2s in HL cell lines as well as in normal liver. The majority of normal tissues and cell lines expressed only low levels of OC2 and OC2s. In contrast, the cell lines Kasumi-1 and HL-60 as well as normal testis showed higher expression of OC2s. We observed that knockdown of OC2 lead to decreased OC2s expression whereas transgenic over-expression of OC2 had no impact on OC2s. Interestingly, over-expressed OC2 was located predominantly in the cytoplasm and not in the nucleus. By transgenic over-expression or knock-down of OC2 and subsequent DNA microarray analysis we identified potential new candidates regulated by these factors.

P89
CRISPR/Cas9-mediated demethylation of *FOXP3-TSDR* in T cells

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Introduction: The methylation status of *FOXP3-TSDR* (Treg specific demethylated region) plays a fundamental role for differentiation and stability of regulatory T cells (Tregs). Transfer of Tregs with a stable suppressive function is considered to be a promising therapy for chronic diseases like inflammatory bowel disease, graft-versus-host disease or type I diabetes. Tet (Ten-eleven translocation) enzymes catalyze the reaction of 5-methyl-cytosine to 5-hydroxymethyl-cytosine and facilitate active DNA demethylation.

Objectives: We aim to actively demethylate the *FOXP3-TSDR* of Jurkat T cells using CRISPR/Cas9 technology.

DOI: 10.1002/eji.201970300

Materials & methods: We electroporated Jurkat T cells with vectors encoding an inactive Cas9 fused with the catalytic domain of the TET1 enzyme and a distinct sgRNA for each vector. We transfected an additional GFP-plasmid to assess transfection efficacy. Viable, efficiently transfected GFP+ cells were sorted. After DNA extraction and bisulfite modification, *FOXP3-TSDR* including 9 CpGs was amplified and DNA-methylation was analyzed by deep amplicon sequencing.

Results: 48 hours after electroporation, 20-30 % of Jurkat T cells were viable and 20 % of total cells were GFP+. DNA methylation of *FOXP3-TSDR* was reduced in a proportion of viable transfected cells compared to untransfected controls.

Conclusion: These first results from a single experiment indicate that active demethylation of *FOXP3-TSDR* appears to be efficient in a proportion of Jurkat cells. This active demethylation could be the basis for induction of Tregs from naïve human T cells using CRISPR/Cas9 technology.

P90

mTORC1-dependent RNA synthesis in cycling T cells

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Adaptive immune responses to infections are marked by rapid proliferation and expansion of lymphocytes. T cells have to quickly increase their biosynthesis of nucleic acids, ribosomes, fatty acids and proteins which in sum makes up blast formation. However, it is not clear how RNA synthesis and cell cycle regulation are connected and what the role of RNA polymerases in this process is. We show that mTORC1-deficient T cells expanded around three times slower in response to persistent antigen. We have also seen that the amount of total RNA in activated T cells is largely controlled by the mechanistic target of rapamycin complex 1 (mTORC1), but not by mTORC2. RNAseq data indicated that these quantitative differences apply to the transcription of all RNA biotypes, with rRNA comprising 83-87% of the T cells" RNA while its processing is not affected by mTORC1. Accordingly, RNA extraction and FISH experiments visualizing 47S precursor rRNA and polyA mRNA levels indicated that activated mTORC1-deficient cells express reduced 47S rRNA and mRNA levels. We have then shown that the transition between the G1 and S cell cycle phases is obstruced in mTORC1-deficient cells, which decelerates their division rate three times. Several metabolic inhibitors had similar effects on RNA synthesis. Altogether our data suggest that global anabolic biomass production by proliferating T cells is under mTORC1 control.

P91

Resident and resting memory T lymphocytes of the bone marrow maintain efficient longterm memory to systemic pathogens

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Introduction: it is a matter of current debate, whether bone marrow is a hub for circulating memory T lymphocytes, and/or the home of a discrete population of bone marrow-resident memory T lymphocytes.

Objectives: Addressing under steady state conditions the maintenance of bone marrow memory T cells, in terms of proliferation, transcription, and/or mobility; Analyzing the repertoire diversity and complexity of bone marrow memory T cells; Dissecting the role of bone marrow memory T cells in response to booster vaccination in previously immune donors in the longterm.

Materials & methods: Human subjects: paired peripheral blood and bone marrow samples from systemically healthy adults; peripheral blood samples from adults before and after MMR vaccination.

Mice: Eight-week-old C57BL/6 male mice either untreated or immunized with LCMV.

Flow cytometry, cell sorting, whole-transcriptome profiling (microarray or RNA-Seq), immunofluorescent staining and confocal microscopy, NGS of TCR CDR3 Vβ were performed.

DOI: 10.1002/eji.201970300

Results: We provide evidence that, despite expressing the putative activation marker CD69, CD4+ and CD8+ memory T lymphocytes of the bone marrow take on a resting transcriptome and are non-proliferative, being in the G0 phase of the cell cycle not expressing KI-67. They dock onto IL-7-expressing stromal cells and express the tissue-resident memory T (TRM) cell signature genes described for TRM cells of other tissues, and thus qualify as *bona fide* bone marrow TRM cells. Their T cell receptor repertoire is significantly enriched for specificities against systemic pathogens/vaccines, which represent persistent, current, and particularly childhood immunological challenges. The enhanced protective memory of bone marrow memory CD4+ T cells is also reflected by their polyfunctionality. It is intriguing that memory CD4+ T cells specific for measles are maintained almost exclusively in the bone marrow, even in aged individuals in which they are not detectable in the blood, confirming that these cells are indeed sessile. If such individuals are vaccinated again, measles-specific TRM are mobilized into the blood and contribute to the secondary immune reaction.

Conclusion: These results demonstrate that bone marrow is home to resting TRM cells, providing efficient longterm memory to systemic pathogens/vaccines, and that these cells are maintained by contacting with stromal cells rather than by homeostatic proliferation.

P92

CD8αα$^+$ Intestinal Intraepithelial Lymphocytes (IEL) Arise from Two Thymic Precursors and Seed the Intestine in a Tight Wave in Early Life

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TCRαβ$^+$ CD8αα$^+$ intestinal intraepithelial lymphocytes (CD8αα IEL) descend from thymic precursors. To better define this IEL precursor (IELp) population, we analyzed their maturation, localization, and emigration. Using rigorous lineage exclusion criteria, we defined two precursors among DN TCRβ$^+$ thymocytes: a nascent PD-1$^+$ population and a T-bet$^+$ population that accumulates with age. Both gave rise to intestinal CD8αα IEL upon adoptive transfer. In adult mice, PD-1$^+$ cells contained more strongly self-reactive clones, localized to the cortex, and were dominant in S1PR1-dependent thymic egress. Gut homing $\alpha_4\beta_7$ was already expressed by these IELp at a thymic stage. To understand the kinetics of CD8αα IEL seeding the intestine, we performed "time-stamp" experiments: We crossed *Cd4*^{CreERT2} with *Rosa26*^{TdT} (stop-floxed tdTomato) mice. In these mice, tamoxifen or its metabolite 4-OHT permanently labels every CD4 expressing cell. As TCRαβ T cells (including CD8αα IEL) go through a CD4$^+$CD8$^+$ stage during thymic development, a single dose of tamoxifen or 4-OHT will label thymic IEL precursors permanently, so that they can be tracked when seeding the gut. Our results indicate that these cells enter the intestine during a narrow time window in early life and that this influx is almost completely shut down by the age of 3 weeks. These data provide an important foundation for understanding the biology of this abundant population of barrier surface T cells.

P93

Role of human and porcine MHC DRB1 alleles in determining the intensity of individual human anti-pig T cell responses

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Background: Differences in quality and strength of immune responses between individuals are mainly due to polymorphisms in MHC molecules. Focusing on MHC class-II, we asked whether the intensity of human anti-pig T cell responses is influenced by genetic variability in the human HLA-DRB1 and/or the porcine SLA-DRB1 locus.

Methods: ELISpot assays were performed using peripheral blood mononuclear cells (PBMC) from 62 HLA-DRB1-typed blood donors as responder and the porcine B cell line L23 as stimulator cells. Based on the frequency of IFN-γ secreting cells, groups of weak, medium, and strong responder individuals were defined. Mixed lymphocyte reaction

DOI: 10.1002/eji.201970300

(MLR) assays were performed to study the stimulatory capacity of porcine PBMC expressing different SLA-DRB1 alleles.

Results: Concerning the MHC class-II configuration of human cells, we found a significant over-representation of HLA-DRB1*01 alleles in the medium/strong responder group as compared to individuals showing weak responses to stimulation with L23 cells. Evaluation of the role of MHC class-II variability in porcine stimulators revealed that cells expressing SLA-DRB1*06 alleles triggered strong proliferation in approximately 70% of humans. Comparison of amino acid sequences indicated that strong human anti-pig reactivity may be associated with a high rate of similarity between human and pig HLA/SLA-DRB1 alleles.

Conclusion: Variability in human and porcine MHC determines the intensity of individual human anti-pig T cell responses. MHC typing and cross-matching of prospective recipients of xenografts and donor pigs could be relevant to select for donor-recipient combinations with minimal anti-porcine immunity.

P94

Influence of *in utero* paracetamol exposition on the fetal liver hematopoietic stem cell development

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Introduction: Paracetamol (APAP) is a commonly used analgesic and antipyretic drug, which is licensed in all three trimesters of pregnancy. However, maternal APAP intake during pregnancy is associated with reduced hematopoietic stem cells (HSC) in cord blood and an increased risk for development of T cell mediated immune diseases, such as asthma in the offspring. Hence, *in utero* APAP exposition may affect fetal T cell differentiation leading to T cell hyperreactivity later in life. Fetal T cell differentiation can be studied *in vitro* using the OP9 cell culture system by transfecting the OP9 stromal cells with the ligand Delta-like 1 (DLL1) to create a thymus like environment.

Question: The aim of this study was to investigate the effect of *in utero* APAP exposition on the T lymphopoiesis of fetal liver HSC *in vitro*.

Methods: C57/BL6 (6-8 weeks) females were allogeneically mated with BALB/c males. On gestational day (GD) 12.5 pregnant mice were intraperitoneally injected with 250mg/kg bodyweight APAP or PBS. APAP induced liver damage was assessed by determination of plasma transaminases activity 24h after APAP challenge. At GD 14.5 fetal livers were harvested, and HSC were isolated by fluorescence activated cell sorting of Sca-1$^+$ c-Kit$^+$ cells. T cell differentiation was induced by coculture of isolated HSC with DLL1$^+$OP9 cells. HSC cocultured with DLL1$^-$OP9 cells were used as controls. T cell differentiation was analyzed by flow cytometry.

Results: Liver damage in APAP challenged pregnant mice was confirmed by increased plasma transaminase levels. Fetal liver HSC were decreased in response to *in utero* APAP exposition. The residual HSC were cocultured with DLL1-expressing OP9 cells which successfully adopted T cell fate, whereas HSC cocultured with control cells showed no T cell commitment. Furthermore, HSC cocultured with DLL1 expressing OP9 cells progressed through the distinct double negative as well as double positive T cell stages, as described in literature. In addition, precursors of regulatory T cells could be identified in fetal liver HSC cocultured with DLL1 expressing OP9 cells.

Conclusion: The cocultured HSC clearly adopted a T cell fate. Future experiments will analyze the changes in HSC development upon maternal APAP intake during pregnancy. The overall aim of our studies is to improve pain medication during pregnancy and to reduce immunological consequences for the offspring which are associated with *in utero* APAP exposition.

P95

Contribution of tissue-resident memory CD8+ T cells to systemic immunity after reinfection

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DOI: 10.1002/eji.201970300

Tissue-resident memory CD8$^+$ T (T_{RM}) cells differentiate from activated effector T cells localizing to infected tissues in the first days after pathogen entry. T_{RM} cells are located at barrier sites and contribute to border control upon secondary infection through direct anti-microbial actions and via recruitment and priming of other immune cells. While central memory (T_{CM}) and effector memory CD8$^+$ T (T_{EM}) cells actively recirculate throughout the body, T_{RM} cells localize to confined tissue sites and do not contribute to systemic immunity. The evolutionary advantage of a highly spatially restricted population of memory T cells is difficult to apprehend as, e.g. in the context of skin infections, pathogens may use distinct anatomical locations as ports of entry.

We have previously shown that expression of the transcription factor Hobit is confined to T_{RM} cells and essential for the formation of these memory cells. In order to visualize the essential steps in the development of T_{RM} cells , we have created a compound knock-in mouse that not only allows for the assessment of Hobit expression but also for determining the fate of T cells that have expressed Hobit at some point during their development. Using reinfection models, we found a sizeable fraction of T cells in the circulation that do not express Hobit but have done so during their developmental history. Phenotypically, these tissue-experienced cells share properties with the T_{EM} population, express CX3CR1 but lack CD62L and are present in the spleen and circulation, but are excluded from lymph nodes. Adoptive transfer experiments with memory CD8$^+$ T cells confirmed the potential of T_{RM} cells to give rise to circulating effector and memory cells upon pathogen rechallenge.

Our findings imply that T_{RM} cells can relocate from tissue to circulation upon pathogen rechallenge and may thereby contribute to systemic immunity. This plasticity of resident memory may pose important implications on the establishment of protective immunity following local infections and vaccinations.

P96

Context dependent analysis of the miR-181 targetome

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Introduction: MicroRNAs are small non-coding RNAs with gene regulatory function. They negatively regulate gene expression by facilitating mRNA decay and/or translational inhibition. How an individual miRNA engages in either pathway *in vivo* in a given context remains unknown. MiR-181a/b-1 is one of the most abundant miRNAs in thymocytes, particularly during thymic development in CD4+/CD8+ double positives. Loss of these miRNAs results in defective development of unconventional T cells, including iNKT cells, MAIT cells and Treg cells.

Objectives: Here, we used miR-181a/b-1-deficient thymocytes as model cells to classify targets of miR-181a/b-1 based on their mode of repression.

Materials & methods: To this end, we performed RNA-Seq in combination with Ribosome profiling on thymocytes, taken from either WT or miR-181a/b-1-deficient mice.

Results: We found that predicted miR-181a/b-1 target mRNAs are mostly repressed through the mRNA decay pathway. However, a certain group of mRNAs are subject to translational repression as well. Our analysis suggests that translational repression affects mostly mRNAs, the 3"UTRs of which, contain 3 or more miR-181 response elements, while the type of response element appears irrelevant.

Conclusion: We concluded that T-cell development represents an easily accessible and *in vivo* model to study miRNA function. Further validation will establish context-dependency of miRNA targeting rules.

P97

scRNA-Seq reveals chimerism of skin T cells after HSCT and identifies human skin Trm

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DOI: 10.1002/eji.201970300

Memory T cells are compartmentalized and tissue-resident (non-recirculating) memory T cells (Trm) have recently been identified as important players in barrier defense in mice. Experimental evidence for the existence of true Trm cells in humans and their function in localized autoimmune pathologies is only beginning to emerge. Besides that, a method for identifying long-term Trm in human skin has not yet been proposed.

Here, we used a clinical intervention situation, i.e. hematopoietic stem cell transplantation (HSCT), to distinguish recirculating T cells from the recipient long-term Trm. Short tandem repeat analyses revealed persistence of recipient skin T cells in patients that had undergone successful myeloablative conditioning. The observed chimeric situation was further investigated by single-cell RNA-sequencing (scRNA-Seq) on hematopoietic cells from a healthy donor and from a patient with recipient skin T cell two years after sex-mismatched HSCT. Single-cell SNP genotyping identified recipient Trm in the skin of a patient at least two years after HSCT which was confirmed by analysis of gender-specific gene expression. Interestingly, pseudotemporal ordering of cells indicated that cells at early stages of tissue adaptation were also present within the persisting recipient T cells. However, they exhibited a unique transcriptional signature linked to tissue residency. Additionally, donor cells outnumbered recipient cells within the clusters of less tissue-adapted cells, suggesting that those might be transiently skin-homing.

In conclusion, we show that SNP genotyping of scRNA-Seq data is a powerful tool to identify skin Trm and that T cells can reside in the human skin long-term. The signatures we obtained from recipient cells will enable us to study true skin Trm in healthy individuals and to compare tissue-adaptation and functions of these cells in greater detail.

Antigen presentation and vaccination (P98-P114)

P98
TNF is required for upregulation of Mincle expression and adjuvant activity in response to mycobacterial glycolipids.

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TNF blockade by neutralizing antibodies or soluble TNFR2-Fc (Etanercept) is an established and highly successful treatment in human autoimmune disorders, such as rheumatoid arthritis and inflammatory bowel disease. However, TNF blockers increase the risk of infections, including reactivation of latent tuberculosis. The C-type lectin receptors (CLR) Mincle, Mcl and Dectin-2 are expressed on myeloid cells and bind mycobacterial cell wall glycolipids, e.g. the cord factor Trehalose-6,6-dimycolate (TDM), resulting in inflammatory gene expression and adjuvant activity. Here we show that TNF is sufficient to strongly upregulate Mincle, Mcl and Dectin-2 in bone marrow-derived macrophages. Importantly, TNF signaling was required for upregulation of these CLR and for secretion of the cytokines G-CSF and IL-6 in macrophages stimulated with TDM, the synthetic adjuvant Trehalose-6,6-dibehenate (TDB), or *Mycobacterium bovis* BCG, as revealed in TNF-deficient macrophages or by addition of Etanercept. Furthermore, Mincle-upregulation *in vivo* and induction of Th17 responses after immunization with the Mincle-dependent adjuvant TDB was completely abrogated in TNF-deficient or Etanercept-treated mice. Together, interference with production or signaling of TNF impairs inducible expression of the CLR Mincle, Mcl and Dectin-2 *in vitro* and *in vivo*, which is functionally relevant for vaccination responses and may contribute to the well-documented susceptibility for infections in patients receiving TNF blockers.

P99
The ubiquitin-like modifier FAT10 lowers type I interferons and enhances interferon-γ after viral infection

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Introduction: The ubiquitin-like modifier FAT10 is expressed in mature dendritic cells and medullary thymic epithelial cells and is strongly induced by interferon(IFN)-γ and tumor necrosis factor. FAT10 is the only ubiquitin-like modifier that targets its hundreds of covalently-linked substrates for degradation by the 26S proteasome. While the conjugation of and proteasome targeting by FAT10 are well characterized, the biological function of FAT10 has remained incompletely understood. Since FAT10 was reported to lower the type I interferon response and enhance virus replication in influenza A virus-infected cells we have investigated whether FAT10$^{-/-}$ mice respond differently to viral infection.

DOI: 10.1002/eji.201970300

Objectives: To compare the type I and type II IFN response of FAT10$^{-/-}$ and wild type mice to infection with influenza A virus (IAV) and lymphocytic choriomeningitis virus (LCMV). We further compared virus titers and disease symptoms after IAV and LCMV infection.

Materials & Methods: FAT10$^{-/-}$, FAT10$^{-/+}$, and FAT10$^{+/+}$ mice were infected i.n. with IAV or i.v. with LCMV-WE, and virus titers, body weight, and survival were monitored over time. The *fat10* mRNA expression was measured in spleen, thymus and lung after viral infection and the concentrations of IFN-α, IFN-β and IFN-γ in the serum were determined. Moreover, the production of type I and type II interferons by splenocytes from virus infected FAT10-deficient and –proficient mice was measured with and without a one-day stimulation of T cells with immobilized CD3 and CD28 antibodies.

Results: *Fat10* mRNA was induced in spleen and thymus 3-7 fold after LCMV infection and 27 fold in lung after IAV infection. Splenocytes of LCMV infected FAT10$^{-/-}$ mice on day 3 post infection showed a 50% reduction of IFN-γ production after a one-day stimulation with CD3/CD28 antibodies *in vitro* on protein and mRNA level, while the production of IFN-α and IFN-β was doubled as compared to FAT10$^{-/+}$ mice. This reduction of IFN-γ production was accompanied by a significant diminuition of mRNA for IL-12 p40 in spleen cells from FAT10$^{-/-}$ mice. Moreover, IAV infected FAT10$^{-/-}$ mice showed reduced IFN-α serum levels. Nevertheless, virus titers, body weight loss, and survival after IAV infection was not affected by FAT10 deficiency.

Conclusion: FAT10 fine-tunes the balance of interferons during viral infection by lowering the type I and enhancing type II interferons.

P100

Establishment of a novel human lymphoblastoid B cell line for comparative analyses of the immuno- and constitutive proteasome

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Introduction: The 20S proteasome is a multi-subunit cellular complex consisting of four rings. Three subunits of the inner rings are catalytically active (β1, β2 and β5). Upon stimulation with IFN-γ or TNF-α, these subunits are replaced by their inducible forms low molecular mass polypeptide (LMP)2 (β1i), LMP7 (β5i) and multicatalytic endopeptidase complex-like (MECL)-1 (β2i). The resulting proteasome containing the induced subunits is named immunoproteasome (IP) compared to the constitutive proteasome (CP). Originally, the IP was found to play an important role in generating antigenic peptides for presentation by MHC class I molecules. More recently, its involvement in expansion, survival and differentiation of T cells has been reported.

Objectives: Even though many studies have shown the importance of IP in immune cells and its role in antigen presentation, it is still unknown whether there are specific substrates processed by the IP or the CP. In order to address this question as well as to investigate the biological impact of IP-selective substrates, a novel cell line for inducible IP expression was generated to allow for a comparable analysis.

Materials & methods: cDNA encoding for human LMP2 and LMP7 was cloned into a lentiviral plasmid which was used to transduce LCL721.174 cells together with the plasmid encoding for the tamoxifen-inducible transcriptions factor Gev16. LCL 721.174 cells are immortalized human B cells with a genomic deletion in the region encoding for LMP2 and LMP7 and therefore do not express IP. Single cell clones were screened for strong transgene expression as well as incorporation into the proteasome complex.

Results: Without tamoxifen neither LMP2, LMP7 nor MECL-1 are expressed whereas the constitutive subunits (β1c, β2c and β5c) are present. Upon tamoxifen addition, LMP2, LMP7 and MECL-1 expression increases and β1c, β2c and β5c expression decreases over time.

Efficient incorporation of the induced subunits into the mature 20S proteasome was confirmed by proteasome purification and nonequilibrium pH gel electrophoresis. Data from current investigations on the effect of IP expression for cell proliferation, sensitivity to protein-misfolding inducing agents and degradation of ubiquitin conjugates will be presented.

DOI: 10.1002/eji.201970300

Conclusion: A novel cell line was established for studying functions and substrates of IP and CP. Successful conversion from CP to IP upon tamoxifen addition was shown and thus allows for comparative analyses.

P101

Deciphering the mechanism(s) of antigen presentation impairment in the colorectal cancer microenvironment

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Colorectal cancer (CRC) is the third most common types of cancer and it constitutes one of the leading causes of death. The pathogenesis of CRC relies on a complex of interactions between developing cancer and surrounding tissue, including the immune system. One of the most abundant tumor-infiltrating cell population is represented by tumor-associated macrophages (TAMs). Although macrophages were regarded as involved in anti-tumor immunity, the majority of studies referring to carcinomas, including CRC, shows that greater infiltration of TAMs is associated with poor prognosis. Moreover, the antigen-presenting molecules MHC-II are not expressed in about two-third of colorectal cancers (CRC) and this is associated with a decrease of tumor-infiltrating T cells and an increase of metastatic potential of CRC.We demonstrated that Helicobacter pylori(Hp) induces macrophages to express/expose the recently identified immune receptor CD300e on the plasma membrane, while it decreases the amount of the antigen-presenting molecules MHC-II, thus compromising the capacity of macrophages to activate CD4 T lymphocytes. Considering the opposite behaviour of CD300e and MHC-II in Hp-infected macrophages and the impact of CD300e activation on the level of the antigen presenting molecule, we postulate that CD300e could act as an immune checkpoint regulating the activation of T cells, via the modulation of MHC-II molecules on antigen presenting cells (APCs), including TAMs of CRC.Immune histochemical analysis performed on human CRC specimens (stage II) showed a clear-cut staining for CD300e in a good proportion of TAMs infiltrating CRC. This is mirrored by a lowered expression of MHC-II, compared to macrophages expressing CD300e in normal mucosa. Interestingly, we also found that monocytes cultured in CRC cells-conditioned medium (CM-CRC) or in presence of CRC decellularized matrix, increase surface expression of CD300e, while decrease the exposure of the MHC-II. Interestingly, after six days, these cells show a pro-tumor macrophage-like phenotype. These data open the possibility that the immune receptor CD300e might be involved in hijacking the ability of macrophages to elicit an effective anti-tumor immune response in CRC. Our data suggest that CD300e and MHC-II could be part of the same pathway and implies that CD300e could act as an immune check point molecule modulating the adaptive immune response via a fine tuning of MHC-II.

P102

Quantitative estimation of Hepatitis B tittres in health care workers of tertiary care hospital in north western region of India

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Background: Health care workers (HCWs) are at high risk for acquiring blood borne infection because of needle stick injury (NSI) and occupational exposures to potentially infectious bodily fluids. Of these Hepatitis B is not only the most transmissible infection, but also the only one that is preventable by vaccination.

Methodology: Our study (pilot study) aimed the status of hepatitis-B screening in HCWs, estimation of Anti-HBs titres, vaccination status for Hepatitis-B and assessing the risk of HCWs for accidental blood exposure in a newly establishing tertiary care hospital of western-india. A total of 200 HCWs enrolled in this study. The HCWs were evaluated for NSI using questionnaire based study and the Hepatitis-B screening and estimation of Anti-HBs titres were evaluated using ELISA-based Kits.

Results: In all, 162 out of 200 HCWs had taken atleast 1 dose of vaccine resulting in a vaccination rate 81%. In this only 159 out of 200 (79.5%) had received 2nd dose whereas only 119 of 162 (73.5%) HCWs completed the primary series of 3 doses and only 11.7% had taken their booster doses. of which Doctors had statistically significant adherence with vaccination doses compared to paramedics (p=0.011). Majority 65.8% had prick/exposure with gloves on, followed by exposure to blood or body fluids to intact skin ie., 34.1%; 82% of the HCWs have taken local measures immediately after the exposure. Only 23 HCWs of the NSI or exposures were reported to the hospital authorities. We found that 4/200 (2%) of the HCWs were reactive for HBsAg; 18(9%) of the HCWs were reactive for Anti-HBc; 166 (83%)were reactive to Anti-HBs. Among our study population of HCWs 34 out of 200 (17%) were

DOI: 10.1002/eji.201970300

having titres < 10 mIU/l; another 34 out of 200 (17%) were having titers 10-100 mIU/ml ; Majority of HCWs (66%) had anti-HBs ≥ 101 mIU/ml as good responders to vaccination.

Conclusion: Since our hospital which is an establishing tertiary care centre the number of HCWs effectively protected with 3 doses of HBV vaccine is not adequate which can be addressed by educating HCWs and keeping a continuous surveillance for vaccination status. Incorrect PEP findings indicate a lack of knowledge which needs to be addressed. Findings from the present study demonstrate there is urgent need to vaccinate and train HCWs to reduce the chance of acquiring HBV infection.

Keywords: health care workers (HCWs),hepatitis B vaccination status,postexposure prophylaxis practices(PEP)

P103

Engineering Flavivirus-like particles platforms to assess the pre-existing Flavivirus immunity

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Introduction: Antibody-dependent enhancement and cross-protection can both result from pre-existing immunity resulting from infections with different flaviviruses. A better understanding of the underlying mechanisms is thus essential for the development of safe vaccines in areas where several flaviviruses cocirculate.

Objective: To assess the extent of Flavivirus cross-reactive immunity induced by Flavivirus-like particles.

Materials and Methods: Flavivirus-VLPs based on the structural genes of Zika virus (ZIKV), Japanese encephalitis virus (JEV), yellow fever virus (YFV), and West Nile virus (WNV) were produced in a mammalian expression system. Balb/c mice were immunized with the different VLPs using a homologous regimen with 3 immunizations of 10ug of VLP mixed with Sigma Adjuvant System. The specific binding antibodies against each virus were quantified by immunoperoxidase monolayer assay (IPMA).

Results: The VLPs were expressed at similar efficiency and could be purified to concentrations of 1 mg/ml. All VLP-immunized animals developed antibodies against the homologous virus that increased with each subsequent immunization. The onset of cross-reactive antibody responses was delayed, but usually detectable after the second immunization and reached titers within the range of the matched response after the third immunization. Neutralizing antibody responses were generally low, and there was limited cross-reactivity.

Conclusion: The robust homologous antibody response against the ZIKV, JEV, YFV and WNV structural proteins together with the strong cross-reactivity against heterologous flaviviruses makes Flavivirus VLPs promising tools to study the effect of pre-existing flavivirus immunity.

P104

Humoral immunity by a recombinant CMV vaccine vector provides strong antiviral immune protection

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Natural Cytomegalovirus (CMV) infection induces the strongest known T-cell memory, which dominates the memory compartment of seropositive people. Therefore, recombinant CMVs are intensively studied as potential vaccine vectors eliciting optimal cellular immunity. Much less is known about their ability to induce protective humoral immunity. Therefore, we generated recombinant mouse CMV (MCMV) vectors eliciting either a predominantly humoral or an exclusively cellular immune response against the same antigen and compared their capacity to protect against a virus challenge.

We have previously shown that MCMV vectors encoding an optimally positioned antigenic peptide induce much stronger T-cell responses than MCMVs expressing the full-length protein with the same peptide, mainly due to optimized proteasomal processing. We used the same strategy to clone MCMV vectors expressing the full-length hemagglutinin (HA) from H1 type A influenza, or an optimally positioned, MHC-I restricted peptide from HA.

While the vector with the optimized epitope induced 5-10x stronger CD8 T cell responses than the vector with the full length HA, only the HA-full-length vector protected the mice against influenza challenge, as evidenced by weight loss

DOI: 10.1002/eji.201970300

monitoring and infectious virus titration from lungs of challenged mice. On the other hand, only the full-length vector induced strong humoral immune responses, as evidenced by the hemagglutination assay.

To validate conclusively that humoral immunity was essential for immune protection by this vector, we immunized B-cell deficient JHT mice with our optimized-epitope vector or the HA-full-length vector and assayed them in challenge assays. We observed no immune protection upon infection with either MCMVs.

Therefore, MCMVs expressing the full-length HA are protective against influenza challenge, and this protection is not due to cellular immune responses. To our knowledge, this is the first report arguing that MCMV vectors may be used to induce robust and protective humoral immune responses.

P105

Cell-Mediated Immunity Induced by two Recombinant Modified Vaccinia Virus Ankara Vaccines Delivering the G Antigen of Nipah Virus

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Introduction: Nipah virus (NiV) is a highly pathogenic zoonotic paramyxovirus (BSL4) that is transmitted to humans from bats directly or via intermediate hosts. NiV infection in humans causes severe acute respiratory syndrome and encephalitis with high mortality rates. The presence of NiV in wildlife hosts, its capacity to infect a wide range of animals and its ease of propagation, makes it an emerging infectious agent of concern. Thus, safe and effective vaccines are needed for prophylaxis in humans and animals. Modified Vaccinia Virus Ankara (MVA), a highly attenuated and replication deficient virus with a favourable safety profile, serves as a potent vector system for the development of candidate vaccines against infectious diseases.

Objective: We developed two MVA candidate vaccines expressing NiV attachment glycoprotein (G), a highly promising NiV-specific immunogen. We aimed to test the ability of the vaccines to elicit NiV-specific T cell responses.

Material & Methods: We generated two recombinant MVA vaccines expressing either the full-length form or soluble form of NiV-G. The cDNA gene sequences encoding the G antigen were generated by DNA synthesis and modified by introducing silent codon alterations that remove termination signals for vaccinia virus early transcription. The target gene was cloned into MVA vector plasmids and introduced by homologous recombination into the MVA genome.

Results: The recombinant MVA viruses expressing NiV-G were genetically stable and replicated efficiently in MVA permissive chicken embryo fibroblasts but not in MVA non-permissive human cell lines. Infected human cell lines however demonstrated efficient NiV-G synthesis. In our vaccine trials, IFNAR-/- mice were inoculated twice with 10^8 pfu of the MVA-NiV-G vaccines over a 21-day period. Immunisations induced NiV-G-specific cell-mediated immunity. We generated a peptide library covering the whole NiV-G protein and identified peptides that stimulated IFNγ-producing CD8 T cells by IFNγ-Elispot assay and intracellular cytokine staining. The two MVA-NiV-G vaccine candidates differed in their ability to generate NiV-G specific CD8 T cell responses, with the vaccine expressing the soluble form of NiV-G stimulating a more robust CD8 T cell response *in vitro*.

Conclusion: We developed two NiV candidate vaccines that generated anti-NiV CD8 T cell immunity. These vaccines await further testing to evaluate their protective efficacy in a mouse model of NiV challenge.

P106

Immune responses and protective efficacy elicited by a recombinant autolyzed *Salmonella* expressing FliC flagellar antigen of F18+ *Escherichia coli*

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Porcine edema disease (ED) caused by $F18^+$ Shiga toxin 2e-producing ***Escherichia coli*** (STEC) has imposed significant economic losses in the swine industry worldwide, resulting in sudden deaths in post-weaned piglets. The flagellin protein of $F18^+$STEC, a structural component of the flagellar filament, is a known virulence factor that mediates adhesion and invasion to porcine epithelial cells. In this study, ***Salmonella*** inactivated by the ***E*** lysis gene and expressing the flagellin (***fliC***) antigen was genetically engineered utilizing a plasmid (pMMP184) carrying an

DOI: 10.1002/eji.201970300

efficient heterologous antigen delivery system. The resulting strain JOL1485 producing FliC was successfully inactivated by the ***E*** lysis gene cassette. Following the lysis procedure, FliC secretion and production of JOL1485 was validated by immunoblot analysis. To evaluate protective immunogenicity elicited by the constructed strain, BALB/c mice were injected with 1×10^8 lysed cells via the intramuscular route. The markedly elevated titers of FliC-specific IgG, IgG1 and sIgA antibodies were observed, indicating a robust Th2-associated humoral immune response was raised in the immunized mice. The proportion of $CD3^+CD4^+$ splenic T cells and proliferative activity were also elevated in ***in vivo*** and ***in vitro*** stimulated mice splenocytes. Further, JOL1485 successfully elicited upregulated gene expression of cytokines IL-6, IL-8, IL17, IL-21, IFN-γ and TNF-α in naïve porcine peripheral blood mononuclear cells (PBMCs). The overall immune response elicited by JOL1485 conferred a significant rise of protection against a lethal virulent $F18^+$STEC challenge whereas all non-immunized mice died following the challenge. Our results demonstrate that ***fliC*** efficiently expressed in the genetically inactivated ***Salmonella*** strain has immunostimulatory and protective effects against a $F18^+$STEC lethal challenge, and may be promising as a potential vaccine candidate against ED infection.

P107

Targets of the T cell response to human herpesvirus 6B

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Introduction: Human herpesvirus 6 (HHV-6) is prevalent in healthy persons, causes disease in immunosuppressed carriers, and may be involved in autoimmune diseases. Recent studies established that HHV-6-specific T cells are present in healthy carriers and are important for effective control of infection. However, information on target antigens, epitopes, and antiviral function is still limited. Such knowledge is desirable for immunomonitoring, adoptive T cell therapy for transplant patients at risk of viral reactivation, and investigations of potential autoimmune pathogenesis.

Methods: In order to identify epitopes that are presented by infected cells to virus-specific T cells, we used a combination of specific T cell expansion by peptide-loaded autologous B cells, single T cell cloning, and effector assays with HHV-6-infected cells as targets. Two types of peptide libraries were used: Libraries of peptides from across the viral proteome that are presented by selected HLAs, and overlapping peptide libraries that fully cover selected HHV-6 proteins.

Results: In a cross-sectional screen, we identified 16 peptides from 12 antigens as CD8 T cell epitopes that are presented by infected cells through HLA-B*08:01. T cells specific for an epitope from IE-2 were particularly frequent, allowing their ex vivo quantification with HLA-peptide multimers. Moreover, we observed very effective lysis of HHV-6B-infected cells. Multiple B*08:01 epitopes were identified in IE-2, the viral DNA polymerase U38, and the major DNA-binding protein U41. These three proteins were chosen for a protein-covering screening to identify further CD8 and CD4 T cell epitopes presented by diverse HLA molecules. Until now, specific T cell clones were established that recognized two CD8 T cell epitopes and four CD4 T cell epitopesin U38 and eight new CD4 T cell epitopes in U41, covering various HLA restrictions.

Conclusions: These results show that the CD8 and CD4 T cell response to HHV-6B targets a wide range of multiple antigens of diverse functional classes presented by infected cells. While many of these elicit low-frequency T cell responses, a minority of epitopes are targeted by T cells that stand out in terms of their ex vivo frequency in carriers. Identification of these targets will serve as a reliable basis for immunomonitoring, identification of protective specificities, adoptive T cell therapy, and vaccine development.

P108

Immunoproteasome inhibition in a murine model of polymyositis

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Introduction: Polymyositis (PM) belongs to the idiopathic inflammatory myopathies (IIMs) which are a group of muscle diseases that present with muscle weakness in combination with inflammation of the muscle tissue. Currently, there are no effective targeted therapies but glucocorticoids and non-specific immunosuppressive drugs are applied. Muscle biopsies suggest an autoimmune pathogenesis as cellular infiltrates are detected in multiple foci within the endomysial parenchyma which consist predominantly of perforin-positive CD8+ T cells. Experimental PM can be induced in mice by vaccination with fragments of the muscular C protein. This model, designated C protein-induced myositis (CIM), allows the investigation of new treatments for PM.

DOI: 10.1002/eji.201970300

Objectives: As immunoproteasome subunits β1i and β5i are up-regulated at mRNA and protein level in myofibers and muscle infiltrating cells in IIMs, immunoproteasome inhibition may hold potential for the treatment of inflammatory myositis. Thereby, we analyzed the effect of immunoproteasome inhibition on the development of polymyositis induced by immunization of mice with the fragment 2 of the myosin binding protein C. Furthermore, we investigated the effect of immunoproteasome inhibition on the generation of cytotoxic T lymphocytes (CTL) epitopes derived from the C protein.

Materials and methods: C57BL/6 mice were immunized subcutaneously by injection of 200 µg of the fragment 2 of myosin binding C protein emulsified in Freund´s complete adjuvant. 2 µg of pertussis toxin in PBS was injected intraperitoneally at the same time. On day 13, 15, 17, 19, 21, 23, 25 and 27 post-immunization the mice were treated subcutaneously with 10 mg/kg ONX 0914 or vehicle (Captisol®). The muscle strength was assessed with a grip strength meter.

Results: Treatment with ONX 0914 significantly reverted the decline of grip strength associated with induction of polymyositis. PM-dependent increases of proteinuria were ameliorated by ONX 0914 treatment. Histological analyses of inflamed muscles and assessments of CTL responses will be presented.

Conclusion: By using the immunoproteasome-specific inhibitor ONX 0914 in a murine model of PM we show that the immunoproteasome may be considered as a potential therapeutic target for the treatment of PM and other autoimmune inflammatory myopathies.

P109

Influence of elevated ambient CO2 on the sensitizing potential of ragweed (*Ambrosia artemisiifolia*) pollen

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Question: The incidence of allergic diseases has been steadily increasing over the past decades causing a significant impact on public health worldwide. This is partly due to man-made climate change, contributing to earlier flowering of allergenic plant species and increasing the duration of the pollen season. Especially elevated CO2 levels have been shown to increase pollen production of ragweed (*Ambrosia artemisiifolia*). The allergenic potential of pollen depends not only on the allergen itself, but also on endogenous adjuvant substances, such as lipid mediators and adenosine. These substances have been shown to skew immune responses and presumably promote allergic sensitization to pollen proteins in humans.

Our aim was to elucidate the influence of elevated CO2 on the composition of pollen adjuvant factors and their potential to induce allergic sensitization.

Methods: Ragweed plants were grown in climate-controlled chambers under elevated (700 ppm) or normal (380 ppm) CO2 levels. Untargeted metabolomics was applied to discover relevant adjuvant factors in pollen from control and CO2 treated plants. Aqueous pollen extracts were used in a mouse model to induce allergic sensitization *in vivo* as well as to stimulate murine B cells, human monocyte-derived dendritic cells and human nasal epithelial cells *in vitro*.

Results: The metabolome analysis revealed differential expression of lipids, flavones, flavanols and sugars, as well as adenosine in CO2 *vs* normal pollen. CO2 pollen were stronger allergy inducers in the mouse model by increasing neutrophil and eosinophil influx into bronchoalvear lavage fluid. This corresponds to lower IL-10 and higher TNFα induction in human dendritic cells compared to the control *in vitro*. CCL22 induction in human nasal epithelial cells was slightly increased when stimulated with CO2 treated pollen. IgE expression was increased in murine B cells upon treatment with CO2 pollen.

Conclusion: Our results suggest that higher levels of CO2 in the environment influence the sensitizing potential of ragweed pollen by changing the composition of endogenous adjuvant factors, especially adenosine, which in turn induce stronger allergic inflammation in mice as well as in the *in vitro* read-out system.

DOI: 10.1002/eji.201970300

P110

Modulation of allergy and vaccine responses through maternal *S. mansoni* infection

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Introduction: Chronic infection with the parasitic helminth *Schistosoma mansoni* is characterized by a modified Th2 response coupled to immunosuppression. This protects the host against overwhelming inflammatory responses against the parasite, but has spillover effects to bystander antigens, such as allergens. There is recent evidence that schistosomiasis during pregnancy similarly influences offspring allergic and inflammatory responses, as well as to vaccines.

Objectives: We intend to understand mechanisms underlying the relationship between chronic maternal helminth infection and altered immunogenicity, particularly as relates to allergy and vaccination.

Materials and Methods: We apply transmaternal murine models of chronic schistosomiasis to investigate the effect maternal immune status upon imprinting immune predispositions in offspring.

Results: We have shown that allergic airway inflammation (AAI) in adult murine offspring from schistosome-infected mothers is strongly modified by the phase of maternal infection, and suppressed when pregnancy was initiated during late chronic stages. Further, this was associated with changes at the fetomaternal interface, including placental transcriptional profile as well altered cytokine production. More recently, we have found altered antigen-specific responses during the early sensitization stages of allergic response in these offspring, as well as other vaccination models, in line with epidemiological trends. These changes relying heavily on vaccine vector and the mode of antigen delivery.

Conclusions: Murine models of maternal schistosomiasis replicate clear immune imprinting upon offspring as reflects human cohort studies. Through studying altered responses to allergic inflammation and vaccine immunogenicity, we have been able to further determine these changes as relate to antigen-specific responses, and our continued work investigates the mechanisms underlying this. Our studies will help to understand the effects of the maternal immune status during pregnancy on immune predisposition in later life, and mechanisms for fine tuning immune responses.

P111

Deciphering the innate immune response elicited by viral vaccine vectors against emerging infectious diseases

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Emerging infectious diseases (EID) were recently named one of the top ten threats to global health by the WHO.For most EID, epidemic outbreak preparedness is limited by the lack of therapeutics and preventive vaccines. Therefore, the WHO identified priority pathogens, including Ebola virus (EBOV) and Middle East respiratory syndrome coronavirus (MERS-CoV), for which vaccines will be developed in the context of the R&D Blueprint Initiative to prevent epidemics. Recently, the Coalition for Epidemic Preparedness Innovations (CEPI) has included the MVA and VSV platform in its portfolio. While vaccines are one of the most impactful public health interventions, exact mechanisms how they confer protective immunity remain inadequately understood. We aim to understandand identify underlying mechanisms, unknown molecules and pathways responsible for vaccine-induced immunogenicity. The identification of VSV and MVA-specific signatures for immunogenicity may be critical to accelerate their development and implementation.

The viral vector vaccine candidates VSV-EBOV and MVA.MERS-S against EBOV and MERS, respectively, were evaluated during Phase I trials, in which we revealed their immunogenicity and safety in human.To elucidate dynamics of distinct innate immune cell populations we are now applying *in vitro* assays. Human PBMCs were infected with VSV-EBOV and MVA.MERS-S. The infection rate, cell tropism and expression of viral antigens were investigated by

DOI: 10.1002/eji.201970300

qPCR, IHC and flow cytometry. Moreover, luminex bead-based assays and flow cytometry were used to quantify immune mediators by cytokine profiles as well as activation status of innate immune cells.

Preliminary results indicate distinct signatures induced by both vaccines. Both candidates showed a cell-specific tropism and induced activation markers on monocytes. For both vectors, the highest viral load was detected 12 h after infection, whereas the anti-viral response measured as pro-inflammatory cytokine secretion showed the highest induction after 1 h and 24 h post infection with VSV-EBOV or MVA.MERS-S, respectively. While mRNA signatures after vaccination have been reported for VSV-EBOV, an ongoing study aim is to find markers for MVA.MERS-CoV induced immunogenicity.

Taken together, our data provide insights into early immune responses induced by VSV-EBOV and MVA.MERS-S. A better understanding of molecular and cellular events in the host response to vaccines may facilitate the rational design of novel vaccines.

P112

Dynamic regulation of human dendritic cell precursor and subpopulations in the blood after yellow fever vaccination

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Introduction: Yellow fever (YF) virus is a plus strand RNA virus and part of the flavivirus family. Vaccination with the live-attenuated YF vaccine strain (YF-17D) provides life-long protective humoral and cellular immunity against infection and is a unique model for studying the immune response to an acute self-limiting RNA virus infection in humans.

Objective: Using this model, we investigated the response of blood dendritic cells (DC) and monocyte subsets to YF-17D in vivo and in vitro to elucidate the early innate immune events which precede the rapid generation of protective adaptive immunity.

Methods: The frequencies and activation status of blood DC subpopulations and precursors (preDCs) were determined by FACS analysis before and 3, 7, 14 and 28 days after YF vaccination. PBMCs, or sorted monocyte and DC subpopulations were infected with fluorescent YF-17D reporter virus in the presence or absence of pathway inhibitors and infection rates and IFN I responses were analyzed.

Results: After vaccination, a significant expansion of preDCs, marked by expression of Axl, CD33 and CD123, was observed on day 14 while conventional DCs (cDCs) and plasmacytoid DCs decreased in frequency and total numbers. Ki67 staining showed proliferation in cDCs and preDCs after vaccination, suggesting recruitment of preDCs and differentiation towards cDCs in the course of viral infection with YF-17D. Upregulation of CD86 was detected in both CD141+ cDC2 cells and preDCs on day 7 indicating that activation precedes the peak of expansion of cDCs and preDCs. All subset of blood monocytes and DCs could be infected with YF-17D in vitro, but cDC1 and cDC2 showed the highest infection rate. Inhibition of TBK1, JAK1/2 or IFNAR increased the frequency of infected DCs demonstrating the importance of IFN I for restricting YF-17D virus replication.

Conclusion: Our results show that YF-17D vaccination leads to expansion and activation of cDCs and their precursors, but infection of cDCs by YF-17D virus is restricted by the strong type I IFN response. Thus, DC function is preserved while allowing limited presentation of endogenous viral antigens.

P113

The molecular basis of hepatitis B vaccine non-responsiveness

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DOI: 10.1002/eji.201970300

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Introduction: After a complete cycle of three vaccinations against the hepatitis B virus (HBV), about 5% of all vaccinated persons do not mount antibody responses and are so-called non-responders. Most people show an anti-HBs antibody titer of more than 100 IU/L many years. Such a high titer is known to be protective against the highly infectious virus. In contrast, the titer of non-responders stays permanently lower than 10 IU/L, which can be a huge problem for health-care workers, who have a high risk to get in contact with contaminated blood. In the case of influenza vaccination, the phenomenon of non-responsiveness increases with advanced age. In contrast, the group of HBV non-responders already includes young and otherwise healthy people. Although the phenomenon is known for many years, the molecular basis of HBV vaccine non-responsiveness is still not known.

Methods: We initiated an observational study to monitor the immune reactions after a HBV vaccination to compare the immune response of healthy non-responders with responders. The immunomonitoring includes a cytometer-based analysis of blood samples to analyze the distribution of various immune cell subsets as well as their activation status. Additionally, the cytokine responses of responders and non-responders will be analyzed. The second arm of the project includes a whole genome sequencing, which allows the search for genetic markers that influence vaccine responsiveness.

Results: The recruitment of healthy volunteers is still in progress. Preliminary data confirm the association of non-responsiveness and certain HLA alleles that is described in the literature. We adapted a bioinformatics approach aiming for cluster analyses of flow cytometry data and first results will be presented during the conference.

Conclusion: Based on the results, we hope to find biomarkers, which would allow the prediction of HBV vaccination non-responsiveness. Additionally, we would like to develop better vaccination strategies in the future.

P114

Yellow fever vaccination as a model to study the immune response to an acute viral infection

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Objectives: Vaccination with the live attenuated yellow fever vaccine (YF-17D) causes an acute self-limiting viral infection, which results in life-long immune protection and thus provides an attractive model to study the innate and adaptive immune responses. This model could be used to identify the intracellular receptors and predict the vaccination efficacy as well as the genetic inter-individual differences in the activation of the innate and adaptive immune responses to YF-17D. This will aid in designing new vaccines against emerging flavivirus epidemics such as Zika and Dengue

Methods: CRISPR-Cas9 gene edited human cell lines deficient for RIG-I-like receptors or the adapter MAVS as well as primary cells from the corresponding knock out mice were tested in infection assays with YF-17D. Cytokine responses and viral replication were determined by qPCR, ELISA and plaque assays.

A cohort of YF-17D vaccines (n = 250) was recruited and bio-samples were taken before and on day 3, 7, 14 and 28 after vaccination. Induction of interferon-induced genes, neutralizing antibody titers, viral load and T cell responses were determined in plasma and PBMCs.

Results: We demonstrated that RIG-like helicases (RLH) are the dominant receptors, which recognize YF-17D. What is more, the cells deficient in MAVS adaptor lose the ability to stimulate interferon after infection with YF-17D.

Individuals vaccinated with the YF-17D strain show significant differences in the titers of neutralizing antibodies, viral load and the expression interferon-induced genes following vaccination.

Conclusions: YF-17D elicits its interferon response via the RLR-MAVS axis triggering both MDA5 and RIG-I. Different structural RNA motifs produced during the replication cycle are thereby activating RIG-I and MDA5.

DOI: 10.1002/eji.201970300

Our vaccination cohort can be stratified by parameters of the innate and adaptive immune response as the basis for further analyses.

Microbiome and mucosal immunity (P115-P133)

P115
IL-33/ST2 signaling orchestrates an immune network to counteract intestinal inflammation
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Introduction: Ulcerative colitis and Crohn"s disease represent two of the most common chronic inflammatory diseases, collectively referred to as inflammatory bowel disease (IBD). IBD is characterized by pathological mucosal damage and ulceration and is known to be a high-risk condition for the development of colorectal cancer. However, the precise etiology of IBD is still unclear. Recently, interleukin (IL-) 33 has emerged as a critical modulator in inflammatory disorders. Even though several studies highlight the IL-33/ST2 pathway as a key factor in colitis, the results remain controversial.

Objectives: In this study we analyzed the role of IL-33 during intestinal inflammation to investigate its potential to act as a new therapeutic target to counteract colitis.

Materials and Methods: Intestinal inflammation was induced by applying dextran sodium sulfate (DSS) in the drinking water into wildtype mice or adoptively transferring hemagglutinin (HA)-specific T cells into transgenic VILLIN-HA mice. To clarify the impact of IL-33 as a potential therapeutic target, we applied recombinant murine IL-33 intraperitoneally during disease progression. To further characterize the immunological functions of IL-33 and ST2, IL-33floxVillinCre mice, which lack the epithelial expression of IL-33, and complete ST2 knockout mice were used. The pathology of the colon was scored and immune cells were analyzed by flow cytometry. Colonic biopsies were obtained and cytokine levels from colonic explants were measured.

Results: We demonstrate that both ST2 deficient mice and mice deficient in epithelial IL-33 expression showed aggravated colon pathologies during DSS-induced colitis compared to wildtypes. To further elucidate the impact of IL-33 we focused on the function of immune cells, which are known to be affected by IL-33. In the DSS-induced as well as in the adoptive T cell transfer colitis model we observed that mice treated with recombinant IL-33 showed reduced intestinal inflammation which was accompanied by less colon pathology and enhanced infiltration of distinct immune cells compared to untreated mice. Additionally, colonic explants showed in both cases less production of pro-inflammatory cytokines after IL-33 treatment in vivo compared to untreated controls.

Conclusion: In summary, our results indicate that IL-33 regulates intestinal inflammation and that the modulation of its signaling pathway represents a promising novel target to counteract inflammatory pathologies.

P116
Use of gnotobiotic mouse models to reveal a microbial impact on intestinal immune tolerance
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Introduction: The risen prevalence of allergic diseases in industrial countries has been attributed to a change in lifestyle factors that affects intestinal microbiota composition. Microbial dysbiosis - particularly early in life - has been

DOI: 10.1002/eji.201970300

associated with atopic diseases later in life. Mice housed under germ-free (GF) conditions also show an impaired intestinal immune system. In particular, GF or antibiotic-treated mice have drastically reduced frequencies of peripherally-induced regulatory T Cells (pTreg) in the gut. Re-colonization of GF mice re-establishes such inducible Treg populations, which are defined by the co-expression of the transcription factors Foxp3 and RORγt. We hypothesize that these cells are key regulators in intestinal immune tolerance. The microbial factors driving this Treg induction remain poorly understood.

Objectives: By using mice with reduced microbial complexities: ASF (Altered Schaedler flora, eight strains), Oligo-MM (Oligo-Mouse Microbiota, twelve strains) and SPF (specific pathogen free, complex microbiota), we aim to decipher which microbial factors and host-microbiome relationships drive this pTreg induction important for intestinal tolerance.

Materials & methods: Murine intestinal samples are compared by using Metatranscriptomics/-genomics and –bolomics approaches to identify microbiota-derived parameters. These results are combined with the analysis of intestinal immune cell characteristics to identify mechanisms of pTreg induction.

Results: First results show that RORγt+ Tregs are reduced in the small intestine of ASF and Oligo-MM mice compared to SPF mice. The abundance of RORγt+ Tregs thereby negatively correlates with microbial complexity. This phenotype is also observable in the spleen indicating a systemic effect of a reduced microbiota on pTreg abundance. By contrast, GATA3+ T Helper 2 cells are increased particularly in the small intestine of ASF and Oligo-MM mice, inversely correlating with microbial complexity and indicating that these mice could be more susceptible to type 2 immune disorders.

Conclusion: Mice with reduced microbial complexities in their intestine show a reduced abundance of pTregs and a spontaneous accumulation of Th2 cells. Thus, reduced bacterial complexities or - microbial dysbiosis more generally - may prone individuals for an increased susceptibility to allergies stressing the importance to identify microbial parameters from these defined consortia impacting on immune cell balance.

P117

Lifelong asymptomatic infections: The role of the microbiota

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Introduction: *Citrobacter rodentium* is an enteric murine pathogen that models human infections with asymptomatic lifelong enteropathogenic *E. coli* (EPEC) infections. It is still largely unknown how some individuals become lifelong asymptomatic carries of EPEC and unknowingly spread the disease, while others can develop an acute, in some cases life-threatening, clinical manifestation. This immunological situation can be studied in germ-free mice, since these animals are unable to eliminate *C. rodentium* and become lifelong asymptomatic carriers of the bacterium.

Material and methods: Bacterial communities essential for the elimination of *C. rodentium* were assessed by analysis of the microbiota composition. 16S rDNA sequencing and MALDI analysis were performed to analyze bacterial population structures. Immunological analysis (FACS, qPCR) were conducted on colonic cells. Intestinal vessels and protein expression were assessed using intravital microscopy and whole mount staining.

Results: The asymptomatic carrier state of germ-free mice can be reversed by conventionalization. Nevertheless, in the absence of a functional immune system, clearance is impaired while survival prevails. Specific bacterial strains isolated from the microbiome of laboratory mice promote cellular migration and angiogenesis, thus boosting a proper immune response.

Conclusions: This finding indicates that without the active involvement of the immune system, the microbiome alone is insufficient to eradicate *C. rodentium* infection. Furthermore, a defined minimal consortium promotes pathogen clearance and immune activation. The results should shed a light on the propagation and defense mechanisms against intestinal infections and are essential for the preventive and therapeutic treatment of these diseases.

P118

Targeting intestinal tight junctions to inhibit onset of arthritis

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DOI: 10.1002/eji.201970300

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The gut-joint axis has drawn intense attention in the past decade. There is growing evidence that dysbiosis of the gut microbiota is associated with the development of autoimmune disease. However, how microbial dysbiosis can impact the transition from asymptomatic autoimmunity to inflammatory disease is mainly unknown. Here, we identified the intestinal barrier integrity as a key factor for this transition. We found that impaired intestinal barrier function precedes the onset of arthritis in collagen-induced arthritis (CIA) mouse model and RA patients. Zonulin as a main regulator of intestinal tight junctions, was elevated in in CIA mice and RA patients before the onset of clinical symptoms. Furthermore, increased zonulin levels reduced the expression of intestinal tight junctions proteins, induced T cells mediated mucosal inflammation, changed gut microbiota and controlled the transmigration of immune cells from the gut into the joints. Therapeutic restoration of the intestinal barrier in the pre-phase of arthritis using butyrate or CB1R agonist inhibited the development of arthritis. Moreover, treatment with the zonulin antagonist Larazotide acetate, which specifically increases intestinal barrier integrity, effectively reduced arthritis onset. In summary, these data support a gut-joint axis in RA, which is based on zonulin-mediated impairment of intestinal barrier function and which is specifically drugable by the zonulin antagonist larazotide.

P119

AhR-dependent non-genomic signaling in macrophages - A multi-PTM-omics and proteomics study

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Introduction: The Aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor, which is activated by exogenous chemicals but also endogenous ligands like kynurenine and metabolites produces by the intestinal microbiota. Besides its function as transcription factor, AhR participates in non-genomic signaling as a part of a cullin 4B ubiquitin ligase complex. The tyrosine-protein kinase Src (c-SRC) is released from the cytoplasmic AhR-complex upon ligand binding in an active form. So far, the contribution of non-genomic AhR-signaling to the receptors immunomodulatory properties is scarcely investigated.

Objectives: This study aims to elucidate the contribution of non-genomic AhR-signaling to the receptors immunomodulatory properties in macrophages. By means of ubiquitomics and phosphoproteomics potential mechanisms governing macrophage activation and function shall be identified.

Material and Methods: Human monocyte derived macrophages or differentiated THP1-cells were activated with LPS in the absence and presence of endogenous (FICZ) and exogenous (BaP) AhR ligands. The proteome, ubiquitome, and phosphoproteome were comparatively analyzed by LC-MS/MS utilizing a label free quantification approach.

Results: Quantitative information for more than 5500 proteins, 5900 ubiquitination sites and 10000 phosphorylation sites were obtained. The integration of proteome and ubiquitome data revealed a non-degradative ubiquitination of the small GTPase Rac1 which is increased in AhR-ligand treated macrophages. This ubiquitination might impair signaling after activation of Toll-like receptors 1, 2 and 6. Furthermore, the levels of IFIT2 and ADAM17 are decreased in AhR-ligand treated cells after LPS stimulation relative to levels in macrophages treated with LPS alone. Interestingly IRF3 phosphorylation was found decreased in AhR-ligand treated cells, too. These events likely contribute to the reduced inflammatory response i.e. impaired TNF-a release previously described.

Conclusions: Taken together, our data indicate non-genomic effects of activated AhR potentially contributing to the regulation of immune responses. In particular, the receptors role in intestinal homeostasis and immunity might– at least partially – depend on these signaling events.

P120

The impact of nasal carriage with *Staphylococcus aureus* and its enterotoxin B on allergic asthma

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DOI: 10.1002/eji.201970300

Introduction: Allergic asthma is a particularly heterogeneous chronic inflammatory condition of the airways that affects an increasing number of patients. Correlations between colonization with *Staphylococcus aureus* (*S. aureus*) and the development of allergic asthma have been recognized but remain incompletely understood. Interestingly, a substantial number of patients with severe asthma not reacting to common allergens show IgE specific for *S. aureus* enterotoxins. Based on this and other findings, *S. aureus* enterotoxin B (SEB) has been proposed to display a central player in the interplay between *S. aureus* carriage and allergic asthma.

Objectives: We are seeking further mechanistic insights into the effects of nasal carriage with *S. aureus* and of SEB on the development and the phenotype of allergic asthma.

Materials: patients & methods: We are employing a mouse model (C57Bl/6) for allergic asthma based on a local antigen challenge following sensitization with the model antigen ovalbumin (OVA). Here, we are analyzing the effects of intranasal treatment with SEB. We are assessing the recruitment of immune cells to the respiratory tract and the production of cytokines and IgE. Furthermore, we are recruiting patients with allergic asthma to determine nasal *S. aureus* carriage as well as nasal and systemic IgE.

Results: Naïve mice not subjected to allergic sensitization or challenge react to intranasal treatment with SEB in a dose-dependent manner. This reaction is mainly comprised of the recruitment of immune cells to the respiratory tract. Interestingly, the recruited cells include eosinophils, which are typically also increased in allergic airway inflammation. Following sensitization and challenge with OVA, mice show immune cell infiltration and Th-2 cytokine production in the respiratory tract as well as presence of specific IgE. Effects of SEB on this allergic reaction are currently analyzed. Furthermore, patient recruitment and analysis of samples is ongoing.

Conclusions: In a mouse model, *S. aureus* SEB alone leads to airway inflammation and thereby most likely will affect local allergic reactions. The detailed analysis of these effects and the determination of *S. aureus* nasal carriage and IgE production in patients with allergic asthma will significantly increase our mechanistic understanding of the correlations between *S. aureus* carriage, SEB-specific Ig-responses and allergic asthma.

P121

Intestinal development and homeostasis require activation and apoptosis of diet-reactive T cells

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Introduction: Immunological tolerance to intestinal bacteria and food antigens is a multilayered process, and its breakdown leads to hyperactivation of mucosal T and B cells and subsequent development of intestinal pathologies. Successful dietary intervention therapies in patients suffering from celiac disease, food allergies, and inflammatory bowel disease (IBD) demonstrate that diet contains immunologically relevant antigens. Yet, our understanding of diet-specific immune reactivity is largely derived from T cell receptor (TCR) transgenic mice and model antigens where tolerance mechanisms comprising clonal anergy, deletion, and induction of regulatory T cells (Tregs) have been shown

Objectives: Here, we explored the impact of physiological dietary antigens on the phenotype and fate of intestinal CD4+ T cells in man and animals.

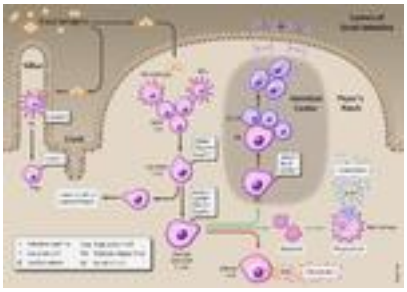
Patients and methods: Cells from PP biopsies of patients with Crohn´s disease and healthy controls were analyzed for the expression of CD4, GL7, annexin V, Helios, Foxp3, by flow cytometry. For animal experiments, germfree (GF) and conventional (SPF) mice were used. Animals were kept on either conventional chow or on food antigen–free diet (protein-free, amino-acid chow) for up to 5 generations. Organs were analyzed histologically, and CD4+ T cells were phenotypically analyzed for markers of activation, tolerance and apoptosis.

Results: Physiological uptake of food-proteins leads to a highly activated CD4+ T cell population in Peyer patches. These cells are distinct from regulatory T cells and develop independently of the microbiota. Alimentation with an antigen-free diet led to an atrophic small intestine with low numbers of T cells, including Tfh cells and decreased amounts of intestinal IgA and IL-10. Food-activated CD44+Helios+CD4+ T cells are controlled by PD-1. Blocking the PD-1 pathway rescued these T cells from apoptosis and triggers proinflammatory cytokine production, which in IL-10–deficient mice was associated with intestinal inflammation. In accordance, patients with Crohn"s disease revealed significantly reduced frequencies of apoptotic CD4+ T cells in Peyer patches as compared with healthy controls.

DOI: 10.1002/eji.201970300

Conclusion: Activation and subsequent apoptosis of food-reactive T cells is required for normal intestinal development and function.

Figure 1



P122

Orchestration of systemic anti-fungal Th17 immunity and immune pathology by a single member of the mycobiome

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Introduction: Th17 cells protect against bacteria and fungi, but also contribute to chronic inflammation. In humans, Th17 responses are particularly important against *Candida albicans,* a mucocutaneous fungal pathobiont. In contrast, the role of Th17 cells for other pathogenic fungal species is unclear. Pulmonary fungus-related disorders and sensitizations are often associated with chronic respiratory diseases such as asthma, COPD and cystic fibrosis. Also these patients show increased Th17 cytokine levels, which correlate with disease severity. However, the capacity of most fungal species to induce human Th17 responses and their potential contribution to pulmonary diseases is currently unclear.

Methods: We used antigen-reactive T cell enrichment (ARTE) for the *ex vivo* analysis of human T helper cell responses against 30 common human pathogenic fungal species.

Results: We show that *C. albicans* is the sole direct fungal inducer of Th17 cells in humans. For all other fungi tested, minor and variable fractions of Th17 cells were detected. Surprisingly, these Th17 cells, but not Th1 cells against the same fungal species, were strongly and selectively cross-reactive against *C. albicans*. Patients with pulmonary inflammation displayed elevated frequencies of cross-reactive *A.fumigatus* Th17 cells, suggesting their specific contribution to lung pathology. In particular in patients with allergic sensitization to *A.fumigatus,* increased Th17 responses strongly correlated with acute ABPA.

Conclusions: Our data identify *C. albicans* as the major fungal inducer of human Th17 responses. We provide a unique example how protective Th17 immunity may simultaneously promote immune pathology when deviated to different target antigens and tissues via heterologous immunity.

DOI: 10.1002/eji.201970300

P123
Mucosal inflammation in XIAP-deficient mice
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Inflammatory bowel disease (IBD) have become a research focus in recent years, due to its increasing prevalence worldwide. Usually, the onset of IBD occurs in young adults and is caused by a multitude of different environmental and genetic factors. However, specific subtypes of IBD, such as the early-onset-IBD (VEO-IBD) occur in pediatric patients and are due to monogenic mutations. One such example is the X-linked lymphoproliferative syndrome type 2 (XLP-2) can be traced back to mutations in a member of the inhibitor of apoptosis protein (IAP) family, X-linked IAP (XIAP). Hematopoietic stem cell transplantation is thus far the only long-term cure available.

Modelling XLP-2 in vivo, we observe in XIAP-deficient mice a mild inflammation in the small intestine, in the terminal ileum. Interestingly, we observe that both the epithelial cell as well as the intestinal immune cells, resident and infiltrating, seem to contribute to the pathogenesis. An interplay of TNF-receptor signaling appears to be the major player involved, in particular with regard to immune cell function and their expression of TNFR2. Furthermore, alterations in commensal bacteria and their metabolites could be detected, potentially contributing to inflammation.

Therefore, it is of highest interest to pinpoint the specific immune cell type mediating the detrimental effects of deregulated immune reactions in XLP-2 and, on a molecular level, which interaction partners of XIAP could be responsible. It is our overall aim, via deciphering the specific role of XIAP in this monogenic early-onset IBD, to contribute to the advances in the field of chronic inflammation in mucosal immunity.

P124
Influence of saccharin on T-cell-mediated contact hypersensitivity in mice
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Introduction: There are many reports showing that gut microbiota modulates various aspects of immune response. Our previous work showed that oral treatment with a broad spectrum antibiotic enrofloxacin prior to hapten sensitization inhibits contact sensitivity (CHS) in mice. Lately, it was shown that artificial sweeteners alter the gut microbiota and induce glucose intolerance in humans and mice.

Objectives: The aim of this study was to determine the influence of saccharin on CHS in mice.

Materials & Methods: Adult BALB/c and C57BL/6 (B6) mice received saccharin in drinking water for eleven weeks prior to CHS induction. BALB/c and B6 mouse strains have been extensively used to study the mechanism of CHS responses. BALB/c mice develop a Th1- whereas, B6 mice Tc1-mediated CHS. To induce CHS, mice were contact sensitized with 5% trinitrophenyl chloride (TNP-Cl) on the shaved skin. Four days later, CHS response was elicited by painting both ears with 0.4% TNP-Cl. Ear thickness was measured before the challenge and then 24h after the challenge using a micrometer. The ear weight and myeloperoxidase (MPO) activity in the ear tissue were tested 24h after the challenge.

Results: BALB/c female mice received saccharin for 11 weeks developed less severe CHS response when compared to control mice receiving water alone. On the contrary B6 female mice received saccharin for 11 weeks developed aggravated CHS response when compared to control mice receiving water only. In both mouse strains CHS reaction in males remained unchanged.

Conclusion: These results suggest that the saccharin influence CHS in mice and this phenomenon is determined by gender.

This work was supported by grant K/DSC/005354 to PK.

DOI: 10.1002/eji.201970300

P125

Multi-omics profiling of MAIT cells reveals specific patterns of antigen-dependent and -independent activation

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Introduction: Mucosal-associated T (MAIT) cells are specialized innate-like T cells involved in anti-bacterial but also anti-viral immunity. They are unique for their ability to recognize bacterial metabolites, inducing an antigen(ag)-dependent activation. Moreover, MAIT cells can also be activated in ag-independent manner. However, molecular mechanisms of MAIT cell activation are not completely understood.

Objectives: To better understand MAIT cell biology, we aimed to define the activation of MAIT cells on the molecular level using global transcriptomics and proteomics.

Materials & Methods: Human peripheral blood MAIT cells were isolated by positive magnetic separation, and co-cultured with *E.coli*-loaded monocytes at a ratio of 1:1 for 16h. MAIT cells were FACS-sorted and lysed for RNA isolation. Paired-end RNA-Seq was conducted using Illumina technology. For global proteomics, separated MAIT cells were stimulated with IL-12/IL-18, anti-CD3/CD28 antibodies or both, lysed for protein extraction, followed by tandem mass spectrometry.

Results: Transcriptomic analysis of activated MAIT cells revealed 86298 differentially expressed transcripts, including 5476 protein-coding genes, 279 lncRNAs, and 8992 newly-assembled transcripts after activation. Ingenuity Pathway Analysis (IPA) of protein-coding genes showed an upregulation of the Th17 and Th1 activation pathway, granzyme B-induced cytotoxicity of target cells and TREM-1 signaling, while the PPAR and the LXR/RXR activation pathways were down regulated.

To further distinguish between ag-dependent and ag-independent activation, MAIT cells we stimulated with IL12/IL18, anti-CD3/CD28 or both, and analyzed by global proteomics. Only a combination of both lead to full activation of MAIT cells, comparable to activation by *E.coli*. IPA showed enrichment of granzyme B-induced cytotoxicity of target cells, Th1 activation pathway and EIF2 signaling as the most significant upregulated pathways. Stimulation of T-cell receptor or by cytokines alone lead to a comparable degree of activation, although pathways related to key effector function i.e. cytotoxicity were stronger induced by a-CD3/CD28 compared to IL-12/IL-18 stimulation.

Conclusion: Taken together, here we present a deep multi-omics profiling of MAIT cell activation, which reveals not only information on relevant signaling pathways, but gives also expression pattern of non-coding RNAs, raising the question about their relevance in this emerging type of innate-like T cells.

P126

Analysing the expression of the murine polymeric immunoglobulin receptor (pIgR) under homeostatic and immune modulating conditions in the respiratory tract

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Introduction: The transfer of secretory immunoglobulin A and M (sIgA, sIgM) through the epithelial cell barrier into the mucosal lumen by the polymeric immunoglobulin receptor (pIgR) is an essential mechanism of mucosal host defence in the respiratory tract. The identification of immunomodulating agents/stimuli that enhance epithelial *Pigr* gene expression might have therapeutic implications with regard to an improved immune exclusion – and thus an augmented respiratory mucosal immunity.

Objectives: The aim of this project is to assess *Pigr* gene expression under homeostatic and immunomodulating conditions in distinctive areas of the upper and lower respiratory tract.

DOI: 10.1002/eji.201970300

Materials & methods: *Pigr* gene expression in the lung, trachea and nasal-associated lymphoid tissue (NALT) of naïve, LPS-treated, IFN-γ-treated, IL-4-treated, germ-free, specific-pathogen-free mice and mice with an undefined microbiome was determined by quantitative real-time PCR.

Results: *Pigr* gene expression gradually decreases from the upper to the lower respiratory tract. LPS-treatment caused increased *Pigr* gene expression in the lung. IFN-γ- and IL-4-treatment led to decreased *Pigr* gene expression in the trachea while lung and NALT showed no alterations. Germ-free mice displayed no significant differences in the *Pigr* gene expression pattern compared to conventional specific-pathogen-free mice, while mice with an undefined microbiome exhibited a decreased *Pigr* gene expression in the NALT.

Conclusions Basal *Pigr* gene expression is site-specific and can be partly modulated by exposition to microbial ligands and proinflammatory stimuli. Inherent differences in the basal *Pigr* gene expression are most probably depending on microbial colonization as well as on yet unknown intrinsic effects.

P127

Interplay between the gut microbiota, immunity and colon cancer

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Introduction: Colorectal cancer has a high incidence in humans worldwide and represents a major cause of mortality. Thus, improved therapeutic approaches are required to reduce the global burden. The gut mucosal homeostasis depends on a sensitive balance between the composition of the microbiota and the host immune response. There is increasing evidence that abnormal immune responses against the enteric microbiota appear to have an essential influence on the development and the emergence of a tumor-supporting microenvironment.

Objective: In this study, we explore the effect of microbial depletion in combination with immunotherapy on the tumorigenesis of colon cancer.

Materials and Methods: Colon cancer was induced in BALB/c mice using the AOM/DSS mouse model or by subcutaneous injection of CT26 colon cancer cells. In both experimental setups mice were treated either with antibiotics in the drinking water, immune checkpoint inhibitor PD-L1 or a combination of both. Stool samples were collected and microbiome analyses were performed using 16S rRNA high-throughput sequencing datasets. In addition, tumor development was monitored and the immunoprofile was determined by flow cytometry and Luminex-technology.

Results: Colon cancer development was affected by antibiotic treatment as well as PD-L1 antibody therapy in both, the AOM/ DSS model where tumor development is dependent on inflammation, as well as the CT26 model where tumor cells are transplanted into mice. As shown by 16S rRNA high-throughput sequencing, antibiotic therapy strongly modulated the bacterial composition. However, when combining both treatments this protective effect was completely abolished. Neither changes in the immune cell composition, nor cytotoxic activity of the antibiotic cocktail on the CT26 tumor cells accounted for the reduced tumor burden.

Conclusions: Our results prove that changes in the microbial composition can influence tumor progression of colon cancer not only locally but also in a systemic manner. Interestingly, we identified that the gut microbiota interferes with the success of immunotherapy. Although the mechanisms still remains to be elucidated in more detail, these finding clearly provide new evidence for the reciprocal impact between the microbiota, tumor cells and the host immune system and offers new insights into this complex relationship.

P129

αvβ8-expression by Batf3-dependent dendritic cells facilitates IgA responses to Rotavirus

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Rotavirus (RV) is one of the leading causes of life-threatening diarrheal diseases among young children. RV-specific intestinal IgA protects from reinfection and is thus the principal effector for long-term immunity. However, very little is known about the mechanisms leading to IgA induction towards viruses at the intestinal site. Intestinal classical

DOI: 10.1002/eji.201970300

Dendritic Cells (cDCs) were shown to facilitate both T cell-dependent and -independent secretory IgA. Whether cDCs can provoke intestinal anti-RV antibody responses in response to RV infection is unclear.

Here, we show that Batf3-dependent cDC1 DCs, but not cDC2 DCs, are critical for the optimal induction of RV-specific IgA responses in the mesenteric lymph nodes (mLNs). Batf3-deficient mice shed RV longer than littermate controls. This correlated with fewer RV-specific CD8+ T cells and a lower fecal RV-specific IgA titer. Compared to littermate controls, Batf3-deficient mice had significantly fewer RV-specific IgA+ B cells in mLNs while total B cell numbers were not affected. The bulk of the RV-specific antibody response was T cell dependent. Sensing of type I interferon on DCs played a redundant role for the induction of normal IgA responses. Optimal RV-specific IgA induction however depended on the selective expression of the TGFβ-activating integrin αvβ8 on cDC1 DCs, while presence of αvβ8 on cDC1 DCs was dispensible for steady-state immune homeostasis.

Together, our results show an essential, TGFβ-dependent role for cDC1 DCs in the generation of the RV-specific IgA response. Given that cDC2 DCs are crucial in driving IgA during steady-state but do not contribute to RV specific IgA responses, we propose that the capacity of DC subsets to induce IgA at the intestinal wall reflects the nature of the stimulating trigger as opposed to an intrinsic capability of a particular DC subset.

P130

The role of IRF-1 during pathogen-induced intestinal Inflammation

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Introduction: IRF-1 is a transcriptional regulator that is mainly activated via IFNγ-STAT1-signaling. IRF-1 activation in immune cells is crucial for anti-microbial defense, predominantly against intracellular pathogens. However, it remains elusive how IRF-1 contributes to mucosal immunity against pathogens located in the intestinal lumen or on mucosal surfaces. Oral infection of mice with the mouse pathogen *Citrobacter rodentium* (CR) is a widely used model for human gastrointestinal infections and inflammatory bowel disease since CR adhering to the colonic epithelial layer induces a characteristic pathology including A/E lesions.

Objectives: We want to elucidate the cell type-specific role of IRF-1 on mucosal immune responses during CR-induced intestinal inflammation.

Materials & methods: To analyze the impact of IRF-1 on mucosal immunity, we conducted CR infection experiments with wildtype (wt) and IRF-1$^{-/-}$ mice and compared disease outcome and bacterial burden. Colonic lymphocytes were analyzed by flow cytometry and in cell culture. Sections of distal colon were histologically scored and stained by immunohistochemistry (IHC). To verify the role of IRF-1 in hematopoietic and non-hematopoietic cells, bone marrow chimeric mice were generated. To confirm the phenotype, IFNγ- and STAT1-deficient mice were infected with CR.

Results: Infection experiments revealed a massive impairment of the antibacterial defense in IRF-1$^{-/-}$ mice indicated by an elevated pathogen burden, increasing weight loss and death of the IRF-1$^{-/-}$ animals around ten days post infection presumably due to systemic bacterial spread. More prominent histological changes in the distal colon were noticed. Flow cytometry of colonic lymphocytes revealed substantial changes in the frequencies and activation stages of immune cell subsets including ILC3s, B and T cells. IHC stainings of colonic cross sections indicated changes in the expression pattern of tight junction proteins. Experiments with bone marrow chimeric mice revealed a direct impact of IRF-1 on hematopoietic cells which in turn influence the non-hematopoietic compartment including the intestinal epithelium. Infection studies with IFNγ$^{-/-}$ and STAT1$^{-/-}$ mice confirmed that the observed phenotype is exclusively IRF-1-dependent.

Conclusion: IRF-1 is required to mount an efficient mucosal immune response against CR through the direct regulation of hematopoietic cells, which have an impact on intestinal epithelial cells.

P131

ROLE OF HUMAN MUCOSAL-ASSOCIATED INVARIANT T (MAIT) CELLS IN AGE-ASSOCIATED *CLOSTRIDIUM DIFFICILE* INFECTIONS

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DOI: 10.1002/eji.201970300

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Introduction: *Clostridium difficile* infection (CDI) can cause life-threating inflammatory responses in the intestinal mucosa in elderly individuals. Their susceptibility is widely undefined but likely involves age-dependent immune responses towards hypervirulent, toxin-expressing strains. Mucosal-associated invariant T (MAIT) cells innately can respond to MR1-presented bacterial antigens of the riboflavin pathway and we recently reported that clinical *C. difficile* isolates activate MAIT cells in healthy individuals. Surprisingly, hypervirulent strains showed moderate riboflavin synthesis but induced superior immune responses indicating the presence of undefined co-stimulatory pathways.

Objective: This project shall complement knowledge of how major virulence factors (toxins) from hypervirulent *C. difficile* affect MAIT cell responses and whether their phenotype changes age-dependently.

Methods: Peripheral blood mononuclear cells (PBMCs) of young and elderly individuals were stimulated with riboflavin-synthesizing *C. difficile* isolates. MAIT cell responses dependent from MR1, IL-12 and IL-18 secreted by APCs and *C. difficile* toxins A, B and transferase (CDT) were studied by flow cytometry. Proteomics was then applied to complement information on the age-dependent molecular phenotype and quantified 5839 MAIT cell proteins in eight individuals.

Results: *C. difficile* isolates induced IL-12 and IL-18 secretion by antigen presenting cells as well as MR1-dependent MAIT cell responses (CD69, IFNγ, Granzyme B, Perforin) equally in elderly and younger individuals. Whereas isolate-specific riboflavin levels seemed not to determine the strength of immune responses, *C. difficile* toxin A and CDT could promote MAIT cell activations. Notably, proteomics of age-matched donors revealed 29 differentially abundant proteins in elderly individuals including CD26.

Conclusion: We provide first evidence for age- and toxin-dependent phenotypes of human MAIT cells and will hypothesize a model for their synergistic role in age-associated CDI.

P132
Optimal neonatal humoral responses to Rotavirus require both cDC1 DCs and T cells
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Rotavirus (RV) is an intestinal pathogen that primarily affects infants and young children. Available vaccines show variable efficacy in developing countries. Dendritic cells (DCs) are crucial for the initiation of immune responses and therefore formidable targets for vaccines. The two major DC subsets, called cDC1 DCs and cDC2 DCs, differ in their capacity of inducing specific adaptive immunity in adults, but their distribution and potentially differential roles in neonates are largely unknown. After confirming that steady state neonates indeed harbored considerable numbers of both cDC1 and cDC2 DCs, we set out to address which DC subsets could influence humoral responses at the intestinal barrier in the context of neonatal RV infection.

Using 5 days old suckling mice from BATF3 homozygous/heterozygous knockout cross-bred litters, we analyzed the role of cDC1 DCs in the initiation of B cell responses during neonatal RV infection. In accordance with our previous findings revealing a relative deficiency in RV-specific IgA+ plasma cells in adult cDC1-deficient mice (unpublished), cDC1-deficient neonates also presented much fewer plasma cells in the mesenteric lymph nodes at day 7 post infection. Strikingly, the effect was much more pronounced in these suckling mice, affecting the total plasma cell pool. In contrast, the RV-induced increase of plasma cells in suckling mice lacking intestinal cDC2 DCs was normal.

We are currently analyzing the nature of RV-induced B cells in neonatal mice. Analysis of TCRa-deficient suckling mice suggests that, like in adults, most of the B cell response depends on T cell help. We are currently investigating whether cDC1 DCs act through T cells for the initiation of the humoral immune response in our setting. We further aim to better understand the requirements for neonatal DC activation: Our current data shows that type I IFN sensing by these DCs is redundant for their function.

DOI: 10.1002/eji.201970300

Taken together, optimal RV-specific B cell responses across ages depend on T cells and cDC1 DCs, but not cDC2 DCs.

P133

***Helicobacter hepaticus* as disease driver in a novel CD40-mediated spontaneous colitis-model**

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The mammalian gastrointestinal tract is shaped by a huge and complex microbiota. Dendritic cells (DCs) in the intestine are constantly exposed to food- and commensal-derived antigens that must be tolerated, while invading pathogens must be eliminated. Therefore, a tight regulation of intestinal tolerogenic and immunogenic DCs is essential to sustain mucosal homeostasis.

Disturbed balances can result in severe inflammatory disorders like Inflammatory Bowel Disease. To investigate the role of the CD40L-CD40 axis in tolerance vs. immunity and the role of DCs therein, we generated a murine model with constitutive CD40-signaling in DCs. CD40-signaling leads to migration of $CD103^+$ DCs from the colonic lamina propria to draining lymph nodes, followed by DC-apoptosis. This loss of $CD103^+$ DCs caused lack of $ROR\gamma t^+Helios^-$ induced regulatory T cells and increase of Th1/Th17 effector cells in the colon, resulting in breakdown of mucosal tolerance and fatal colitis.

We used sera from these mice to isolate fecal antigens recognized by mice with colitis, but not control mice and studied changes of the microbiota during disease development. We detected *Helicobacter hepaticus (Hh)*-specific antibodies in transgenic mice and could protect them from early disease onset by rendering them *Hh*-free. Upon *Hh*-reinfection of transgenic mice, rapid disease onset with increase of Th1/Th17 effector cells in the colon was observed. Our data suggest that *Hh* is the disease driver in a CD40-mediated spontaneous colitis-model, allowing us to study its modulation of the host immune response with impact on disease onset, progression and outcome.

Natural killer and innate lymphoid cells (P135-P159)

P135

Innate Lymphoid Cells in the female genital tract and their role in chlamydial infection

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Chlamydia trachomatis is the most common bacterial agent of sexually transmitted disease. In women, *C. trachomatis*-infection can cause pelvic inflammatory disease, which may result in scarring and thereby infertility. A mouse model of human *C. trachomatis* infection has been established, where infection with *C. muridarum* in female mice causes comparable symptoms and sequelae. Previous *in vivo* studies have found that the immune response to primary *C. muridarum*-infection involves the infiltration of neutrophils and monocytes starting 1 to 2 days post-infection (dpi). After about 7 dpi, T cells accumulate at the site of infection.

Innate Lymphoid Cells (ILCs) play an important role in tissue defense, repair and homeostasis. They are present in epithelial tissues but little is known about their distribution in the female genital tract and their potential role in genital infections. To better understand the role of ILCs in genital tract infections - with respect to immune response, bacterial clearance and tissue damage - we infected mice with *C. muridarum.* Genital tracts of naïve and infected mice were analyzed for the distribution of the ILC subsets during infection and cytokine production in response to the pathogen. The results show that in naïve wt mice cNK cells represent the most prominent cell population within the ILCs in the genital tract. ILC1 and ILC2 cells are also detectable although their total cell numbers in naïve wt mice are much lower than the cNK cell number. ILC3s are barely detectable and may not play an important role during chlamydial infection. Four days post infection the total cNK cell number increases 5.5-fold indicating that they might play a role at early stages of chlamydial infection. At later stages the numbers of cNK cells decrease and reach baseline levels at 14 dpi. Interestingly, at later time points of infection the total number of cNK cells in the genital tract increases again. ILC1 numbers remain stable initially. Intriguingly, until 30 dpi the total cell number of ILC1 cell increases about 10-fold. The number of ILC2 cells does not vary significantly during chlamydial infection at any time. These results identify

DOI: 10.1002/eji.201970300

composition of ILCs in the murine genital tract and a response to chlamydial infection. They suggest that ILCs may partake in anti-bacterial defence and tissue damage, possibly through interaction with myeloid cells.

P136

Identification and functional characterization of epigenetic signature genes in innate lymphoid cell lineages

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Introduction: Epigenetic modifications such as DNA methylation play an essential role for imprinting specific transcriptional patterns in cells. In $CD4^+$ T helper (Th) cells, specific demethylated regions have been identified that are critical for the identity, function and lineage stability of the different Th subsets. Considering the striking similarities between Th cell lineages and innate lymphoid cell (ILC) populations, regarding the dependency on specific transcription factors and expression of signature cytokines, we hypothesized here that also ILC populations can be functionally characterized by unique modifications in their genomic DNA methylation patterns. **Objectives:** In this project we aim to identify epigenetic markers for ILC subpopulations. These markers will be characterized under homeostatic and inflammatory conditions and used to study the functional role of the associated genes.

Materials & Methods: ILC were sorted from mice by both surface markers and transcription factors. Single-stranded DNA libraries were generated for next generation sequencing after bisulfite conversion. Methylomes of ILC 1, 2, 3, LTi and NK cells were established and subsequent usage of the software *metilene* enabled us to identify differentially methylated regions (DMRs) among the lineages. To correlate the methylomes with the transcriptome of the ILC populations, we also conducted RNA-sequencing. To assess the impact of inflammation on the epigenetic status, ILC were sorted from liver of IL-33/ConA treated mice or the gut of mice infected with *C. rodentium* and further analyzed by bisulfite sequencing.

Results: Our epigenetic analysis revealed a closer relationship between ILC1 and NK cells, as well as between ILC3 and LTi, whereas ILC2 appear to be more distinct. 52 DMRs exhibiting substantial methylation difference between ILC subsets were selected as candidates for specific epigenetic markers. Combination of methylome and transcriptome data detected strong correlations between the methylation status of marker regions and the expression level of the associated genes, indicating their relevance for ILC biology. Importantly, initial data suggest that the epigenome of ILC is critically shaped by inflammatory reactions.

Conclusion: The discovery of ILC signatures will enable us to characterize ILC in different locations or under inflammatory conditions *in vivo* as well as to identify molecular mechanisms which control the differentiation and function of ILC.

P140

Ontogeny of innate lymphoid cells in the human intestine

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Introduction: Gastrointestinal diseases are frequent in children, which have a higher susceptibility to infections as well as to immune-mediated diseases such as food allergies. We have previously shown that immune cell populations including NK cells undergo sizeable changes in the first year of life, however, little is known regarding other innate lymphocytes (ILCs) such as ILC2s. ILC2s play a role in tissue repair as well as enhanced type 2 responses in allergies.

Objectives: In this study we aimed to characterize the ontogeny of intestinal ILCs focusing on ILC2s.

DOI: 10.1002/eji.201970300

Methods: Human intestinal tissues were collected from patients who underwent surgeries to correct gastrointestinal congenital abnormalities, reconstruction of ileostomy or tumor resection. Lymphocytes from human infant and adult small intestines were isolated, both from the epithelium and the lamina propria. ILC populations were analyzed using 15-parameter flow cytometry (CD16, CD3, CD56, CD69, c-Kit, CD161, CD103, CRTH2, CD14, CD19, BDCA2, CD1a, CD123, CD34, CD127, NKp44, Live/Dead, CD45).

Results : Innate lymphoid cells were defined as viable Lineage (Lin)$^-$CD127$^+$CD161$^+$ lymphocytes. ILCs were detected in epithelial layers as well as in lamina propria layers of infant intestines, with the latter containing the highest numbers of ILCs. ILCs in general were most numerous early in life and decreased over age. Specifically intestinal ILC2 populations (Lin$^-$CD45$^+$CD127$^+$CD161$^+$CRTH2$^+$) changed rapidly in the first year of life, with large numbers present in infant intestines at birth and afterwards promptly decreasing. Infant intestinal ILC2s highly expressed CD69 and moderately expressed CD103, two intestinal tissue-residency markers. Furthermore intestinal ILC1s (defined as Lin$^-$CD45$^+$CD127$^+$CD161$^+$CRTH2$^-$c-Kit$^-$) and ILC3s (defined as Lin$^-$CD45$^+$CD127$^+$CD161$^+$CRTH2$^-$c-Kit$^+$) populations also decreased with age, but were still detected in adult intestines.

Conclusion: Populations of innate lymphoid cells in the intestine undergo rapid changes in the first year of life with high numbers of ILC2s at birth that subsequently decrease during infancy. This dynamic changes in innate lymphoid cell populations might be relevant in development of tolerance, tissue (re)modelling as well as host defense and are to be further investigated.

P144

Evaluation of Natural Killer cell cytotoxicity against medulloblastoma in vitro and in vivo.

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Question: Medulloblastoma is the most common malignant brain tumor in the pediatric population. Although conventional treatments improve survival, the chances of recovery remain low and result in severe neurological sequelae, especially in young children. CD3-CD56 + Natural Killer cells are able to recognize and kill tumor cells, particularly during the loss of class I MHC molecules. Their cytotoxic potential has already been demonstrated in vitro and in vivo with certain brain tumors but few studies concern medulloblastoma. Our aim was to evaluate the cytotoxic effect of allogeneic NK cells on medulloblastoma cells.

Methods: Three lines of medulloblastoma subgroups (DAOY, D283 and D341) were analyzed and compared to the K562 leukemia cell line, known to be very sensitive to NK cells. The NK cells were selected from blood of healthy donors by immunomagnetic selection, and amplified in the presence of IL-15. After expansion, their cytotoxic potential was evaluated by different tests targeting each step involved in the process of tumor lysis: the phenotype of tumor cells by flow cytometry (expression of B7 molecules, CD56, 73, PVR and MICA/B), conjugation by flow cytometry and confocal microscopy, the degranulation by granzyme B elispot and finally the death of the target cells, by necrosis, late apoptosis and cell lysis (by flow cytometry and adenylate kinase measurement). The impact of co-culture with tumor cells on the expression of inhibitory or activating receptors was also analyzed. Nk cells were next injected in medulloblastoma xenografted nude mice and the survival was followed.

Results: 3 medulloblastoma lines expressed CD56, PVR and CD105. The expression was heterogeneous for B7H1, H3 and H4, CD73, and MICA/B. The 3 lines were able to conjugate with the NK cells and to induce their degranulation. A necrosis percentage of between 30% (D283 and D341) and 38% (DAOY) could be measured after 4 hours of incubation. Percentages of cell lysis between 32 and 77% could be detected for the DAOY and D341 lines respectively. After co-culture, an increase in expression of the activating receptors NKG2D and DNAM1 could be observed while the expression of the inhibitory receptors KIR2DL1 and 2DL5 remained stable. These results suggested that NK cells were able to exert effective cytotoxicity against medulloblastoma. The in vivo survival study of medulloblastoma xenografted nude mice showed an increased survival of the NK cell treated group.

P145

Anti-TNFα/azathioprine therapy modulates the Natural killer cell compartment in pediatric inflammatory bowel disease

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DOI: 10.1002/eji.201970300

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Introduction: Natural killer [NK] cell subsets have recently been found to play an important role in inflammatory bowel diseases [IBD]. We studied NK cell subsets before and after initiation of an anti-TNFαtherapy under co-medication with azathioprine [AZA] in pediatric IBD.

Materials and Methods: The study was approved by the local ethic committee (ethics protocol #347_15B) and written content was obtained. A total of n=25 pediatric IBD patients (Crohn"s disease, CD/ulcerative colitis, UC) and 12 healthy controls (HC) were recruited. Blood samples and intestinal biopsies were collected before initiation of the anti-TNFα therapy and in the longitudinal course with a follow up of 12 months. Flow Cytometry (CD3, CD8, CD16, CD56, CD62L, CCR9,b7 integrin), immunofluorescence staining (CD16, CD56), and NanoString (NanoString Technologies, Seattle, WA, USA) technique was performed. The data were compared to the clinical data derived from the medical files.

Results: In blood samples analysed before initiation of anti-TNFα therapy in patients already receiving AZA, flow cytometry revealed decreased numbers of peripheral CD56brightCD16+[CD, p=0.002/UC, p<0.0001], CD56dimCD16+[CD, p=0.049/UC, p=0.027] and γδ T-cells [CD, p=0.42/UC=0.024] in patients with IBD compared to HC. Double positive CD56+CD16+cells were reduced in intestinal biopsies treated with AZA [CD, p=0.0152/UC, p<0.0001]. In colon specimen collected before initiating anti-TNFα therapy, NanoString analysis revealed an azathioprine dependent mRNA upregulation of *IDO-1 (7*-fold for CD and 4-fold for UC)*, CD274 (*4-fold for CD and 6-fold for UC), *CXCL9 (11*-fold for CD and 2.5-fold for UC), *GBP5 (9*-fold for CD and 6-fold for UC) and *CSF3R (*5.5-fold for CD and 6-fold for UC) compared to non AZA-treated patients. CD-patients reaching a calprotectin <100 mg/kg after initiation of anti-TNFαtherapy had a significant higher relative individual increase of peripheral blood CD56brightCD16+cells beginning at 6 [1.4±0.7 vs 0.80±0.4, p=0.048] and 12 months [1.9±0.8 vs 0.7±0.2, p=0.0005].

Conclusions: Frequency of NK cells in peripheral blood and intestinal mucosa are modulated by anti-TNF treatment and Azathioprine. In spite of a co-medication with AZA an increase of peripheral circulating CD56brightCD16+ cells was observed in patients achieving clinical and biochemical remission. Th• is cell subset may serve to monitor the therapy response in pediatric IBD.

P146

Dissecting Innate Lymphoid Cell Phenotypes during Colitis-Induced Colorectal Cancer in mice

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Introduction: Innate Lymphoid Cells (ILCs) are a heterogeneous family of lymphocytes having distinct biological functions rapidly triggered upon microenvironmental changes. Natural Killer (NK) cells represent the founding member of the ILC family and have long been recognized as an early line of defense against neoplastic development. Recently, a role for ILCs in promoting inflammation, as well as cancer growth, has emerged.

Objectives:We aim to investigate the dynamic changes of ILC phenotypes taking place during the pathogenesis of colitis-induced colorectal cancer (CRC) in mouse models. Due to their sensitivity towards microenvironmental changes, elucidation of ILC complexity can reveal novel molecular pathways for CRC pathogenesis.

Materials and methods:The combinatorial expression of surface markers and transcription factors allows the identification of prototypical ILC subsets. In our experiments, ILC were isolated from murine intestine in the onset of acute colitis, chronic inflammation and from tumors. Conventional mouse models consisting of DSS-induced colitis and AOM/DSS colon cancer were used. Next, 16-color flow cytometry panel was established to dissect tissue-resident and tumor-infiltrating ILCs (TI-ILCs). Data were then analyzed by using high-dimensional cluster analysis. Subsets were assessed for selective expression of cell-specific surface markers, transcription factors and effector molecules.

Results:Our data shed light on common and specific phenotypic patterns for ILCs associated with normal and inflamed colon, as well as, with tumor. In particular, we observed a major shift from type 2 to type 1 ILCs associated

DOI: 10.1002/eji.201970300

with the transitions from healthy to inflamed tissues and, finally, to tumor. The vast majority of TI-ILCs mainly comprised NK cells and other type 1 ILCs, drawing the attention to their implications in cancer progression.

Discussion: Phenotypic shifts in ILC distribution present in inflamed intestinal tissues and within the tumor infiltrate suggest an active role of these cells during CRC pathogenesis. Thus, a deeper understanding of TI-ILCs and the signals involved in shaping their effector functions can lead to unveil novel mechanisms underlying CRC progression.

P147

Influence of diet-induced obesity on liver NK cell-mediated contact hypersensitivity

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Introduction: Our previous work showed that liver NK cells can mediate contact hypersensitivity (CHS) reaction in mice. Further, we found that mice fed high-fat diet (HFD) for 8 weeks develop aggravated CHS reaction when compared to animals kept on normal diet (ND) prior to CHS induction and elicitation.

Objectives: The purpose of this study is to determine mechanisms by which DIO may affect CHS in RAG1$^{-/-}$B6 mice.

Materials & Methods: To investigate the influence of DIO on NK cell-mediated CHS, RAG1$^{-/-}$B6 mice were maintained on HFD or ND for 8 weeks. Then, the animals kept on HFD or ND were split into two groups, one sensitized with DNFB and the second one treated with solvent alone. Further, liver mononuclear cells (LMNC) were cultured with DNP-BSA antigen prior to IFN-γ measurement in culture supernatants. To phenotype NK cells involved in CHS, LMNC were stained with fluorochrome-conjugated antibodies and analyzed using flow cytometry. Additionally, the subcutaneous and visceral fat tissues were collected and weighed and then cells obtained by mechanical dissociation were cultured to determine adipocytokine production.

Results: LMNC from HFD mice restimulated *in vitro* with DNP-BSA produced more IFN-γ than LMNC from ND mice. Flow cytometry analysis of LMNC showed increased percentage of NK1.1^{+} INF-γ^{+} cells in HFD-fed mice compared to ND mice. Subcutaneous and visceral fat tissue from mice on HFD weighed more and produced more IL-6 but less IL-10 when compared to ND mice.

Conclusion: Our data suggest that feeding with HFD for 8 weeks aggravates NK cell-mediated CHS in RAG1$^{-/-}$B6 mice by inducing INF-γ-producing NK cells.

Supported by grant from National Science Center 2016/23/N/NZ6/01488 to PK

P148

A viral inhibition assay to assess the anti-viral effect of different NK cell populations during HCV infection

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Introduction: Natural killer (NK) cells are able to recognize virus-infected and tumor cells and have been described to be important for the early control of HCV infections. Effector functions of NK cells are tightly regulated through an array of activating and inhibitory receptors, by which NK cells can be divided in several subpopulations with distinct anti-viral potential. Killer cell immunoglobulin-like receptors (KIRs) are one of the main NK cell receptor families and have been associated with the resolution of HCV infection. Previous studies suggested a protective effect of the activating NK cell receptor KIR3DS1 as well as the inhibitory KIR2DL3 on the outcome of HCV infection.

Objectives: The aim of this project was to establish an *in vitro* viral inhibition assay to assess the anti-viral activity of different NK cell populations during HCV infection.

Methods: In order to study the contribution of different NK cell populations towards inhibition of HCV replication, a luciferase-based inhibition assay in a HCV cell culture system was established. Huh7 cells were infected with a Jc1

DOI: 10.1002/eji.201970300

reporter virus carrying a gaussia luciferase. Primary human NK cells from donors homozygous or heterozygous for the respective KIR of interest were sorted or used in bulk and subsequently characterized in regards to their ability to suppress HCV replication. Furthermore, KIR-Fc fusion constructs were used in order to boost viral inhibition by NK cells towards Jc1 infected Huh7 cells.

Results: The HCV inhibition assay enabled to analyze the anti-viral potentials of different NK cell populations. NK cells from KIR3DS1/KIR3DS1 homozygous donors as well as sorted KIR3DS1+ NK cells were able to significantly suppress viral replication by activating the anti-viral potential against HCV infected Huh7 cells. In contrast, we did not observe a similar anti-viral effect of KIR2DL3+ NK cells. In addition, co-incubation with KIR3DS1-Fc constructs also allowed KIR3DS1- NK cells to inhibit viral replication more efficiently.

Conclusion: Taken together, the luciferase-based HCV replication assay provides a valuable tool to study NK cell-mediated viral inhibition in cell culture, enabling us to investigate the role of different NK cell populations in HCV infection in more detail. The study demonstrates a high potential of KIR3DS1+ NK cells to suppress viral replication *in vitro*, as well as the ability to modulate anti-viral responses through KIR-Fc constructs.

P150

Infection with BK Virus causes upregulation of HLA-F on immortalized renal tubular cells

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Introduction: Natural killer (NK) cells recognize altered and infected cells through a plethora of receptors including killer-cell immunoglobulin-like receptors (KIRs) and have been implicated in shaping the outcome of various human diseases.

BK Virus is a highly prevalent human polyomavirus with seroprevalence up to 80%. Under immunosuppression, e.g. after kidney transplantation, BK Virus reactivates and may cause BK Virus associated nephropathy (BKVAN). BKVAN results in reduced graft function or graft loss. How BKV reactivation is controlled and why only a portion of renal transplantations can"t control BKV replication is unknown. Interestingly, Trydzenskaya *et al.* showed that kidney transplant recipients develop BKVAN more frequently if they do not encode for the activating KIR3DS1 receptor.

The recent observation that open conformers of the non-classical HLA class I molecule HLA-F serve as ligands for KIR3DS1 now allows for the first time to investigate the role for KIR3DS1+ NK cells during BK Virus infection.

Methods: HLA-F surface expression was assessed using flow cytometry on immortalized human renal proximal tubule epithelial cells (hRPTEC/tert1) infected with BKV (Dunlop strain) and mock-infected controls. KIR3DS1 binding to infected hRPTEC/tert1 cells and uninfected control cells was assessed using recombinant human KIR3DS1/CD158e2 Fc Chimera proteins.

Results: hRPTEC/tert1 cells showed an increased expression of HLA-F after infection with BKV compared to mock-infected controls Expression of HLA-F varied during the course of BK Virus infection with an increase over time.

Upregulation of HLA-F resulted in increased binding of KIR3DS1-Fc constructs to infected cells.

Conclusion: This study provides first evidence for a role of KIR3DS1 and HLA-F during BKV infection.

P154

The role of NK cells in drug-induced liver injury

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DOI: 10.1002/eji.201970300

There are more than 1000 known drugs that can cause liver damage and it is one of the most common reasons why these drugs need to be withdrawn from the market. These drug-induced liver injury (DILI) patients show asymptotic transaminase elevations and various histological changes in hepatic architecture, such as hepatitis, cholestasis, necrosis and fibrosis. If these diseases are not recognized early enough and the medication is discontinued immediately, chronic liver disease or acute liver failure can occur, which is still a major cause of liver transplantation. However, the mechanism leading to DILI still remains unclear. Here, we show that after drug exposure, primary human hepatocytes as well as the hepatocyte cell line Huh7 upregulate activating NK cell ligands. This results in enhanced NK cell activation leading to more cytotoxicity and INFγ production. The presented data demonstrate that NK cells might modulate DILI through direct killing of hepatocytes and INFγ production.

P155

ILC1s in Imiquimod-induced Psoriasiform Dermatitis

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Introduction: Innate lymphoid cells (ILCs) are an essential component of the immune system, mediating tissue homeostasis and inflammation. ILCs can be divided into three groups, based on the transcription factors they depend on and the cytokines they produce. NK cells and ILC1s are classified as group 1 ILCs as they constitutively express the transcription factor T-bet and are able to produce Th1 cytokines. Unlike NK cells, which are found to circulate throughout the body, ILC1s display a tissue-resident phenotype, defined by the expression of a unique pattern of chemokine receptors and adhesion molecules (e.g. CD49a, CXCR6, CD69).

Question: Inappropriate activation of ILCs has been linked to the pathogenesis of inflammatory disorders. As the diverse functional properties of ILC1s in the skin remain poorly defined, we were keen to delineate how this subset contributes to chronic inflammatory skin conditions such as psoriasis.

Methods: To study the role of ILC1s in the course of inflammation, the well-established mouse model of Imiquimod-induced psoriasiform dermatitis was used.

Results: Topical treatment with Imiquimod cream led to a substantial accumulation of $CD49a^+$ ILC1s within the first four days of application. Intriguingly, this subset was a major source of TNF-α with an overall contribution of more than 40% among TNF-α producing lymphocytes. Furthermore, dermal ILC1s were characterized by the expression of the chemokine receptor CXCR6 and CD44. Other markers associated with bona fide ILC1s such as CD69 and TRAIL could not be detected, revealing an unconventional phenotype. Mechanistically, expansion of dermal ILC1s was dependent on IL-12, since local cytokine application led to a pronounced increase in number, whereas injection of neutralizing antibodies prompted the opposite. Interestingly, systemic administration of anti-asialo-GM1 or anti-NK1.1 antibodies did not reduce dermal ILC1 numbers, while NK cell abundance was completely abrogated. In line, *in vivo* labeling revealed a minor contribution of circulating ILC1s to psoriasiform skin inflammation, confirming their tissue-resident nature.

Conclusion: Our findings suggest an underappreciated role of ILC1s in the development of psoriasis.

P157

Alloantibody formation against NK cell antigens in patients after solid organ transplantation

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Introduction: Chronic antibody-mediated rejection (ABMR) is known to play a key role in graft survival, dysfunction or rejection and is still a major obstacle in transplantation success. Recent studies have shown the importance of non-

DOI: 10.1002/eji.201970300

HLA antigens in the formation of donor-specific antibodies (DSA) in graft rejection. A pilot study revealed the presence of allospecific antibodies against antigens encoded in the NK cell gene complex (NKC) and leukocyte receptor gene complex (LRC) in 23% from a total of 92 plasma samples from liver and kidney recipients. The data generated by this previous study propelled us to broaden our search for alloantibody formation against NK cell antigens.

Objective: To investigate the frequency and relevance of alloantibody formation against NK cell antigens and their role in transplantation outcome in patients after solid organ transplantation.

Materials and Methods: Recombinant proteins for the predicted most relevant NK cell antigens were produced and coupled to differentially colored-multiplex beads. The labelled beads were used to screen patient plasma for the presence of antibodies using FACSCanto II flow cytometer.

Results: Overall, allospecific antibodies against various NKC- and LRC-encoded receptors such as NKG2C, KIR2DL2/DS2, KIR2DS1/DL1 and LILRB3 were found in 20% of Kidney recipient patents (n=15), 19% of liver recipient patients (n=42) and 17% of lung recipient patients (n=41). Table 1 describes in more detail the absolute numbers for each post-transplantation sample that tested positive for at least one NK cell antigen-specific antibody.

	Number of Patients per Antigen				
	KIR2DS1/DL1	**KIR2DS2/DL2**	**NKG2C**	**LILRB3**	**Overall**
Kidney (n=15)	1	1	1	1	3 (1)
Liver (n=42)	2	3	3	2	8 (1)
Lung (n=41)	0	5	5	3	7 (5)

Table 1: Overview of recipient-patients screen for alloantibody formation against NK cell antigens. The values refer to the absolute number of patients tested positive for the presence of specific-antigen alloantibody formation defined by each column. The overall values refer to the total number of positive organ-specific transplanted patients who tested positive for at least one type of antibody. The number in brackets refers to the number of patients out of all that showed positive results for more than one NK cell antigen-specific antibody.

Conclusion: The data indicate a high degree of potential mismatch in NK cell diversity between donor and recipient in the case of solid organ transplantation. Further analysis is being performed to evaluate the functional consequences and clinical relevance of these antibodies in transplantation outcome.

Supported by DFG grant SFB738/A5

P158

Expanding human gamma delta T cells for a potential therapeutic use in multidrug-resistant infections

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Multi-drug resistant infections are an increasing threat for human health and economy, yet there is no sufficient strategy in place to face the increasing burden of superbug infections. In this project we attempt to expand human gamma delta (gd) T cells for a potential therapeutic use to fight multidrug-resistant infections.

gd T cells are unconventional T cells with adaptive and innate immune characteristics. Vδ2 T cells, a subset of γδ T cells found in human peripheral blood possess cytotoxic properties towards cancer and infected cells and can be easily expanded by phosphoangtigens and bisphosphonates, such as HMBPP and zolendronate, respectively. The aim of this project was to assess the capacity of *in vitro* expanded γδ T cells to respond to infectious agents in terms of pro-inflammatory activities. In our study HMBPP and zolendronate were able to expand γδ T cells to a similar extent in accordance with previous reports. Furthermore, we showed that the HMBPP-expanded γδ T cells maintain the capacity to produce TNFa and IFNg cytokines upon infectious agents challenge and cytotoxicity towards infected cells, whereas zolendronate expanded γδ T cells cease cytokine production and show attenuated cytotoxic properties towards infected cells, which we consider as an exhaustion phenotype. Moreover, the abrogation of cytokine production in γδ T cells is due to the intrinsic dysfunction of γδ T cells induced by zolendronate. This exhaustion

DOI: 10.1002/eji.201970300

phenotype is neither associated with exhaustion marker expression nor change of memory phenotype. Our study suggests that HMBPP is a more potent stimulus for expansion of functional γδ T cells. We speculate that the exhaustion of γδ T cells may be due to either zolendronate-induced inhibition of certain metabolic pathways, memory induction associated with chromatin alternation or functional transition associated with energy balance in γδ T cells. Metabolomic, transcriptomic and epigenetic analysis will be further performed to decipher the underlying mechanisms of the exhaustion phenotype.

P159

Ubiquitin and ubiquitin-like modifiers modulate NK cell-mediated recognition and killing of tumour cells

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Introduction: PVR and Nectin2 are members of immunoglobulin-like cell adhesion molecules and are up-regulated on tumour cells, including Multiple Myeloma (MM) cells. They are both able to bind the activating receptor DNAM1 expressed on Natural Killer (NK) cells, and this contributes to NK cell-mediated killing of tumour cells. Transcriptional regulation of PVR and Nectin2 expression has been reported by several studies;however a role for post-translational modifications has not been investigated yet.

Objectives: We focused our attention on Ubiquitin (Ub) and SUMO pathways to evaluate their contribution in the regulation of DNAM1 ligands on MM cells.

Materials and methods: PVR and Nectin2 expression was analysed by flow cytometry and confocal microscopy. Inhibition of the SUMO and the Ub pathways were achieved by means of Ginkgolic Acid and PYR41 treatments, respectively. The SUMO pathway was also blocked by shRNA targeting the E2 SUMO conjugating enzyme, UBC9. PVR and Nectin2 modifications were investigated by PLA combined with confocal microscopy. NK cell activation was assessed by Cr^{51}cytotoxicity assay, and MM adhesion to Bone Marrow Stromal Cells (BMSCs) was evaluated by flow cytometry and confocal microscopy. Protein expression levels were compared by western blotting before and after treatment with the specific proteasome inhibitor epoxomicin.

Results: We demonstrated that PVR and Nectin2 were mainly located in intracellular compartments both on plasma cells from MM patients and on MM cell lines. Inhibition of the SUMO pathway promotes the redistribution of the intracellular pool of PVR, resulting in the up-regulation of PVR but not Nectin2 surface expression. This, in turn, renderedMM cells more susceptible to DNAM1-dependent NK cell cytotoxicity and increasedthe ability of MM cells to adhere to BMSCs. On the other hand, inhibition of the Ub pathway decreasedNectin2 degradation and promotedits relocation to cell surface, thus enhancing MM cell susceptibility to NK cell cytotoxicity. We are currently investigating Nectin2 contribution to MM adhesion to BMSCs. Finally, we found that PVR is directly SUMOylated, while Nectin2 undergoes ubiquitination. We also extended these findings to other malignancies.

Conclusions: Our results demonstrate previously unknown mechanisms ofPVR and Nectin2 regulation, revealing that post-translational modifications represent a potential target of intervention in order to increase susceptibility to NK cell-mediated lysis.

Tumor immunology and microenvironment (P160-197, P402)

P160

Investigating mitochondria morphology during T cell exhaustion

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Introduction. Mitochondria morphology tightly regulates several aspects of T cell life. Indeed, fragmented mitochondria sustain *i)* cytosolic calcium accumulation during T cell activation[1], *ii)* T cell migration and invasiveness[2] and *iii)* a fast rate of clonal expansion[2]. Further, mitochondria morphology regulates nutrient utilization during differentiation, influencing the effector/memory fate choice[2,3]. During T cell exhaustion, most of these processes, such as T cell motility, expansion, and nutrient utilization, are strongly downregulated[4]. However, no information is currently

DOI: 10.1002/eji.201970300

available on the changes to which mitochondria undergo during exhaustion and whether they may be partially responsible for such dysfunctional state.

Objectives. We propose to investigate how mitochondria morphology is affected in exhausted T cell inside the tumor-microenvironment. Further, we aim at understanding to what extent an altered mitochondria shape may drive some of the defects found in exhausted T cells, and if its pharmacological modulation could rescue this dysfunctional state.

Materials & Methods. We are using MC38-derived murine tumor model to perform both *in vivo* and *ex vivo* studies on tumor-infiltrating lymphocytes. Further, functional *in vitro* studies are being performed on both murine and human primary T cells.

Results. Our results indicate that Drp1, which is the main pro-fission protein, is strongly down-regulated in PD-1$^+$ exhausted T cells, leading to mitochondria alterations. Mechanistically, we found that PD-1 impairs two kinases responsible for Drp1 activation, i.e. mTOR and ERK, this in turn impairing T cell effector functions. Further, motility paralysis exhibited by exhausted T cells is driven by a down-regulation of the ERK-Drp1 axis.

Conclusions. Our preliminary analysis suggest that exhausted T cells show a defective ability to fragment their mitochondria network due to Drp1 down-regulation. We are now moving to investigate how artificial manipulations of the mitochondria morphology could be exploited to rescue the defects found in exhausted T cell.

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P161

Toll-like receptors in tumor progression and as targets in PDAC treatment

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Introduction: Pancreatic ductal adenocarcinoma (PDAC) is a type of cancer with a poor prognosis and a high mortality rate. It is characterized through a functional tumor microenvironment which promotes carcinogenesis, tumor progression and also therapeutic resistance. This microenvironment contains numerous stroma cells, including activated fibroblasts/stellate cells, endothelia cells and diverse immune cells.

TLRs are components of the innate immune system connected to PRRs. Controversially they are not only expressed by immune cells but also by cancer-as well as stroma cells. Interestingly, tumor promoting activity has been shown for TLR7 and TLR9. Additionally TLR7 is also highly expressed within the tumor tissue. In particular, it could be demonstrated in vitro as well as in mouse models, that stimulation of TLR7 increases the pancreatic tumor growth while its inhibition leads to an attenuation of tumor cell proliferation. This observation seems to indicate that endogenous RNA may stimulate TLR7 due to differences in pancreatic tumor cell RNA-methylation pattern.

Objectives

- Investigating the function and activation mechanism of TLR7 in context of PDAC, including tumor-derived or self-RNA acting as endogenous ligands
- Identification of potential TLR7 inhibitors

Materials and methods: Isolated RNA of pancreatic cancer cell lines, human and murine pancreatic tumors as well as control tissues will be analysed by NGS and HPLC to identify alterations in their methylation patterns. Additionally, the immunostimulatory potential of isolated nucleic acids will be investigated by stimulating immune cells and measuring cytokine production via ELISA.

To identify potential TLR7 antagonists, human TLR7-expressing HEK 293 cells with an inducible SEAP reporter gene and human PBMCs were stimulated with different small molecules.

DOI: 10.1002/eji.201970300

Results: We synthesized and analysed several small molecules for TLR7 inhibitory potential. Indeed, we could identify one antagonist, which was able to inhibit cytokine release induced by specific TLR7 ligands in human PBMCs. We could confirm this result in hTLR7 HEK cells as well. The chemical structure of the antagonist mostly resemble purine bases. Stimulation with different single nucleosides or deoxynucleosides showed no inhibition.

Conclusion: It is known that thymidine homopolymer phosphorothioate oligodeoxynucleoside, can inhibit the activation of TLR7. Based on these facts we will chemically modify different antagonists to increase their potency.

P162

TLR7/9 ligands potentiate malignant lymphoproliferation in mice with oncogenic *Myd88$^{p.L252P}$* mutation-associated lymphoma

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Introduction: Diffuse large B-cell Lymphoma (DLBCL) is the most frequent Non-Hodgkin-Lymphoma, yet 30-40% of cases remain uncured and especially patients with the *MYD88$^{p.L265P}$* mutation have a poor prognosis. MyD88 transmits Toll-like receptor (TLR) signals via the so-called "Myddosome" multi-protein complex, which forms spontaneously in *MYD88$^{p.L265P}$* DLBCL cells and constitutively activates NF-κB, promoting cell survival. The Staudt group recently showed that in human DLBCL cells, *MyD88$^{p.L265P}$* forms a so-called "supercomplex" with the B cell receptor (BCR) and TLR9. Whether this supercomplex signals to NF-κB ligand-autonomously or is driven by TLR ligands *in vivo* is unknown.

Objectives: We investigated whether such a ligand-dependent signalling promotes lymphoproliferative effects *in vitro* and *in vivo*. To monitor tumor emergence in *MYD88$^{p.L265P}$* lymphoma we investigated B-cell distribution *in vivo* by PET/MR imaging and optical imaging.

Materials & Methods: We used a mouse model with Cre-mediated B-cell-specific expression of *Myd88$^{p.L252P}$* (murine homolog of human *MYD88$^{p.L265P}$*) and overexpression of *BCL2 IRES GFP*. Isolated splenic B-cells were analysed for IL-6 (ELISA) upon *in vitro* stimulation with R848 (TLR 7/8 agonist) and CpG (TLR9 agonist). Mice were challenged *in vivo* with CpG injections, glucose metabolism and proliferation were measured by ^{18}F-fluorodeoxyglucose (FDG) and ^{18}F-fluorothymidine (FLT) PET/MR imaging. Additionally, we performed whole body GFP scans.

Results: R848 and CpG stimulation strongly triggered survival, proliferation and IL-6 production of *Myd88$^{p.L252P}$* B-cells. *In vivo* CpG stimulation lead to decreased survival, lymphadenopathy and increased glucose metabolism in lymph nodes (LNs). PBS-injected controls developed milder disease even 6-12 weeks later. Remarkably, *Myd88$^{p.L252P}$ BCL2 IRES GFP* mice older than 27 weeks developed abdominal tumors in 8/11 cases. Additionally, GFP imaging of these mice compared to *BCL2 IRES GFP* controls revealed increased GFP signal in abdomen, spleen, liver and LNs.

Conclusion: We conclude that TLR7/9 ligands can fuel proliferation of *Myd88$^{p.L252P}$* cells *in vitro* and in peripheral lymphoid organs *in vivo*. We suggest that TLR ligands, possibly in tandem with BCR antigens, thus promote the emergence of single B-cells that expand to clonal tumors. Thus blockade of nucleic-acid sensing TLRs might synergize with BCR blockade in targeting B-cell lymphomas with *MYD88$^{p.L265P}$* mutation in patients.

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Visualization of breast cancer derived extracellular vesicles and their impact on adaptive immunity

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Objective Triple negative breast cancer (TNBC) is among the most difficult cancer subtypes to treat and continues to cause a high number of cancer-related death annually. Extracellular vesicles (EVs) are part of the cellular communication of all eukaryotic and prokaryotic cells. They transfer cell type-specific cargo and have important

DOI: 10.1002/eji.201970300

implications in disease. Upon treatment of cancer cells with low-dose chemotherapy, released EVs are able to transfer phenotypic traits to other cancer cells. New treatment strategies for TNBC, like inhibitors of the ER stress pathway might impact on EV biogenesis, cargo delivery and response of cells in the cancer microenvironment. T cells, which are part of the tumour-associated immune response, also produce and respond to EVs – including functional uptake as well as direct binding on surface molecules. Our aim is to identify differences in breast cancer-derived EVs upon treatment with inhibitors of the ER stress as well as low-dose chemotherapy with particular emphasis on altered T cell immunity.

Methods Different breast cancer cell lines, known to be responsive to ER stress inhibitors and low-dose chemotherapy were used as source of EVs, which were prepared by sequential ultracentrifugation. Integrity of different EV sub-types were studied by electron microscopy (TEM) and Nanoparticle Tracking Analysis (NTA). The ImageStream (ISX) technology was used to visualize CFSE labelled EVs. T cells from healthy blood donors will be activated in serum free medium with suboptimal and optimal doses of different stimuli in the presence of labelled breast cancer-derived EVs. T cell proliferation, cytokine production and vesicle uptake will be studied.

Results Using 4 different TNBC cell lines, we were able to decrease the amount of EV depleted growth serum needed from 10% to 2%. Breast cancer cell released EVs were positive for CD63 and TSG-101 as determined by TEM and showed the expected size distribution and total particle count by NTA. Cell derived EVs were visualized by direct labelling with CFSE without immobilization on beads using the ISX technology. Preliminary data suggest altered EV release from ER stress inhibitor treated breast cancer cells.

Conclusion Serum reduced culture conditions of breast cancer cell lines were successfully implemented**.** Cell free strategies to visualize extracellular vesicles could be established by the use of imaging flow cytometry. The impact of EVs on T cell immunity is still under investigation.

P165

Phagocytic Macrophages of the Primary Tumor are Associated with Lymphogenous Metastasis in Prostate Cancer

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Prostate cancer (PC) is the second most frequently diagnosed malignant tumor and the sixth leading cause of death for cancer in the male population. Every year, over 899,000 new cases are diagnosed in the world, which is 14% of all cases of cancer. The recruitment of migratory hematopoietic cells, including tumor-associated macrophages, to the supporting stroma is essential in the progression and metastasis of tumors. One of the main macrophages function is phagocytosis of apoptotic cells, that can induce secretion of key factors implicated in tumor progression. Besides the lack of specificity and sensitivity of modern diagnostic methods leads to the search for new factors that make it possible to predict the development of metastasis in a PC patients.

To explore the role of phagocytic macrophages in prostate cancer lymph node metastasis immunohistochemical (IHC) analysis was performed.

IHC staining of the 37 human prostate cancer paraffin embedded and cutted tissue samples (13 with and 24 without lymph node metastasis) was done using mouse antibody to CD68 (1:50; Zytomed Systems). Large phagocytic "foamy" and small non-phagocytic cells were counted in 10 fields of each tissue samples using Leica DMRE light microscope with x100 magnification. GraphPad Prism 7.03 program was used for Mann Whitney U-test.

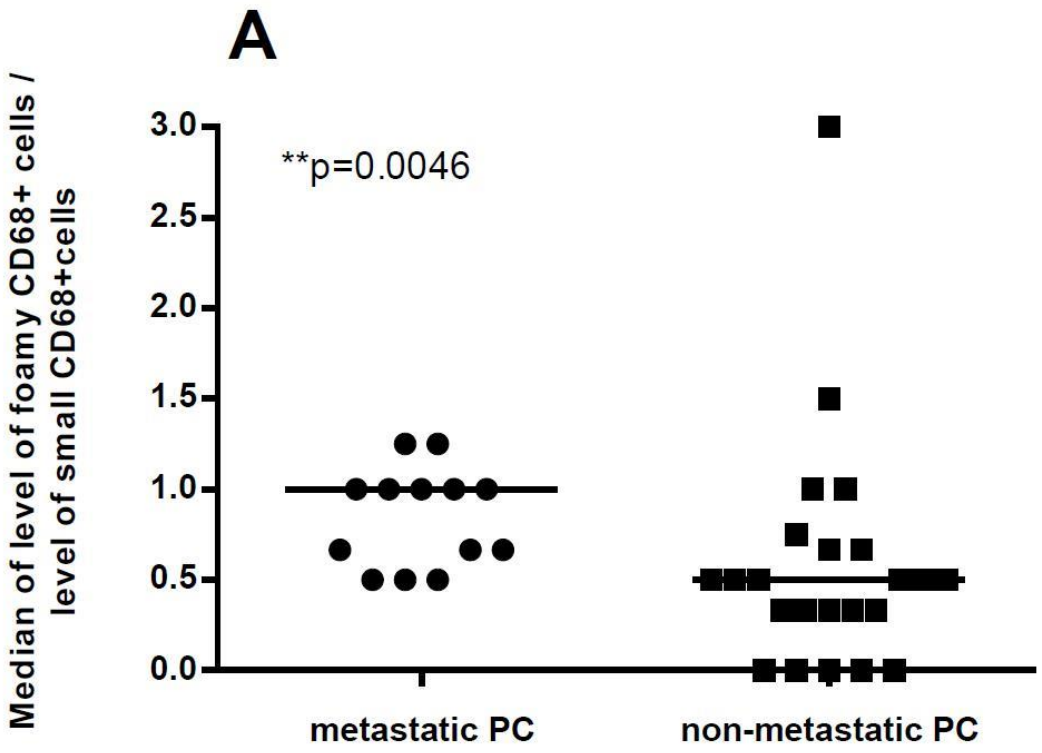
The number of large phagocytic CD68+ cells in relation to small CD68+ cells was significantly higher in samples of tissues of the primary prostate tumor of patients with lymph node metastases compered to non-metastatic patient samples (fig. A).

This marker may be useful for improving the diagnosis of metastatic prostate cancer, as well as predicting the development of lymph node metastases during biopsy in patients with primary prostate cancer after extensive research.

DOI: 10.1002/eji.201970300

Fig.A. The relative level of "foamy" phagocytic CD68+ cells to small non-phagocytic CD68+ cells in prostate cancer tissue of patients with and without lymph node metastases.

Figure 1



P166

Effector functions of γδ T cells and their potential in cancer immunotherapy

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γδ T-cell based immunotherapy is a promising concept for the treatment of malignancies either alone or in association with new immunotherapeutical approaches as immune checkpoint blockade therapy. Aiming to improve the effectiveness of future γδ T-cell based immunotherapies against malignancies, we analyzed the expression and biological significance of inhibitory checkpoints molecules PD-1, CTLA-4, LAG-3, TIGIT and TIM-3 on colon cancer infiltrating and circulating γδ T cells. The analysis showed higher levels in tissue-resident (either normal or tumor) γδ T cells, as compared to circulating γδ T cells, while MFI analysis showed an increase of their expression from circulating cells toward infiltrating cells, reaching maximum intensity in tumor infiltrating cells. Moreover, γδ T cells infiltrating invasive tumors had higher percentages and expression of immune checkpoint molecules compared to non invasive tumors.

Blocking PD-1/PD-L1 interaction *in vitro* by Nivolumab increased the cytotoxic activity of γδ T cells against tumor cells, sustained the viability of the effector γδ T cells by reduction of their apoptosis rate but without promoting their proliferation, and induced the differentiation of γδ T cells toward a central memory phenotype. In addition, the blockade of PD-1 and CTLA-A4 signalling by Nivolumab and Ipilimumab induced IFN-γ and TNF-a production by γδ T cells upon stimulation with colon cancer cells, and restored their antitumor activity.

DOI: 10.1002/eji.201970300

Our results clearly demonstrate that γδ T cells in the tumor microenviroment are conditioned by tumor cells to express inhibitory molecules and the modulation of these molecules restores the antitumor efficacy of γδ T cells. Therefore, combination of γδ T cell based therapies with checkpoint blockade may prove a useful strategy to improve the efficacy of cancer immunotherapy.

P168

Bispecific antibodies enhance tumor-infiltrating T cell-cytotoxicity against primary HER2 expressing-high-grade differentiated ovarian tumors

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Epithelial ovarian cancer displays the highest mortality of all gynecological tumors and causes around 140,000 deaths annually in women worldwide. With a 5-year-survival rate of only < 40% after first line therapy ovarian cancer remains a highly lethal tumor entity to this day. A significant problem for an effective treatment is the recurrence of the disease even after successful surgical treatment – caused in many cases by a resistance against the current platinum based chemotherapeutic standard regime. Commonly, human epidermal growth factor receptor (HER) 2 expression is detected in 22-66% of the patients and did not correlate with patients" survival. Many attempts to improve ovarian cancer patients" outcome by adding additional drugs failed. Therefore, the development of new therapeutic strategies is of major clinical interest. One strategy could be the introduction of bispecific antibodies (bsAb) which enhance cytotoxic activity by selectively targeting immune cells to tumor-associated antigens.

In our study, we isolated primary cancer cells out of tumor tissue and ascites from high-grade serous ovarian carcinoma patients and characterized them. We observed an amplification of the genes human telomerase RNA gene and c-MYC by FISH-technique. A polyploidy of 60-90% together with a high expression of pan-cytokeratin and epithelial cell adhesion molecule (EpCAM) classified them as cancer cells. Interestingly, all *ex vivo* isolated ovarian tumors expressed HER2 analyzed by flow cytometry. HER2 expression is stable after culturing tumor cells. Trastuzumab treatment in ovarian cancer is not established partially due to the high tumor heterogeneity which leads to lower response rates. A very promising strategy to overcome certain limitations of mAb and occasionally described HER2 resistance mechanisms can be the use of bsAb. Our results revealed an impaired cytotoxicity of T cells (including γδ T cells) isolated out of the tumor in comparison to T cells out of the blood of the same patients. Importantly, bsAb efficiently enhance cytotoxicity of peripheral blood- and tumor-infiltrating T cells against HER2-expressing primary ovarian cancer cells.

Taken together, our results revealed that HER2 expressed on high-grade differentiated ovarian tumors can be an efficient tumor antigen for bispecific antibodies targeting HER2-expressing ovarian cancer cells to T cells.

P169

Type I interferons modulate tumorigenic activity of neutrophils via altered emergency granulopoiesis

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Introduction. Cancer development is determined not only by the tumor cell features, but also by the surrounding non-malignant stroma. Stroma consists of a great variety of cells, including neutrophils, that participate in immune responses against cancer. The role of neutrophils in tumor development is controversial as they can show pro- or antitumoral properties depending on activating cytokines. Nevertheless, the source and kinetics of activation of protumoral neutrophils remains an open question. It is unclear if they are derived through the altered differentiation in hematopoietic organs or generated only upon arrival to the tumor tissue. Gaining insights into IFN-mediated modulation of neutrophil tumorigenicity should provide a basis to identify target molecules involved in tumorigenic stimulation.

Objectives. Concerning the essential role of neutrophils in the regulation of tumor progression, and that IFNs have been shown to suppress tumorigenic activity of these cells, we explored the mechanisms and timing involved in the switch between anti- and pro-tumor activity of neutrophils.

DOI: 10.1002/eji.201970300

Methods. We compared emergency granulopoiesis in bone marrow and spleen of WT and type I IFN-deficient (*Ifnar1*$^{-/-}$) mice during head-and-neck tumor progression. Composition and localization of the hematopoietic niches in these organs was assessed. We also analyzed differences in maturation, mobilization and migration of neutrophils, and their pro- or antitumoral properties.

Results. We observed that hematopoietic cell numbers in bone marrow and spleen steadily increased along tumor progression in both WT and *Ifnar1*$^{-/-}$ mice. This correlated with the increased level of G-CSF in plasma. The amounts of LT-HSC were comparable in both mice types, while ST-HSC, MPP and CMP were significantly elevated in WT mice, suggesting the role of IFN in the initiation of progenitor maturation. The amount of immature and mature neutrophils in BM showed no significant differences in both types of mice. Nevertheless, peripheral neutrophil counts were higher in *Ifnar1*$^{-/-}$ mice.

Conclusion. Type I IFNs affect not only functionality of tumor-associated neutrophils, but also their development. The availability of IFNs modulates myelopoietic niches in bone marrow and spleen, and influences the kinetics of myeloid progenitor maturation and neutrophil release. The exact mechanisms of this phenomenon have to be evaluated to understand the kinetics of the neutrophil tumorigenic polarization.

P170

Impact of neutrophils on γδ T cell-cytotoxicity towards pancreatic tumor cells

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Neutrophils can directly kill cancer cells, and support other immune anti-tumoral strategies. On the other hand, neutrophils can also exert pro-tumorigenic activities *via* the production of factors which promote cancer growth, angiogenesis and metastasis formation. An increased number of neutrophils in the blood of cancer patients has been associated with poor clinical outcome.

Increased γδ T cell-infiltration is present in many tumors, and γδ T cells have attracted much attention for potential application in cancer immunotherapy, partly due to their HLA-independent recognition of antigens. In this study, we analyzed the reciprocal interactions between neutrophils, pancreatic cancer cells and T lymphocytes with a special focus on their interplay with γδ T cells.

Recently, we have shown that zoledronic acid (ZOL)-activated neutrophils inhibit the proliferation of resting γδ T cells *via* ROS, arginase-1 and serine protease production. Here, we demonstrate that also the cytotoxic activity of resting γδ T cells (alone or within leukocytes) against pancreatic tumor cells can be inhibited by neutrophils. The neutrophil-mediated inhibition of γδ T cell-cytotoxicity was more pronounced after activating neutrophils with ZOL, an approved drug in clinical use for bone fragility disorders and cancer-associated bone disease. Our results revealed that the uptake of ZOL by neutrophils induced the release of serine proteases which inhibited cytotoxic function as well as IFN-γ and TNF-α production by resting γδ T cells.

Interestingly, we observed a different impact of neutrophils on γδ T cell-cytotoxicity when γδ T cells were directly activated by their selective antigens or by bispecific antibodies instead of an indirect activation by ZOL. Direct activation of γδ T cells by phosphorylated antigens or by targeting γδ T cells to tumor-antigen-expressing pancreatic cancer cells with bispecific antibodies enhanced the cytotoxic activity, cytokine and granzyme B production of resting γδ T cells, which overcame the suppression by ZOL-activated neutrophils. In addition, culturing neutrophils with autologous short-term activated γδ T cells, which already continuously produce IFN-γ, TNF-α and granzymes, augmented rather inhibited γδ T cell-cytotoxicity against pancreatic cancer cells.

This study demonstrates that the balance of anti- and pro- cancer activity of neutrophils is influenced by the particularly delicate interplay that exists between neutrophils and T lymphocytes.

P171

Activation of Liver X Receptor upregulates the expression of the NKG2D ligands MICA and MICB in Multiple Myeloma through different molecular mechanisms.

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DOI: 10.1002/eji.201970300

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Introduction: Natural Killer (NK) cells have an important role in immunosurveillance of Multiple Myeloma (MM) progression, and their activity is enhanced by combination therapies able to regulate the expression of specific activating ligands. Liver-X-Receptors (LXRs) are nuclear receptors important regulators of intracellular cholesterol and lipids homeostasis. Moreover, they have regulatory roles both in cancer and in immune response. Indeed, they can regulate inflammation and innate and acquired immunity. Furthermore, LXR activation directly acts in cancer cells (e.g. prostate, breast, melanoma, colon cancer, hepatocarcinoma, glioblastoma and MM) that show an accumulation of cholesterol and alteration of LXR-mediated metabolic pathways.

Objectives: Here, we investigated the role of LXR and cholesterol on the expression of the Natural Killer (NK) cell-activating ligands MHC class I chain-related molecule A and B (MICA and MICB) in MM cells.

Materials and methods: Human MM cell lines and MM plasma cells [$CD38^+CD138^+$ (PCs) derived from bone marrow of MM patients] were treated with synthetic LXR agonists GW3965 (GW) (or LXR-623) for 72 hours. NKG2DLs cell surface and intracellular expression were analyzed by flow-cytometry and confocal microscopy. mRNA levels were analyzed by Real-Time PCR.

Results: The results shown in this work indicate that MM cells are responsive to LXR activation, which induces changes in the intracellular cholesterol content. These changes correlate with an enhanced expression of MICA and MICB in human MM cell lines and in primary malignant plasma cells, two ligands of the Natural Killer Group 2D (NKG2D/CD314) activating receptor expressed in cytotoxic lymphocytes, rendering MM cells more sensitive to recognition, degranulation and killing by NK cells. Mechanistically, we observed that LXR activation regulates MICA and MICB expression at different levels: MICA at the transcriptional level, enhancing *mica* promoter activity, and MICB by inhibiting its degradation in lysosomes.

Conclusion: The present study provides evidence that activation of LXR, by enhancing NKG2D ligand expression, can promote NK cell-mediated cytotoxicity and suggests a novel immune-mediated mechanism involving modulation of intracellular cholesterol levels in cancer cells.

Keywords: Multiple Myeloma, LXR, Cholesterol, Natural Killer, NKG2D.

P173

Tumor associated Natural Killer cells in prostate cancer are endowed with decidual-like phenotype and pro-angiogenic function

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Question: Prostate cancer (PCa), represents the second frequent male cancer in the world. Immune cells, as a consequence of their plasticity are able to acquire pro-tumor/pro-angiogenic phenotype and function. Natural killer (NK) cells are cellular mediators of the innate immunity, primarily involved in tumor recognition and elimination. Altered NK phenotype and functions have been observed in different tumors, including PCa. Here, we phenotype and functionally characterized peripheral blood NK cells (tumor associated NK: TANK) from PCa patients and investigated their interactions with endothelial cells and macrophages, compared with subjects with benign prostatic hyperplasia (BPH) and age-matched healthy controls (HC).

Methods: NK cell phenotype and functional characterization were performed by multicolor flow cytometry for surface antigens on peripheral blood samples of HC, BPH, and PCa patients. Conditioned media (CM) from HC, BPH and PCa TANKs were used for functional studies of angiogenesis on human umbilical-vein endothelial cells (HUVEC), studies for macrophage recruitments (migration assay by Boyden chambers) and polarization. Molecular studies were performed by real time PCR (qPCR) on TANKs isolated from BPH and PCa patients and macrophages exposed to CM of BPH and PCa TANKs. Results from clinical samples were also validated in NK cells from HC donors, polarized with conditioned media of 3 different prostate cancer cell lines (PC-3, DU-145, LnCap).

DOI: 10.1002/eji.201970300

Results: BPH and PCa TANKs exhibit the CD56+CD9+CD49a+CXCR4+ pro-angiogenic/decidual-like phenotype. These results were confirmed in an *in vitro* model of healthy-donor derived NK cells, exposed to CM of 3 different prostate cancer cell lines (PC-3, DU-145, LnCap), where we also observed downregulation of IFNγ, TNFα and Granzyme B and increased production of pro-angiogenic factors (CXCL8, Angiopoietin1, and Angiogenin). By secretome analysis, we found that BPH and PCa NK cells can release pro-angiogenic factors, like CXCL8, and cytokines/chemokines involved in macrophage recruitment. CM from BPH and PCa TANKs were able to support angiogenesis *in vitro*, by inducing the formation of capillary-like structures on HUVEC, recruiting the THP-1 activated macrophages and polarizing them towards the M2-like macrophage subtype.

Conclusions: Our data provide the rational that TANK cells from PCa patients are switched toward a pro-angiogenic/pro-tumor phenotype and function.

P174
Impact of microbiota in antitumor vaccination
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Gut microbiota plays an important role in many diseases including cancer (Zitvogel *et al*., 2015). Recent evidence has shown that some bacterial strains modulate responses to numerous forms of cancer therapy, in particular immunotherapy (Routy *et al*., 2017). Tumors are characterized by specific mutations that they acquire during their development and progression which, when translated into proteins, give rise to neoantigens (Yarchoan, *et al*, 2017). These can be recognized by T cells and may be utilized for the development of personalized vaccines directly targeting patient"s neoantigens. We are analyzing the influence of gut microbiota on a particular approach to neoantigen vaccination, the Electro Gene Transfer (DNA-EGT). It has been previously demonstrated that EGT increases the efficacy of DNA vaccination (Aurisicchio *et al*., 2014). Therefore, we have started to characterize the interaction between microbiota and DNA-EGT vaccination, studying gut composition and the impact of microbiota on immune response and tumor growth. Our data suggest that this kind of vaccination is affected by microbiota, as shown by significant reduction of cytokine CD8 production in ATB-treated and vaccinated mice. Moreover, we have observed that, surprisingly, treatment with antibiotics cause a delay in tumor growth in association with a tumor neoantigen vaccine delivered by EGT. Preliminary NGS analyses showed that after antibiotics treatment there is a selection of bacteria strains in favor of Gram+. These bacteria produce short chatty fatty acid (SCFA) that link microbiota and immune response and can act as pro or anti-inflammatory molecules depending on environment or cellular subsets (Bultman, 2017). We are currently analysing different immune populations and microbiota composition at different time points to better understand how specific microbiota can affect tumor growth.

Cancer and the gut microbiota: An unexpected link, Zitvogel *et al.,* 2015

Cancer vaccination by electro-gene-transfer, Aurisicchio *et al.,* 2014

Gut microbiome influences efficacy of PD-1–based immunotherapy against epithelial tumors, Routy *et al., 2017*

Interplay between diet, gut microbiota, epigenetic events, and colorectal cancer, Bultman, 2017

Targeting neoantigens to augment antitumour immunity, Yarchoan *et al.,* 2017

P175
Tumor Associated Macrophages: toward a better understanding of their cancer driver capability in Malignant Pleural Mesothelioma.
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DOI: 10.1002/eji.201970300

Introduction: Malignant Pleural Mesothelioma (MPM) is an aggressive cancer characterized by chronic inflammation driven by the presence of non-degradable asbestos fibers. MPMs are known to be rich in Tumor-Associated Macrophages (TAMs), which are a major source of inflammatory mediators. In a gene expression analysis of human mesothelioma tumor surgical samples we identified two highly upregulated genes: *GPNMB*, coding for glycoprotein non metastatic B (GPNMB), and *SPP1*, coding for osteopontin (OPN). Both proteins were found by our group upregulated also in tumor-conditioned macrophages (1).

Question: The aim of the project is to investigate the biological role of OPN and GPNMB produced by cancer cells or by macrophages on tumor growth.

Methods: We used MPM biological samples (tumors, plasma) and a murine mesothelioma model with three cell lines AB1, AB12, and AB22, mimicking the different histotype of human MPM: sarcomatoid, biphasic, epithelioid.

Results: OPN and GPNMB were found expressed in human MPMs (IHC); their circulating levels (ELISA) were significantly higher in patients than in healthy donors. In vivo experiments confirmed that murine mesothelioma are highly infiltrated by immune cells, especially macrophages. OPN was strongly expressed by the three cell lines while GPNMB levels were very low, unlike human MPMs; accordingly, we silenced OPN and overexpressed GPNMB in AB1, AB12, and AB22 cells to study the role of these proteins in vivo. Ongoing experiments showed that the silencing of OPN strongly decreases tumor growth in vivo, while the overexpression of GPNMB increased tumor progression in some mice.

Conclusion: Our results demonstrate that OPN produced by MPM cells has a strong tumor-promoting activity. Similar results are noted with GPNMB-expressing MPM cells but need to be further confirmed. These two proteins may serve as target for therapeutic interventions in MPM.

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P176

Homing to suppress – the role of Treg trafficking during colon cancer

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A persistent and chronic inflammation of the colon, as it occurs in patients suffering from ulcerative colitis (UC), can predispose the tissue to cancer formation. The underlying immunological mechanisms that cause cancer induction and progression in UC patients are not well understood. Our previous studies already pointed out an important impact of regulatory T cells (Tregs) on the pathogenesis of colon cancer, as we showed that the transient ablation of tumor-infiltrating Tregs improves CD8+ T cell mediated anti-tumoral immunity. The modulation of Treg trafficking to the colon therefore might be a promising approach to specifically decrease Treg frequencies in the colon and thereby boosting anti-tumoral immunity. One potential candidate in the regulation of Treg homing during CAC might be the G-Protein coupled receptor 15 (GPR15), a recently identified colon-homing receptor. In a mouse model for colon cancer, we identified enhanced expression of GPR15 on tumor-infiltrating Tregs. The induction of colon cancer in GPR15-KO mice resulted in reduced tumor growth that was associated with a reduced frequency of Tregs in the tumorous tissue, as well as enhanced anti-tumoral CD8+ T cell responses. In conclusion, GPR15 presents a promising novel therapeutic molecule to target tumor-infiltrating Tregs during colon cancer.

P177

Role of the tetraspan MS4A4A on macrophage-mediated immune responses to pathogens and tumor growth

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DOI: 10.1002/eji.201970300

Introduction: Monocytes and macrophages (MØs) are key components of several pathological conditions, undergoing profound functional reprogramming influenced by the microenvironment. Several markers have been reported to identify specific MØs subsets and activation profiles. Recently, we described the tetraspanin MS4A4A as part of the transcription signature of M2-MØs. MS4A4A is selectively expressed in tissue resident MØs and is upregulated during M2/M2-like polarization, being also highly expressed in tumor-associated M2-like MØs. The pattern recognition receptor Dectin-1 was identified as a molecular partner of MS4A4A. Indeed, MS4A4A-deficient MØs present an impaired Dectin-1-dependent crosstalk with NK cells, leading to uncontrolled metastatic spreading in the lung.

Question: Several aspects of MS4A4A immunobiology remain unclear. Our goal is to define the relevance of MS4A4A in MØ biology for effective immune responses to pathogens and in tumor biology.

Methods: In the tumor setting, we are taking advantage of MØ-deleted Ms4a4a mice to define its role in experimental carcinogenesis models (AOM-DSS colon cancer, urethane-induced lung cancer). In the host defense setting, we are testing in vitro the biological relevance of MS4A4A for human monocyte-derived MØs immune response to swollen A. fumigatus conidia, and we are investigating the relevance of MS4A4A polymorphisms (SNPs) in Aspergillus fumigatus infection through genetic association studies in human cohorts of immunocompromised patients after hematopoietic stem-cell transplantation.

Results: In the tumor setting, M*Ø-Ms4a4a-/-* mice have shown no increased predisposition for the development of colon tumors in the AOM-DSS model. However, preliminary data demonstrates that these mice present a slightly higher number and dimension of neoplastic lesions in the urethane-induced lung carcinogenesis model. Regarding the role of MS4A4A in infection, we discovered that the MS4A4A M178V (AàG) SNP increased the susceptibility to invasive aspergillosis development in immunocompromised patients. Preliminary *in vitro* data shows that non-stimulated MØs with a GG or AG genotype express lower levels of MS4A4A than MØs with an AA genotype.

Conclusion: A better understanding of MS4A4A expression and function in macrophages will be essential to pave the way to the use of this molecule as a therapeutic target or prognosis marker in both tumor and infection context.

P178

The oncosuppressive role of RNASET2 gene in a mouse tumor syngeneic model

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Question: The human *RNASET2* gene has been reported by our lab to act as a powerful tumor suppressor gene in two independent *in vivo* xenograft-based murine models of human ovarian cancer. *RNASET2* encodes for an evolutionarily conserved, pleiotropic extracellular ribonuclease, whose secretion by cells in the tumor microenvironment is likely involved in tumor suppression. This *in vivo* oncosuppressive role is carried out by RNASET2-mediated recruitment into the tumor microenvironment of M1-polarized macrophages. We recently reported by *in vitro* assays a role for human recombinant RNASET2 in macrophage chemotaxis and polarization. To investigate the role of murine RNASET2, we performed a syngeneic in vivo assay.

Methods: Groups of BALB/c mice were injected subcutaneously with 2x106 of C51 colon carcinoma syngeneic wild-type cells, RNASET2-expressing C51 clone cells (Full vector) and non RNASET2-expressing C51 clone cells (Empty vector), and we analyzed in vitro and the tumor growth rate was followed for up to 3 weeks. At 2 and 3 weeks post-tumor injection, 3-6 mice of each group were sacrificed and IHC and FACS analyses were performed on tumor masses for immune cell investigation. The markers studied were: F4/80, CD86 for M1, F4/80, CD206 for M2, Gr-1, CD11b for myeloid-derived suppressor cells (MDSC), CD4, and CD8.

Results: Full-length murine RNASET2 C51 cells-injected mice showed delayed tumor growth in immunocompetent mice. Interestingly, large tumors developed in mice inoculated with empty vector-transfected cells or with C51 wild-type tumor cells, whereas cells expressing the wild-type full-length form of RNASET2 were clearly suppressed in their tumorigenic potential. At 2 weeks post-tumor inoculation, M1 macrophages were highly recruited in the tumor expressing the RNASET2, with concomitant inhibition of influx MDSC, whereas at 3 weeks there was a relevant expansion of CD8+ T cells in these tumors.

DOI: 10.1002/eji.201970300

Conclusions: These results support the role of RNASET2 as an evolutionary conserved tumor suppressor gene endowed with the ability to inhibit cancer growth *in vivo* in an immunocompetent experimental colon carcinoma murine model through recruitment of both innate immune M1 macrophages and adaptive CD8+ T cells.

P179

Targeting bHLH domain of HIF-1 is not sufficient to inhibit endogenous HIF-1 transcriptional activity

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Introduction: Hypoxia is a prominent feature of the tumor-microenvironment in many solid tumors and Hypoxia Inducible Factor-1 (HIF-1) is a crucial molecule in this phenomenon (1). During hypoxia, HIF-1 binds hypoxia response element (HRE) sequence in the promoter of different genes, especially those involved in angiogenesis and epithelial-mesenchymal transition (EMT) mechanism; mainly through basic helix-loop-helix (bHLH) domain (2). Considering the critical role of in bHLH-HRE interaction, targeting of bHLH might be a promising approach to inhibit HIF-1 activation in hypoxia.

Objectives: We inserted an inhibitory bHLH (ibHLH) domain in pIRESII-EGFP vector. The ibHLH domain consisted of both HIF-1a-bHLH and HIF-1b-bHLH sequences, capable of competing with HIF-1 in binding to HRE sequence.

Materials & methods: HEK293T cells were transiently transfected with pIRES2-EGFP and ibHLH-pIRES2-EGFP vectors and treated with 200 µM of cobalt chloride ($CoCl_2$) for 48h to induce hypoxia. Real-time PCR was applied to evaluate the effect of ibHLH on activation of the downstream genes of HIF-1.

Results: Hypoxia was successfully induced in HEK293T cell line, since $CoCl_2$ significantly increased the gene expression of VEGF, Vimentin, and Beta-catenin. Both control and ibHLH vectors decreased the hypoxia-depended gene expression of VEGF, N-cadherin, vimentin and Beta-catenin genes. However, ibHLH failed to be effective on respective genes when compared to the control vector.

Conclusion: Despite the critical role of bHLH domain in binding HIF-1 to HRE sequence of different genes, it seems that targeting bHLH is not sufficient to inhibit endogenous HIF-1 transcriptional activity.

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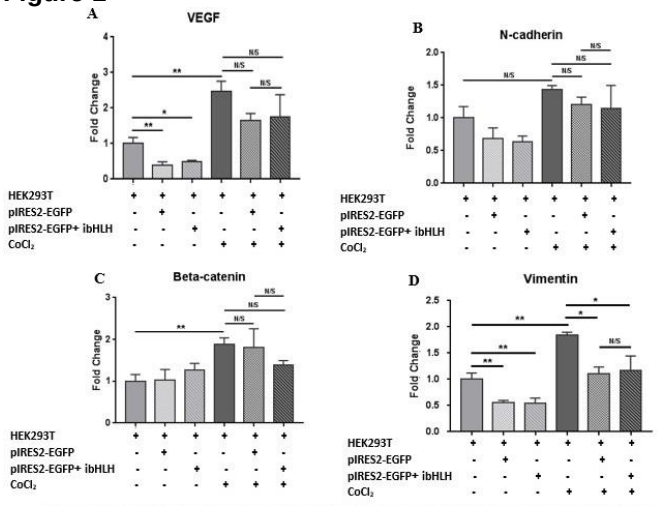
Figure legend:

Figure 1. The effect of ibHLH on the hypoxic-depended expression of VEGF (A), N-cadherin (B), Beta-catenin (C), and Vimentin (D). Data represent the mean (± standard deviation, SD) of Fold Changes from two independent experiments, each performed in duplicate. Statistical analysis was performed on **Log2** Fold Change, using One-way ANOVA, Bonferroni. Error bars indicate SDs. (*P<0.05, **P<0.01, N/S: Not significant).

DOI: 10.1002/eji.201970300

Figure 1



P180

Effects of parenteral nutrition on the immune system in patients with head and neck squamous cell carcinoma

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Introduction: Oncologic patients often face malnutrition, particularly in the case of head and neck squamous cell carcinoma (HNSCC), due to the site of the lesion that may hinder swallowing. Malnutrition is associated with immunosuppression and increased incidence of infections and mortality, and improving the nutritional status with support regimes, such as parenteral nutrition (PN) can restore immune competence.

The bags destined for PN contain mostly lipids and are enriched with essential fatty acids, specifically polyunsaturated fatty acids (PUFAs) ω3 and ω6. It is now clear that lipids, including PUFAs, influence the immune system: ω3 have an anti-inflammatory effect, thus repressing the immune response, whereas ω6 promote Th1-like responses.

Although current guidelines suggest caution in the use lipid emulsions of ω6 in patients with uncontrolled inflammatory responses, such as those underlying autoimmune diseases, these may instead be advisable in patients unable to develop an adequate antitumoral response.

The goal of clinical research is the personalization of therapies which include supportive therapies, such as nutritional support. It is indeed possible to predict that, in the near future, the individualized choice of specific nutrients could allow the patient not only to deal with chemotherapy with less toxicity, but also to promote advantageous immune responses and, therefore, survival.

Objectives: We have studied the effects on the immune system of two different formulations of PN, one based on olive oil (enriched in ω6 fatty acids) and one on fish oil (enriched in ω3 fatty acids), on the immune system of patients in the final stages of HNSCC.

Materials & methods: Peripheral blood was obtained before the initiation of NP (T0), and after 1 week (T1). Deep immunophenotyping through polychromatic flow cytometry was performed, including detailed cell counts and in vitro stimulation of innate and adaptive immune cells.

Results : The results show that already after 1 week of PN with the olive oil-based formulation the immune system is poised for antitumoral, with a significant reduction in immune-suppressive T regulatory cells and an increase in IFNγ-secreting Th1 lymphocytes.

DOI: 10.1002/eji.201970300

Conclusions: The data suggest that in patients with terminal cancer, the choice of PN can impact the immune system favouring anti-tumor responses.

P181

Cisplatin suppresses stabilin-1 mediated clearance of EGF by tumor-associated macrophage

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Question. Tumor-associated macrophages (TAMs) are a major component of innate immunity supporting primary tumor growth and metastasis. Chemotherapy is one of the main treatment strategies for solid tumors, while chemoresistance is the major limitation of the chemotherapy for patients with various types of cancer. In the present research we investigated the influence of chemotherapeutic drug cisplatin on clearance function of TAMs.

Methods. For the endocytic uptake of EGF and acLDL we used flow cytometry analysis. Confocal microscopy was used for the analysis of stabilin-1-mediated internalization and endocytic trafficking of EGF and acLDL in CHO cells and in modeled TAMs. We performed whole-transcriptome next-generation sequencing (NGS) of RNA samples obtained from our modeled TAMs, differentiated in the presence of MCF-7 or Colo206F supernatants. Reactome database was used for identify the pathways which are involved in membrane transport, including endocytosis. Validation of sequencing data by real-time PCR was performed for selected genes implicated in the endocytic uptake: DNM3, STX8, DENND1A and EHD1.

Results. For the first time we demonstrated that stabilin-1 ectopically expressed in CHO cells mediates endocytic uptake of EGF, key growth factor stimulating progression of breast and colorectal cancer. In the model of primary human TAMs differentiated in the presence of conditioned supernatants of breast cancer (MCF-7) and colorectal cancer (Colo206F) cell lines, we have demonstrated that cisplatin decreases stabilin-1-mediated internalization and endocytic trafficking of EGF as well as general scavenger receptor ligand acLDL, without affecting gene expression of scavenger receptor stabilin-1. Molecular mechanisms of cisplatin effect on TAMs were uncovered using high throughput RNA sequencing. Gene set enrichment analysis identified that cisplatin contributes to defects in endocyting machinery reducing membrane biogenesis and vesicular transport. Significant suppression of DNM3, STX8, DENND1A and EHD1 genes expression by cisplatin was confirmed by RT-PCR.

Conclusions. We suggested, that suppression of receptor-mediated clearance of tumor-supportive factors, such as EGF, by chemotherapeutic drugs may potentially lead to the tumor progression and/or relapse as a mechanism of macrophage-mediated chemoresistance.

P182

Cytokine induced alterations in microRNAs and immune relevant markers in hematological diseases

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Background: MicroRNAs (miRNA) have been studied in recent years as they emerged as possible targets for the treatment of hematological malignancies. Hematological diseases comprise a family of diverse stem cell as well as immune cell malignancies including Myelodysplastic syndrome (MDS) and Acute myeloid leukemia (AML). Communication of cells in the stem cell niche, is regulated by different processes among them are the action of microRNAs and cytokines/chemokines. Cytokines are known to modulate marker profiles of immune cells and can modulate the microRNA profile or machinery. The role of aberrantly expressed microRNAs (miR-15, -29, -34, -143, 181b) in MDS and AML for cytokine-induced immune regulation is unknown and therefor investigated in this study.

Methods: Different human leukemic cell lines (THP-1 and HL-60) as well as MDS-L cells were treated with IFNgamma, TNFalpha, IL-1beta and their combinations. The expression of HLA-I/II, B7-H1, B7-H3, CD40, CD54 and CD85j was analyzed by flow cytometry. Conventional RT-qPCR was used to investigate the expression of B7-H1, B7-H3 and IL-1beta mRNA, stemloop qPCR to analyze various microRNAs (miR-34a, miR-324, miR-15a, miR-29a, miR-143 and miR-181b). Furthermore, miRNA mimics were used to overexpress miR-143 and miR-181b in THP-1 cells.

DOI: 10.1002/eji.201970300

Allogeneic CD4 T cell co-culture assays were used to study the impact of miR overexpression on T cell immune responses.

Results: All cell lines investigated showed no alteration of B7-H3 and CD85j expression upon cytokine treatment whereas all other molecules were affected at least in one of the cell lines. MHC-II and CD54 showed the strongest alteration in expression. Combinations of IFNgamma and TNFalpha led to synergistic effects for B7-H1, CD40 and CD54. Among the investigated miRNAs, miR-29a showed the most significant upregulation upon IFNgamma treatment of up to 6fold in THP-1 cells, 2fold in HL-60, whereas MDS-L cells showed no regulation for the same miR. Overexpression of miR-143 and miR-181b did not led to altered proliferation neither cytokine production of CD4 T cells in allogeneic co-culture assays.

Conclusions: We identified cell type specific cytokine induced alterations in immune relevant molecules on hematopoietic precursor cells. MiR-29 as previously suggested responds to cytokine treatment in AML cells, not MDS. Allogeneic CD4 T cell effector functions were unaltered upon stimulation with THP-1 cells overexpressing miR-143 and mir-181b.

P183
Myelodysplastic syndromes – the role of microRNAs and T cells
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Introduction: Myelodysplastic syndromes (MDS) are a group of bone marrow failures leading to underdeveloped and consequently immature and nonfunctional blood cells. Genetic and epigenetic alterations as well as changes in the bone marrow environment are some of the pathophysiological causes. MicroRNAs are known to play an important role in almost all biological processes among them are hematopoiesis and T cell functions. MicroRNA-34a, -143, and -181b are differentially expressed in unsorted bone marrow samples from MDS patients as compared to healthy controls.

Objectives: The aim of the study is to investigate the influence of differentially expressed microRNAs in MDS on the hematopoietic stem cell and the cells of the stem cell niche including T cells. Furthermore, we want to identify the biological targets of these microRNAs to reveal new possible therapeutic strategies and/or prognostic markers.

Material & Methods: We transfected microRNA-mimics polymer based and plasmids (miR-34a, -143 and -181b) by the use of a lentiviral system into the leukemic cell line THP-1 and the MDS cell line MDS-L. These miR-transfected cells were used in co-culture assays with age mached healthy dondor T cells. Additionally we transfected the donor T cells polymer based with miRs. To identify the role of these microRNAs on the stem cell level we used miR-transfected cell lines and primary cells in i) proliferation and cell cycle analyses, ii) colony forming unit (CFU) assays and iii) in PMA driven monocyte differentiation studies.

Results: We successfully transfected THP-1 and MDS-L transient and stable and primary T cells transient with miR-34a. MiR-transfected T cells showed no difference in polyclonal activation by anti CD3/CD28. MiR-transfected THP1 and MDS-L cells had neither impact on allogeneic T cell co-culture activation. However, miR-34a overexpression had functional consequences on THP-1 cell proliferation. The successful differentiation of THP-1 and MDS-L into monocytes like cells yielded in an adherent cell type. Additionally, CFU assays were successfully established with murine and human bone marrow material.

Conclusion: Although miR-34a does not affect T cells directly, it has impact on proliferation of hematopoietic precursor cells. The established cell differentiation protocols as well as the CFU assay serve as starting point to investigate the influence of microRNAs on the hematopoietic stem cell level in future analyses.

P184
Mast cells influence ovarian tumor growth *in vitro* and *in vivo*
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Introduction Ovarian cancer has the highest mortality rate among female reproductive tract malignancies, with a 5-year survival rate of only 41%. A complex network including the interaction between the tumor itself and immune cells

DOI: 10.1002/eji.201970300

regulates the tumor micromilieu and thus its establishment and growth. The participation of mast cells (MCs) on ovarian tumor pathophysiology and tumor angiogenesis is poorly understood.

Objectives As MCs possess both anti-tumorigenic and pro-tumorigenic capabilities we aimed to understand their effect on tumor cell proliferation and tumor growth employing *in vitro* and *in vivo* approaches.

Materials and methods Wound healing assays using human (OVCAR-3 and SK-OV-3) and murine tumor cell lines (ID8 and ID8 F3) were conducted. Mast cells (human: HMC-1; murine: MC/9) were given directly to the tumor cells or a transwell system was employed to determine the effect of MC-derived soluble factors.

$5x10^6$ ID8 cells or ID8 F3 cells (deletion of p53) were injected subcutaneously into the flanks of 8-10 weeks old female C57BL6/J mice as well as mast cell-deficient B6.Cg-KitW-sh/HNihrJaeBsmJ mice (C57BL/6J background; $Kit^{W\text{-}sh}$). Control mice received 0.1 ml PBS. The tumor development was recorded weekly by high frequency ultrasound. The mice were sacrificed 14 weeks after tumor application.

Results We observed a diminished proliferation of human ovarian tumor cells upon cell-cell contact with HMC-1 mast cells or their supernatant. The *in vivo* application of ID8 cells into mast cell-deficient $Kit^{W\text{-}sh}$ resulted in significantly increased tumor growth when compared to C57BL6/J mast cell sufficient mice.

Conclusion Our *in vitro* results suggest that mast cells have a direct influence on ovarian tumor cell proliferation. *In vivo*, we indeed confirmed that the absence of host MCs is related to abnormally rapid tumor growth of ovarian cancer cells. We are currently investigating possible MC-derived mediators of this important effect. We suggest that MCs have a negative effect on ovarian tumor growth and may serve as a new therapeutic target.

P185

Phenotype of tumor-associated macrophages can be used in predictions of metastasis of prostate cancer

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TAMs (tumor-associated macrophages) are innate immune cells, which are present in prostate cancer (PC) tissue. TAMs play major role in tumor progression, invasion, neoangiogenesis, metastasis and resistance to therapy. Prostate cancer patients often have long time periods between curative intent surgery or radiation therapy until the time of biochemical recurrence or metastatic relapse is detectable but the disease at this stage can be incurable with the current treatment options. We believe that correctly identifying the phenotype of TAMs and the corresponding molecular biomarkers can predict the aggressiveness of PC, response to chemotherapy and development of postoperative metastasis. Tumor tissue samples were obtained from 40 PC patients who underwent radical prostatectomy. The samples were assessed by immunohistochemistry techniques followed by confocal microscopy. We have available data from the immunohistological analysis of several biomarkers, such as, CD68+, known as a pro-inflammatory marker in macrophages and Stabilin, which is a receptor protein with multiple functions such as receptor scavenging or angiogenesis.

P186

Role of γδ T cells in inflammatory bowel disease and colitis-associated cancer

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Inflammatory bowel disease is a complex chronic inflammatory disease of the human gut with no clear etiology. Colitis-associated cancer (CAC) is a CRC subtype that is associated with inflammatory bowel disease (IBD); it is difficult to treat and has high mortality.

DOI: 10.1002/eji.201970300

Because of the role of γδ T cells remain unclear in IBD progression and in the transition to CAC, we investigated their percentage, phenotypical features and effector functions in the intestinal mucosa of at early onset, long standing IBD patients and CAC patients, as compared to healthy subjects.

We found a reduced frequency of Vδ1 T cells in tissue from early and late IBD patients, and in contrast an increased frequency of Vd2 T cells in the gut of late IBD patients with effector memory and terminally-differentiated phenotypes. CAC tissue contained verily low percentages Vδ1 and Vδ2 T cells, but the latter expressed elevated levels of IFNγ, TNF-α and IL-17. Luminex analysis of supernatants obtained from biopsies of long standing IBD patients demonstrated the presence of elevated levels TNFα, IFNγ and IL17.

Moreover, in IBD patients with sustained chronic inflammation, we identify a yet unappreciated role of Vδ2 T cells in TNFα, IFNγ production suggesting that they also participate to the chronic inflammatory process. Conversely, both the frequency and the cytokine production ability of Vδ1 T cells was dramatically reduced. Gene expression profile highlighted that patients with sustained IBD have overexpression of pro-inflammatory cytokine genes. Highly suggestive of a possible linear progression existing from chronic intestinal inflammation to CAC, the study of Vδ2 T cell subsets in IBD and in CAC may provide the basis for the development of novel therapies with the aim to block the progression from IBD to CAC.

P187

Omics-Analyses of B16 and MC38 tumors and their Tumor-associated macrophages (TAM)

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Introduction: Tumor growth and progression are based on mechanisms to avoid efficient anti-tumor immune responses. In order to develop new innovative therapeutic strategies to target tumors, a detailed understanding of tumor- and micro milieu-specific mechanisms contributing to inefficient anti-tumor immune responses is essential.

In contrast to colon adenocarcinomas, melanomas are characterized by high-rate glycolysis resulting in tumor acidification which leads to a cAMP-mediated expression of the transcriptional repressor ICER in tumor-associated macrophages (TAM), ultimately resulting in functional polarization of macrophages towards a non-inflammatory M2 phenotype.

Objectives: The main objective is to analyze the cellular and molecular mechanisms regulating the induction of ICER in melanoma-associated macrophages (MAM) leading to the development of non-inflammatory M2-like macrophages.

Material & methods: To characterize the underlying glycolytic-dependent mechanisms of melanoma-specific immunoevasion comparative metabolome and secretome analyses of *ex vivo* purified melanomas and colon adenocarcinomas were performed. Further, we conducted transcriptome and proteome analyses of B16 melanoma and MC38 colon adenocarcinoma infiltrating TAMs.

Results: Melanomas are characterized by a high glycolytic activity which leads to an acidification of the tumor microenvironment. Our findings implicate that in this microenvironment MAMs in contrast to colon adenocarcinoma macrophages are polarized towards a non-inflammatory M2-phenotype in a cAMP and ICER-dependent manner.

Our data show that cAMP-signaling pathway related genes are upregulated in melanoma but not in colon adenocarcinoma infiltrating macrophages.

By Omics analyses we revealed that melanomas and colon adenocarcinomas differ both in their secreting enzymes and in their distribution of metabolites. These metabolomic analyses exposed that B16 melanoma is defined by a high turnover of DOPA. Investigations in dopamine affecting the macrophage phenotype showed their polarization towards a non-inflammatory phenotype as well.

Conclusion: Taken together, our findings identify that tumor acidification leads to a G-protein-coupled receptor and ICER-dependent polarization of tumor- associated macrophages towards a non-inflammatory M2 phenotype. Also strong metabolization of DOPA in melanomas seems to have an anti-inflammatory effect on macrophages.

DOI: 10.1002/eji.201970300

P188

Effects of CTLA-4 blockade in Ptch1$^{+/-}$ medulloblastoma immune infiltrate

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Introduction. Experimental and clinical studies provided compelling evidence that overcoming negative costimulatory regulators (checkpoints) results in effective therapy against a wide range of malignancies. CTLA-4 (CD152) blockade lowers threshold for T cell activation, sustaining priming and differentiation of anti-tumor CD4 and CD8 effector cells. *Ptch1+/-* heterozygous knockout mice, the mouse model for Gorlin syndrome, are a valuable preclinical model of medulloblastoma (MB), the most common brain tumor in children. Multimodal treatment, including surgical resection, chemotherapy and radiation, results in good cure rates for MB. However, the risk of recurrence and serious long-term side effects underscores the need for new therapeutic approaches. To date, there have been few successful immunotherapy settings targeting MB, prompting for studies on its microenvironment and effects on infiltrating immune cells.

Objectives. To assess the effects of CTLA-4 blockade on MB immune infiltrate.

Materials & methods. MB tumors derived from *Ptch1$^{+/-}$* mice were serially transplanted subcutaneously in syngeneic immune competent WT C57Bl/6 mice. When tumors reached the average volume required, animals were randomized and treated with anti-CTLA-4 mAb (clone 9D9, 100 mg/mouse i.p., every 5 days for three times) or control antibody. Tumor volumes were measured three times a week using a caliper. At sacrifice, tumor fragments, draining lymph nodes, spleens and colon samples were collected for histology, multi-parametric flow cytometry analyses or snap frozen for molecular analysis.

Results. Immune cells infiltrating tumors in control mice were quite rare (0.5-1.2% of CD45$^+$ cells) and mainly characterized by the presence of myeloid cells, identified by CD11b, CD11c, F4/80, Ly6G and Ly6C expression, and T cells. Most of CD4 and CD8 T cells were expressing CTLA-4, PD-1 and PD-L1 at high levels. A consistent percentage of CD4 cells were Foxp3$^+$ regulatory T (Treg) cells, also expressing checkpoint receptors. Preliminary results showed that CTLA-4 blockade improved the presence of CD45$^+$ cells, specifically of T cells, with a drastic increase in CD8, a consistent reduction of Treg cells, and a mild effect on CD4 cell number. Modulation of PD-1/PD-L1 was also observed.

Conclusion. Preliminary results showed the efficacy of the treatment with the anti-CTLA-4 blocking antibody in inducing a quantitative and qualitative improvement of the immune cell infiltrate in MB tumors.

P189

T cell-cytotoxicity towards pancreatic cancer cells is modulated by TRAIL-receptor 4

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One of the deadliest cancers with a mortality rate almost equal to its incidence rate is pancreatic ductal adenocarcinoma (PDAC). The poor prognosis is due to late diagnosis, acquired immune evasion mechanisms, highly aggressive growth and resistance to current therapeutic treatments. Previously, we have shown that CD8+ αβ T cells as well as different γδ T cell subsets infiltrate the PDAC tissue. Interestingly, the abundance of γδ T cells was reported to have a positive prognostic impact on survival of cancer patients. Since γδ T cells utilize TRAIL for killing of tumor cells in addition to granzyme B and perforin, we investigated the role of the TRAIL-/TRAIL-R system in γδ T cell-cytotoxicity towards PDAC cells. Interaction of TRAIL with TRAIL-R1/-R2 can induce cell death and is believed to be important for the immune surveillance of tumors, while interaction with TRAIL-R3/-R4 negatively regulates TRAIL-induced apoptosis.

DOI: 10.1002/eji.201970300

Coculture of several PADC cells with γδ T cells, but not αβ T cells, resulted in a moderate lysis of tumor cells. The lysis of Colo357- and PancTuI cells was independent of TRAIL as it was not inhibited by the addition of neutralizing anti-TRAIL antibodies or TRAIL-R2-Fc fusion protein. In accordance, knockdown (KD) of death receptors TRAIL-R1 or TRAIL-R2 in Colo357- and PancTuI cells had no effect on γδ T cell-mediated cytotoxicity.

Interestingly, however, KD of a decoy receptor TRAIL-R4, which robustly enhanced TRAIL-induced apoptosis in PDAC cells, almost completely abolished the γδ T cell-mediated lysis of Colo357 cells. The inhibition of cytotoxicity was associated with a reduced granzyme B release by γδ T cells, and mediated by an enhanced cyclooxygenase (COX)-2 expression and increased release of COX-2 metabolite prostaglandin E2 by TRAIL-R4-KD cells.

Importantly, reduced granzyme B release by γδ T cells cocultured with TRAIL-R4-KD Colo357 cells was partially reverted by bispecific antibody [HER2xCD3] and led in consequence to an enhanced tumor cell-lysis. Likewise, inhibition of COX partially enhanced γδ T cell-mediated lysis of TRAIL-R4-KD cells. The combination of bispecific antibody and COX-inhibitor completely restored the lysis of TRAIL-R4-KD cells by γδ T cells.

In conclusion, we uncovered an unexpected novel role of TRAIL-R4 in PDAC cells. In contrast to its known pro-tumoral and anti-apoptotic function, TRAIL-R4 augments the anti-tumoral cytotoxic activity of γδ T cells.

P190

Aberrant up-regulation of iNOS/NO system is correlated with an increased abundance of Foxp3+ cells and reduced effector/ memory cell markers expression during colorectal cancer: Immunomodulatory effects of cetuximab combined with chemotherapy.

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Background and aims: Colorectal cancer (CRC) remains the most cancer type related to chronic inflammation, however the mechanisms that link inflammation to CRC development and progression are still poorly understood. Our study aimed to investigate one of the prominent inflammatory response in cancers, iNOS/NO system.

Methods: In this regard, we evaluated the link between the iNOS/NO system and CRC progression, its relation with the host *immune responses and its response to* cetuximab combined with chemotherapy using different approaches and techniques (Griess method, ELISA, Immunofluorescence, HE staining, Immunohistochemistry, primary cell culture..).

Results: We found that the nitrite levels were *nearly twice higher* in metastatic CRC plasma and culture supernatants from PBMCs and tumor explants compared with those without metastases and healthy controls. Interestingly, we showed that the highest iNOS expression and NO levels are present in the damaged CRC tissues that have highest *leukocyte infiltration*. Our findings highlight the implication of iNOS/NO system in tissue alteration and leukocyte invasion. Thus, we observed imbalance between effector/memory T cell markers and Treg transcription factor (Foxp3). Accordingly, we detected higher IFNγ and T-bet expression and levels in *colorectal tumor tissues* at *early stage*. In contrast*, consistent with* iNOS and Foxp3 expression, TGFβ, CTLA-4 and IL-10 were significantly related to the tumor stage progression. Furthermore, our study revealed that Cetuximab combined with chemotherapy treatment markedly down-regulates iNOS/NO system as well as IL-10 and TGFβ levels*.* Altogether, we stipulated that cetuximab can potentiate the efficacy of chemotherapy, particularly by iNOS/NO system and immunosuppressive cytokines modulation.

Conclusion: Thus, we suggest that iNOS/NO system may represent an attractive candidate biomarker for monitoring CRC progression*, malignity* and response to treatment.

P192

Unveiling the role of mast cells in colitis-associated mouse colon cancer

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DOI: 10.1002/eji.201970300

Introduction: Mast cells (MCs) are unique tissue-resident sentinels that secrete a diverse array of mediators that can stimulate, modulate, or suppress the immune responses. They are recruited in several types of tumor, including the colorectal cancer. However, their role during carcinogenesis and tumor growth hasn't been clearly elucidated yet.

Objectives: The aim of this study is to evaluate the significance of MCs in colon tumor progression from colitis to cancer using a mouse model of colitis-related colorectal carcinoma (CRC).

Materials & methods: To examine the role of MCs in intestinal tumorigenesis, dextran sodium sulfate (DSS) in drinking water was used to induce Colitis to C57BL/6 mice while colon tumors were induced by intraperitoneal injection of azoxymethane (AOM). We assessed MCs number and localization by a combined use of cytofluorimetric, histochemical and confocal microscopic analysis. Expression of MC proinflammatory cytokines was examined by intracellular fluorescent staining.

Results: In comparison to healthy control mice, a higher number of c-kit$^+$ and FcεRI$^+$ double positive MCs were detected in colitis-affected colon and in adjacent normal mucosa of AOM/DSS treated-mice. MCs were further increased in the tumor tissue as also confirmed by toluidine blue staining that revealed a high density of these cells in both mucosal and submucosal regions. Interestingly, compared to MCs located in tumor-free colonic tissue, infiltrating MCs showed an activated phenotype. Indeed, they were able to produce higher amount of interleukin (IL)-6 and tumor necrosis factor α (TNF-α), which represent key cytokines in CRC progression. No major differences were observed for IL-9 and IL-17 production.

Conclusion: Using conventional model of colitis-induced CRC, we showed an intra-tumoral accumulation of MCs characterized by an activated phenotype in term of cytokine profile, thus suggesting their contribution in the pathogenesis and progression of the carcinoma, fostering the idea of MC targeting as a way to impair tumor growth.

P193

Pro-tumoral role of Complement activation in murine sarcoma models

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Cancer related inflammation (CRI) plays a fundamental role in fuelling tumor appearance and development. Although the important contribution of complement activation to inflammation, its role in CRI still remains understudied.

Recently our group demonstrated the pro-malignant role of complement activation in models of mesenchymal (3-MCA-induced) and epithelial (DMBA/TPA-induced) inflammation-driven skin carcinogenesis, showing that mice deficient for the central complement component C3 were protected from tumor development.

Further experiments revealed the deposition of C3-cleavage products on vessels and tumor cells of sarcoma tumor tissues. The C3 deposition on tumor cells was also observed *in vitro*, both on 3-MCA-derived sarcoma and on different murine/ human cancer cells. Both *in vitro* and *in vivo* experiments suggested that the activation of the Classical/Lectin pathways was involved in this process.

Analysis of C3-downstream mechanism(s) of protection in two different sarcoma murine models showed that C3aR- but not the C5aR1- or C5L2-deficient mice were protected from tumor growth in a transplantable model of sarcoma (MN-MCA1), as well as in the 3-MCA-induced carcinogenesis model. *C3-/-* reduced tumor growth was associated with reduced macrophages, showing a M1-like phenotype, and enhanced activated effector CD8+ frequencies in tumor, suggesting that these cells could play a role in the protective phenotype observed in C3-deficient mice. Similar results were obtained with *C3aR-/-* mice, suggesting that the C3a/C3aR axis was involved in the pro-tumoral role of complement in sarcoma models.

All together our results indicate that complement is an essential component of tumor-promoting inflammation in sarcoma influencing macrophage and T cell functional activation.

P194

Spatial oxidation of L-plastin and reversibility by the Thioredoxin 1 system is critical for actin-based functions of T-cells and tumor cells

DOI: 10.1002/eji.201970300

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A pro-oxidative tumor micromilieu is a key determinant in down-modulation of anti-tumor immune responses. In contrast to tumor cells, which highly upregulate anti-oxidant systems, T-cells possess very low levels of anti-oxidants. This makes T-cells at the tumor site vulnerable to immunosuppressive pro-oxidative effects, e.g. by thiol oxidation on proteins. Particularly, Thioredoxin 1 (Trx1), an important component of the cellular antioxidant defense system, is critical for maintaining proteins in the reduced state, thereby ensuring proper cellular responses. Thus, to understand the molecular basis for the failure of anti-tumor T-cells responses and success of the tumors in a pro-oxidative micromilieu, the regulation of redox sensitive proteins and their interplay with the anti-oxidant systems need to be investigated.

In our study, we uncovered that L-plastin (LPL), an actin-bundling protein that plays a role in various immune cell functions and tumor progression, is reversibly regulated by Reactive Oxygen Species (ROS) induced thiol oxidation on *Cys101* and *Cys42*. The reduction is mediated by the Trx1 system, as shown by Trx1 trapping, Trx1 knock-down or blockage of Trx reductase 1 (TrxR1) by auranofin in various tumor cell lines. Mechanistically, thiol oxidation of LPL diminished its actin-bundling capacity and actin-based cellular functions of tumor cells. Ratiometric imaging using an LPL-roGFP-Orp1 fusion protein and a dimedone-based proximity ligation assay revealed that LPL oxidation in tumor cells occurred primarily in actin-based cellular extrusions, where the Trx1 system is largely excluded. In line with this, at the cellular level, ROS induced oxidation of LPL was more prominent in T-cells which have lower Trx1 levels than tumor cells. Currently, we are investigating the spatial oxidation of LPL in resting and stimulated T-cells in response to several physiological stimuli. Our preliminary findings point towards involvement of spatial LPL oxidation on physiological functions of T-cells as well as their pathological suppression depending on the amount and location of oxidized LPL. Altogether, these findings highlight a delicate balance between the antioxidant capacity of T-cells, oxidation levels on specific proteins and cellular functions. The interplay between the antioxidant state of T-cells and global protein oxidation needs to be further addressed to understand the importance of redox modulation for T-cell functions.

P195
Macrophages involvement in neuroendocrine tumor behaviour and progression

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Neuroendocrine tumors (NETs) are rare neoplasms showing a wide spectrum of clinical behaviors. Therapeutic options available for NET treatment are rarely curative and mostly palliative, as NETs frequently show resistance to pharmacological therapy. Cancers develop in complex tissue environments, which they depend on. Tumor-associated macrophages (TAMs) are a major cellular component of the tumor microenvironment. Two polarized state of macrophages are described in literature: M1 (anti-tumor promoting effects) and M2 (pro-tumor promoting effects).

The study of tumor microenvironment could provide new insights into the behaviour of pulmonary and pancreatic NETs, more effective therapeutics strategies that could overcome pharmacological resistance and provide new immune markers.

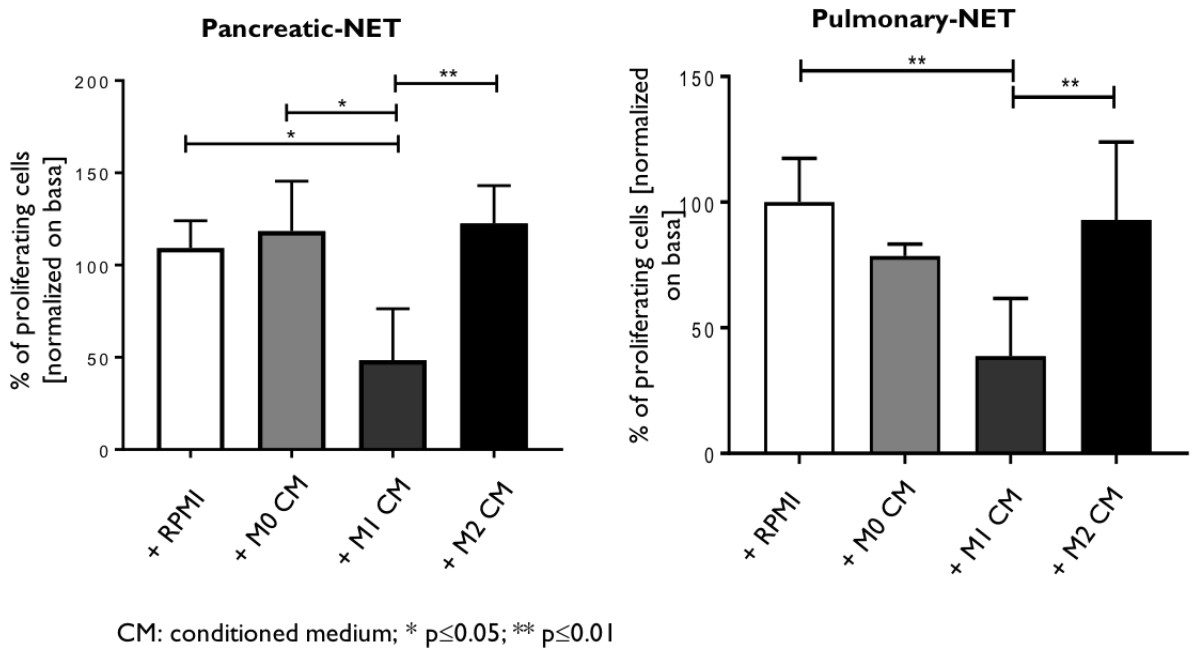
Monocytes, purified from buffy coat, where differentiated for 7 days with M-CSF. M0 macrophages were incubated, for 24h, with IL-4 or with IFN-γ and LPS to obtain M2 or M1 polarized macrophages respectively. To assess the effects of M1, M2 macrophages on biological behavior of NET, primary cells and NET cell lines QGP1 (pancreatic-NET) and H727 (pulmonary-NET) where cultured with macrophages conditioned medium (CM). Cell cycle was evaluated by facs analysis. The effects of tumor cells CM on macrophages were evaluated by Real-Time analysis for M1, M2 markers.

DOI: 10.1002/eji.201970300

We found out that M1 macrophages significantly decreased cell growth, viability and cell cycle of QGP1 and H727 cell lines. Interestingly, the CM of NET cell lines promoted the differentiation of macrophages into an M2 phenotype, after 24h of culture. Data of primary NET cells derived from patients, showed that CM of M1 macrophages strongly decreased cell proliferation of both pancreatic and pulmonary NET cells. Co-culture of QGP1 or H727 cells with macrophages showed that only M1 macrophages are able to induce apoptosis and necrosis of tumor cell lines. Preliminary results on angiogenesis showed that CM of M1 macrophages is able to decrease VEGF protein in NET cell lines and pulmonary NET cells form patients.

M1 macrophages have a potent anti-tumor effect, capable of affecting proliferation and tumorigenicity of NET cell lines. Interestingly, NET cell lines contribute to promote a M2 phenotype of macrophages, suggesting a potential involvement of tumor microenvironment in the behaviour of pulmonary and pancreatic NETs.

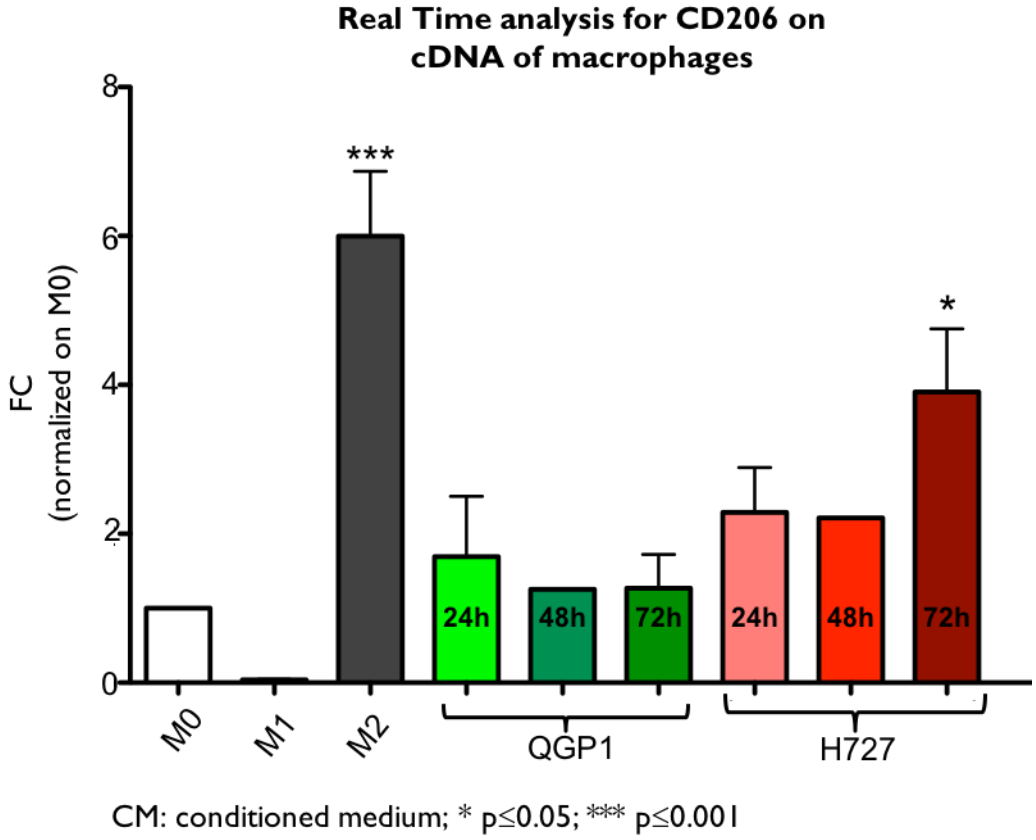
Figure 1



CM: conditioned medium; * p≤0.05; ** p≤0.01

Figure 2

DOI: 10.1002/eji.201970300



P196

IL17-Signalling and Effector T-cell function in the microenvironment of *Pancreatic Ductal Adenocarcinoma*

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Introduction: Pancreatic ductal adenocarcinoma is a tumor type showing highly desmoplastic properties and a pronounced stroma, supressing antitumoral immune cell activity and increasing resistance against chemotherapy, resulting in poor prognosis for patients[1]. Recent publications suggest a role of pro-inflammatory cytokine IL-17 not only in the context of autoimmune diseases, but also in development of pancreatitis and the shift from pancreatic intraepithelial neoplasia (PANins) to the onset of PDACs[2,3]. In this context, CD8+ T-cells[4,5] are of special interest, because of their double role, either as IL-17-producers (Tc17) cells promoting tumor development or their antitumoral role as cytotoxic T-lymphocytes (CTLs), which might open up new avenues of treatment by modulation of these immune cells in combination with classical antitumor therapies6.

Objectives: We focus on the effects of Tc17 cells and CTLs in the tumor microenvironment to identify the direct and indirect effects of IL-17 signalling on pancreatic cancer cells and other potential interaction partners in the tumor stroma. Furthermore, we employ immunomodulatory treatment of T-cells to reduce IL-17 secretion and increase an antitumoral CTL phenotype.

Materials and Methods: Antigen-based xenograft tumor mouse models, either subcutaneous or by ultrasound-guided orthotropic injection and a genetic mouse model (KPC) are used in conjunction with In vitro methods and analysis of human patient samples.

DOI: 10.1002/eji.201970300

Results: Our data shows a positive correlation between tumor onset, growth and the frequency of Tc17 cells in the tumorenvironment. Additionally, Tc17 cells that were changed to a CTL-like phenotype by immunomodulatory treatment promote tumor rejection and reduce tumor burden inVivo.

Conclusion: CD8+ T-cell-based IL-17 signalling as a potential promotor of tumorigenesis can be ameliorated by immunomodulatory treatment of T-cells, reducing overall IL-17 producer levels and turning pro-tumorigenic Tc17 cells into antitumoral CTL-like cells.

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P197

IFN-γ and TNF-α production by T cells in HNSCC stroma promotes a distinct transcriptional signature with immunosuppressive properties by tumor-enriched mesenchymal stem cells

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Question: Mesenchymal stem cells (MSC) are enriched in Head-Neck squamous cell carcinoma (HNSCC) and display immunosuppressive properties, thus favoring tumor immune escape (Liotta F., 2015). The identification of the molecular programs involved may suggest novel therapeutical targets.

Methods: HNSCC specimens were enzyme-digested. Tumor infiltrating lymphocytes (TIL) were analyzed by flow cytometry to investigate their phenotypic and functional properties. MSC were derived from HNSCC and bone marrow (BM) and expanded *in vitro*. Transcriptome data were generated via microarray technology. Quantitative PCR and flow cytometry were used to validate transcriptome data. T cell proliferation was evaluated via 3H-TdR incorporation assay.

Results: We observed an accumulation of IFN-γ and TNF-α producing T cells in HNSCC specimens. T regulatory (Treg) cells were also enriched if compared to peripheral blood (PB). To investigate how IFN-γ and TNF-α affect the immunosuppressive potential of MSC, we obtained transcriptome data from resting or IFNg+TNFa stimulated BM- and HNSCC-MSC. Principal component analysis showed that BM- and HNSCC-derived MSC have a distinct transcriptional signature either resting or after cytokine stimulation, thus suggesting that are distinct cell subsets. Looking for genes selectively induced by cytokine treatment that may confer immunosuppressive potential to MSC we found IDO1, PD-L1 (CD274) and IL4I1. We confirmed microarray data by qPCR or flow cytometry. Selective inhibition of these three pathways during In vitro MSC-T cell cocultures highlighted their immunosuppressive capacities.

Conclusions: HNSCC-TIL are enriched in Treg cells, while T effector cells-derived IFN-γ and TNF-α potentiate MSC immunosuppression via activating several non-redundant pathways. Altogether, these mechanisms favor HNSCC immune-evasion.

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DOI: 10.1002/eji.201970300

P402
Diversity of CD4 blood T-cell clonality predicts longer survival with CTLA4 or PD-1 checkpoint inhibition in advanced melanoma
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Introduction: Recognition of cancer antigens drive clonal expansion of cancer-reactive T cells, which is reflected by restricted T-cell receptor (TCR) repertoires. Several studies showed that pre-existing tumor-specific T cells are associated with better outcome of cancer patients under anti-CTLA4 or PD-1 checkpoint inhibition.

Objectives: To understand how tumor escapes anti-tumor immunity

Materials & methods: We analyzed tumor-associated T-cell repertoires from patients with advanced melanoma upon the therapeutic blockade of cytotoxic T-lymphocyte-associated protein 4 (CTLA4) or programmed cell death 1 (PD-1). TCR Vβ-gene spectratyping allowed us to quantify restrictions of T-cell repertoires and, further, diversities of T-cell clones.

Results: Surprisingly, the blood TCR repertoires were variably restricted in CD4 and extensively restricted in CD8 T cells of patients with advanced melanoma, and contained clones in both T-cell fractions prior to the start of immunotherapy. A greater diversification especially of CD4 blood T-cell clones before immunotherapy showed statistically significant correlations with long-term survival upon CTLA4 or PD-1 inhibition. Analysis of TILs and corresponding blood available in one patient indicated that blood clonality may at least partially be related to the clonal expansion in the tumor microenvironment. In patients who developed severe immune-related adverse events (IrAEs), CD4 and CD8 TCR spectratypes became more restricted during anti-CTLA4 treatment, suggesting that newly expanded oligoclonal T-cell responses may contribute to IrAEs.

Conclusion: Our data propose that the diversity of T-cell clonality in the blood may reflect broader T-cell recognition of tumorwhich can induce effective anti-tumor response under checkpoint inhibitions, and thus be a prognostic biomarker for anti-CTLA4 or PD-1 treatment.

Immunometabolism and immunosignaling (P198-P240)

P198
Altered Metabolism in Differentially Educated NK Cell Populations
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Introduction: Natural killer (NK) cells are important in controlling intracellular pathogens via cytotoxic action. Interestingly, the cytotoxic potential of an NK cell can be altered through interactions of inhibitory receptors with their ligands in a process called education. The NK cells receiving these inhibitory signals are termed educated and are more effective at killing than their uneducated counterparts but the mechanisms leading to this potentiation are still not fully understood. Recent work has revealed that metabolism and calcium signalling are crucial in the function of NK cells indicating a possible link to education.

Objectives: We sought to find whether the education state of human NK cells was linked to their metabolic profile and if differences in calcium signalling were associated with education or cellular metabolism.

Methods: Uptake of the metabolic substrates 2-NBDG and BODIPY FL C16 were compared between educated and uneducated NK cell populations. Both 2-NBDG, a fluorescent glucose analgoue and BODIPY FL C16, a fluorescent palmitate, were measured using flow cytometry. Oligomycin and 2-DG were used for blockade of oxidative phosphorylation and glycolysis respectively and CD107a expression was used to assess NK cell function. Calcium signalling was measured using fluorescent microscopy of the ratiometric calcium dye Fura-2.

DOI: 10.1002/eji.201970300

Results: It was found that both BODIPY and 2-NBDG had increased uptake in educated compared to uneducated NK cells. Interestingly, comparison of NK cells educated via KIR or NKG2A showed that NKG2A educated NK cells were the main contributor to this difference. Metabolic blockade of oxidative phosphorylation significantly decreased the degranulation of NK cells with a greater decrease seen in KIR educated compared to NKG2A educated NK cells while only small differences were seen following blockade of glycolysis. Increased calcium flux was seen in educated NK cells compared to uneducated but it is yet unknown whether this difference is directly linked to metabolic changes.

Conclusion: These results indicate that metabolism plays a role in the functional differences seen between educated and uneducated NK cells. They further indicate that NKG2A educated NK cells may remain more functionally competent than KIR educated if oxidative phosphorylation is restricted. Understanding metabolic programming in NK cell education may unveil future targets to manipulate NK cell function for use in a clinical setting.

P200

Dietary intake regulates the circulating inflammatory monocyte pool

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Caloric restriction is known to improve inflammatory and autoimmune diseases. However, the mechanisms by which reduced caloric intake modulates inflammation are poorly understood. We found that short-term fasting reduced monocyte metabolic and inflammatory activity and drastically reduced the number of circulating monocytes in humans and mice. Regulation of peripheral monocyte numbers was dependent on dietary glucose and protein levels. Specifically, we found that activation of the low-energy sensor 5'-AMP-activated protein kinase (AMPK) in hepatocytes and suppression of systemic CCL2 production by peroxisome proliferator-activator receptor alpha (PPARα) reduced monocyte mobilization from the bone marrow. Importantly, while caloric restriction improves chronic inflammatory diseases, fasting did not compromise monocyte emergency mobilization during acute infectious inflammation and tissue repair. These results reveal that caloric intake and liver energy sensors dictate the blood and tissue immune tone and link dietary habits to inflammatory disease outcome.

P201

TCAIM-induced mitochondrial cristae formation inhibits metabolic reprogramming and CD8$^+$ T cell effector differentiation

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To induce full effector cell differentiation upon activation T cells need to undergo a metabolic reprogramming from catabolic pathways with ATP production by mainly oxidative phosphorylation towards anabolic processes for nucleotide, amino acid and fatty acid synthesis. To do so T cells shift to aerobic glycolysis and glutaminolysis for building block and ATP synthesis. This metabolic shift relies on mitochondrial cristae resolution and fission. However, what mechanisms control activation-induced mitochondrial dynamics is less clear. We have previously shown that the mitochondrial protein TCAIM (T cell activation inhibitor, mitochondrial) is highly expressed in naïve and quiescent T cells but downregulated upon activation and effector cell differentiation. Notably, T cells with reinforced *Tcaim* expression could not get activated or reject allogeneic skin grafts. This diminished activation was associated with reduced mitochondrial redistribution and mROS production, as we showed for CD4$^+$ T cells. We now investigated

DOI: 10.1002/eji.201970300

whether TCAIM interferes with CD8$^+$ effector T cell differentiation too and examined the mechanism by which TCAIM controls mitochondrial functions.

Flow cytometry analysis of polyclonally stimulated naïve CD8$^+$ T cells isolated from mice with a T cell specific *Tcaim* overexpression (KI) or knockout (KO) confirmed an inhibitory role of *Tcaim* expression for CD8$^+$ T cell effector differentiation and Granzyme-B or IFN-g production. Metabolite and RNA Seq analysis of wild type and *Tcaim* KI CD8$^+$ T cells indicated an abrogation of activation induced metabolic shift towards glycolysis and glutaminolysis, as metabolites like lactic acid, serine or sphingosine as well as mRNAs of crucial anabolic regulators e.g. *Ldha*, *Hif1a*, *c-Myc* or *Gls2* were reduced. Interestingly, electron microscopy revealed tight cristae formation and inhibition of activation-induced mitochondrial fission upon *Tcaim* KI. In contrast, *Tcaim* KO caused highly fragmented mitochondria even in unstimulated naïve cells. To examine how TCAIM controls mitochondrial dynamics we performed combined co-immunoprecipitation and MS analysis and found proteins involved in cristae formation and mitochondrial fusion (e.g. MIC60, SAM50) as TCAIM interaction partners.

Taken together, we identified activation-induced TCAIM downregulation as an important mechanism for induction of mitochondrial cristae dissolution and fission, and thus metabolic reprogramming during effector T cell differentiation.

P202

Leptin induces TNFa-dependent inflammation in acquired generalized lipodystrophy and combined Crohns disease

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Introduction: Leptin, a fat-derived adipokine, has been suggested to modulate intestinal inflammation in mice. However, clinical evidence regarding its immune stimulatory potential in human Crohn"s disease remains sparse.

Objectives and methods: We here describe a case of a 21-year-old patient with the solitaire combination of acquired generalized lipodystrophy and combined Crohn"s disease (AGLCD) featuring a complete lack of visceral and subcutaneous adipose tissue, absent leptin production and severe intestinal inflammation, who received daily injections with 2.5 mg recombinant N-Methionylleptin (rLeptin). Using mass and flow cytometry, immunohistochemistry as well as metabolic Seahorse analyses, we characterized the *in vivo* effects of rLeptin substitution on the patient"s immune cell composition and function and compared our results to a cohort of healthy donors and Crohn"s disease patients.

Results: Deep immune profiling by mass and flow cytometry revealed extensive functional and metabolic changes in the immune cell compartment of the AGLCD patient when compared to healthy controls or patients with Crohn"s disease. Substitution with recombinant leptin altered the immune cell metabolism, differentiation and function of human T, NK and myeloid cells ultimately resulting in increased TNFa production and aggravating Crohn"s disease in the AGLCD patient, which could be reversed by anti-TNFα therapy

Conclusions: Our results suggest that leptin might play a crucial role in human immune cell homeostasis and contributes to intestinal autoimmunity in a TNFα-dependent manner.

P204

AhR-driven immunometabolic program in type-1 dendritic cells restrains antitumor responses

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Introduction: The immune system has a key role in controlling tumor initiation and growth. Conventional dendritic cells (DCs) are immune cells critical for innate and adaptive immune responses, and they include cDC1 and cDC2 subtypes. cDC1s are critical for CD8$^+$ T-cell priming early in an antitumor response, and this function is affected by environmental signals. The transcription factor Aryl hydrocarbon Receptor (AhR) is an environmental "sensor" of specific metabolites, both endogenous and exogenous in nature, *via* association with other cell-intrinsic transcription factors. Emerging data have shed light on an unexpected role of AhR in fostering tumor escape mechanisms.

DOI: 10.1002/eji.201970300

Objectives: We assessed the impact of AhR deletion in cDC1 on the immune response to tumors.

Materials & methods: cDC1 were differentiated from C57BL/6J bone marrow cells after FLT3L treatment for 9 days. Cytokine production in cDC1 and in cDC1–$CD8^+$ T-cell co-coltures was analyzed by intracellular staining. $Ahr^{fl/fl}$ XCR1-Cre and $Ahr^{fl/fl}$ control mice were inoculated subcutaneously with fibrosarcoma tumor cell line. Tumor growth was monitored daily for 30 days.

Results: Whole genome analysis revealed that AhR is expressed in mature $CD24^+CCR7^+$ cDC1 to greater extent than in cDC2 and pDCs. In particular, in mature cDC1, AhR in combination with the cDC1-specific factor IRF8 drived the expression of immunosuppressive indoleamine 2,3-dioxygenase (IDO1). *Ido1* promoter analysis revealed two AICEs (AP1-IRF Composite Elements) motifs, confirmed by ChIP-seq studies as capable of binding IRF8 in cDC1. Selective genome editing of AICE1 and AICE2 motifs by CRISPR CAS9 technology suppressed IDO1 expression in cDC1, confirming that these two elements were required for IDO1 expression in mature cDC1. Accordingly, AhR deletion in cDC1 abrogated IDO1 expression. Surprisingly, we found that deficiency of either $Ahr^{-/-}$ or $Ido1^{-/-}$ in $CCR7^+$ cDC1 strongly potentiated IL-12 and TNF-α productions by cDC1. Therefore, in a cDC1–T $CD8^+$ co-colture system, we demonstrate that *Ahr* deletion in cDC1 greatly increases IFN-γ, Granzyme B and Perforin production. Notably, in an *in vivo* mouse model, selective AhR deletion in XCRI expressing cDC1 accelerated spontaneous immune rejection of an otherwise progressive fibrosarcoma cell line.

Conclusion: Overall, these data point to AhR as a new immune inhibitory target in cDC1, which can be targeted pharmacologically to overcome immune tolerance and resistance to immunotherapy.

P205

Why do not all end-stage kidney disease patients get gout? Hyperuricemia impairs β2 integrin-mediated neutrophil migration.

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Question: Acute gouty arthritis is triggered by the deposition of monosodium urate (MSU) crystals in joints, inducing an acute inflammatory response, which is characterized by the infiltration of neutrophils. Elevated serum uric acid (UA) levels, known as hyperuricemia (HU), are common in patients with chronic kidney disease (CKD), a major risk factor for gouty arthritis. In addition, only a minority of patients with advanced CKD-related HU experiences gouty arthritis. We hypothesized that HU affects neutrophil chemotaxis in acute gouty arthritis during renal failure.

Methods: *Mice study*: Six week old Alb-creERT2/Glut9lox/lox (ki/ki) or Glut9lox/lox without active cre (control) mice were injected with tamoxifen and placed on a chow or high-fat diet with inosine. After 22 days, all the mice received an intrascrotal injection of MSU crystals per mouse 4 hours prior to exteriorization of the cremaster muscle. Rolling flux fraction, leukocyte adhesion and rolling velocities were analyzed as well as extravasated leukocytes counted on HE stained cremaster muscles. ***Human study***: Human neutrophils were isolated from healthy individuals and pre-incubated with or without soluble uric acid (sUA) prior to stimulation with human CXCL8. The expression levels of LFA-1, MAC-1 and mAB24 (one b2 integrin activation marker) were quantified by flow cytometry.

Results: ***Mice study*:** After 22 days, all ki/ki mice fed with inosine developed HU. Only mice on high fat diet developed HU+CKD. Intravital microscopy revealed that HU mice have an increased leukocyte rolling velocity in vessels, a reduced leukocyte intravasal adhesion and extravasation compared to healthy mice. These effects were even more amplified in HU+CKD mice. ***Human study*:** In blood neutrophils isolated from healthy individuals, sUA impaired CXCL8 induced β2 integrin activation.

Conclusions: Our data shown HU and CKD synergistically modulate leukocyte rolling, adhesion, and extravasation towards the site of MSU crystal-induced inflammation. And one molecular mechanism is sUA-induced impaired β2 integrin expression on neutrophils and prevents neutrophil activation. This might be a molecular explanation for the previously unexplained clinical phenomenon of an unexpectedly low prevalence of gouty arthritis despite persistent HU in CKD.

DOI: 10.1002/eji.201970300

P206

RelA/NF-kB and STAT3 transcription factors cooperate in trans-activating the human IL-17A proximal promoter in response to CD28 individual stimulation.

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Introduction CD28 is an important costimulatory receptor for T lymphocytes that, in humans, may delivers TCR-independent signal leading to the up-regulation of pro-inflammatory cytokines. We have recently reported that CD28 autonomous signalling induces the expression of IL-17A in peripheral CD4+ T lymphocytes from healthy donors, Multiple Sclerosis and type 1 diabetes patients. **Objectives** Due to the relevance of IL-17A in the pathophysiology of several inflammatory and autoimmune diseases, the aim of this work was to characterize the mechanisms and signalling mediators responsible for CD28-induced IL-17A expression.

Patients & methods Primary CD4+ T cells isolated from the peripheral blood of healthy donor (HD) were stimulated with agonistic anti-CD28 antibodies and the expression of IL-6 and IL-17A (Real-time PCR, ELISA), the nuclear translocation of tyrosine phosphorylated STAT3 (pSTAT3) and RelA/NF-kB (western blotting) as well as their specific recruitment on the human IL-17A proximal promoter (chromatin immunoprecipitation, ChIP assays) were analysed.

Results: CD28-mediated up-regulation of IL-17A gene expression depends on RelA/NF-kB and IL-6-associated STAT3 transcriptions factors. In particular, we found that CD28-activated RelA/NF-kB induces the expression of IL-6 that, in a positive feedback loop, mediates the activation and nuclear translocation of pSTAT3. pSTAT3 in turn cooperates with RelA/NF-kB by binding specific sequences within the proximal promoter of human IL-17A gene, thus inducing its expression. Finally, by using specific inhibitory drugs, we also identified class 1A phosphatidylinositol 3-kinase (PI3K) as a critical upstream regulator of CD28-mediated RelA/NF-kB and STAT3 recruitments and trans-activation of IL-17A promoter.

Conclusion: Our findings reveal a novel mechanism by which human CD28 may amplify IL-17A expression in human T lymphocytes and provide biological bases for immunotherapeutic approaches targeting CD28-associated class 1A PI3K to dampen IL-17A- mediated inflammatory response in autoimmune/inflammatory disorders.

P207

Self-RNA sensing and signaling-independent anti-influenza activity of the pathogen recognition receptor RIG-I: novel targets for antiviral intervention strategies

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Introduction: Influenza A virus (IVA) infections cause substantial morbidity and mortality during seasonal and pandemic outbreaks worldwide. However, established antiviral therapeutics are of limited efficacy. RIG-I is a cytoplasmic virus sensor recognizing IVA dsRNA structures. Activated RIG-I triggers IFN signaling (IS) and hence antiviral actors like the oligoadenylate-synthetase (OAS) - RNase L system.

Objectives: A K270A point mutation (PM) in human RIG-I mediates the inactivation of its IS ability, while its ability to compete with the viral polymerase remains (Weber *et al*., 2015). To investigate the impact of the two antiviral RIG-I functions in an IVA infection *in vivo*, we have created two mouse strains: with a RIG-I PM (K271A) and RIG-I deficiency (KO), respectively.

Secondly we hypothesize that IFN mediated downstream activation of RNase L leads to formation of self-RNA fragments which enhance RIG-I – IS via a positive feedback loop. These fragments have the potential to serve as new adjuvants for IVA therapy.

DOI: 10.1002/eji.201970300

Methods: The RIG-I PM and KO mice were generated with CRISPR/Cas9 on a C57BL/6J background by the LTK at the university of Zurich. We bred homozygote mice and investigated IS with ELISA and RIG-I protein integrity.

For investigation of RNase L activity, we use in vitro RNA degradation assays with cell lysates. For RNase L activation, we use the natural activator 2"-5" OA or the Glycolysis intermediate F-1,6-bP (Suhadolnik *et al.* 1990), which suggests a link between Glycolysis and RNase L activity. RNA integrity is investigated with agarose gel electrophoresis and RIG-I – IS induction by RNA with an IFNβ reporter gene assay.

Results: We bred homozygote mice of both lines which show no markedly health restrictions. Importantly, the RIG-I PM leads to an ablation of RIG-I mediated IS.

Further, we found that RNase L from cell lysates mediates RNA degradation. Resulting RNA enhances the activity of an IFNβ reporter.

Conclusion: We established a RIG-I PM mouse with altered RIG-I - IS. Mice will be infected with different IVA strains and variation in the antiviral activity of RIG-I will be validated by histologic and molecular-biologic methods.

Secondly, we can show that RNase L activation via F-1,6-bP in cell lysates and intracellular leads to RNA degradation. Resulting RNA has the ability to stimulate RIG-I – IS, supporting the existence of a positive feedback loop and a link between metabolism and RNase L activation.

P208

A highly conserved tyrosine residue within the SH2 domain of Lck regulates T-cell development and activation

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Introduction: Upon TCR engagement, the Src-family kinase Lck phosphorylates the ITAMs of the TCR, thereby initiating signaling events required for T-cell activation and T-cell development. It is textbook knowledge that regulation of Lck activity occurs via phosphorylation of two critical tyrosine residues, Y394 and Y505. Phosphorylation of Y505 is controlled by the protein tyrosine kinase Csk and the tyrosine phosphatase CD45. Phosphorylation of Y505 by Csk induces an intramolecular interaction between pY505 and the SH2 domain of Lck, thus generating a "closed" and inactive enzyme. Conversely, dephosphorylation of Y505 by CD45 and trans-auto-phosphorylation of Y394 "opens" and activates Lck. In addition to these two well-characterized tyrosine residues, Lck possesses a third highly conserved tyrosine, Y192, located within the SH2 domain whose precise function in T cells is unknown. Recently, it has been proposed that phosphorylation of Y192 alters the association between Lck and CD45 thereby leading to Lck inactivation in Jurkat T cells due to hyperphosphorylation of negative regulatory Y505 (Courtney et al., Mol Cell, 67: 498ff, 2017).

Objectives: Aim of the study was to assess the *in vivo* function of Y192 in non-transformed T cells.

Materials & methods: Lck^{Y192E} and Lck^{Y192F} knock-in mice were generated in which we assessed thymic development, the cellular composition of the secondary lymphatic organs and TCR-mediated signaling events.

Results: Whereas, Lck^{Y192F} knock-in mice showed no alteration of T-cell maturation and function, Lck^{Y192E} knock-in mice displayed a severe block of thymic development (at DN3) and severely impaired TCR-mediated signaling (similar to Lck-Ko mice). Despite an unaffected subcellular localization, co-immunoprecipitation studies further showed an impaired binding of Lck^{Y192E} to CD45 (likely being responsible for hyperphosphorylation of Y505). Surprisingly however, *in vitro* kinase assays revealed normal kinase activity of the Lck^{Y192E} mutant.

Conclusion: Taken together, our data suggest that despite being hyperphosphorylated on Y505 (and hence presumably believed to be closed and inactive) and despite being incapable to initiate TCR signaling the Y192E Lck mutant still retains its kinase activity. Hence, altered interactions with signaling components (e.g. the TCR) might underlie the severe signaling defect of Lck^{Y192E} T cells. Thus, studies are currently underway to assess the Lck^{Y192E} interactome in resting and activated T cells.

DOI: 10.1002/eji.201970300

P209

Inhibition of cross-talk of JAK- and MAPK- pathways in monocytes by small molecule JAK-inhibitors renders their cellular function

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Introduction. Monocytes are immunological key players bridging natural and acquired immunity. JAK2 is the central regulator of GM-CSF-signaling in monocytes, supporting anti-inflammatory programming. JAK/STAT-blockade by new small molecule inhibitors promises new treatment options in inflammatory disease, but in parallel, also impacts monocyte biology.

Objectives: In this study, inhibition of JAK2-dependend pathways and their consequence on monocytic functions were investigated. The goal was to detect side effect mechanisms of JAK inhibitor use.

Material and Methods: Healthy human primary monocytes were analyzed for cytokine expression and phenotype after stimulation with GM-CSF and pre-treatment with non-toxic dosages of 1–1000 nM of JAK inhibitor tofacitinib. Furthermore, dose-dependent inhibition of JAK/STAT-phosphorylation and cross-talk to MAPK-signaling in monocytes was investigated. Subsequently monocyte capacity to induce Foxp3$^+$ regulatory T-cells in co-cultures with autologous naïve T-cells was analyzed.

Results: GM-CSF-activated monocytes induce Foxp3$^+$ Tregs when co-cultured with autologous naïve T-cells. Pre-incubation of monocytes with tofacitinib up to 100 nM supports this anti-inflammatory program, illustrated by reduced TNFα and IL-6 secretion, and up-regulation of mannose receptor CD206. Dosage above 1000 nM tofacitinib abrogates anti-inflammatory program in monocytes, still inhibiting TNFα and IL-6, but also inhibiting induction of Tregs in co-culture. Analyzing signaling pathways during tofacitinib treatment of monocytes reveals that above 1000 nM tofacitinib, GM-CSF-induced JAK2 activation is completely blocked. Furthermore, activation of STAT5 and MAPK pathways via ERK1/2 and p38 MAPK are also blocked revealing intense cross-talk activation in monocytes by GM-CSF-signaling through JAK2.

Conclusion. Small molecule inhibitors of JAK pathways are attractive treatment options for chronic inflammatory diseases because they can block many cytokines in parallel. Tofacitinib e.g. dose-dependently facilitates reprogramming of monocytes to a more regulatory phenotype. Dosage above 1000 nM reverts this beneficial effect at least in monocytes and clinical use will reveal whether clinical side effects will arise.

P211

Macrophage phosphoproteome analysis reveals MINCLE-dependent and -independent mycobacterial cord factor signaling

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Immune sensing of *Mycobacterium tuberculosis* relies on recognition by macrophages. Mycobacterial cord factor, trehalose-6,6"-dimycolate (TDM), is the most abundant cell wall glycolipid and binds to the C-type lectin receptor (CLR) MINCLE. To explore the kinase signaling linking the TDM-MINCLE interaction to gene expression, we employed quantitative phosphoproteome analysis. TDM caused upregulation of 6.7% and suppressed 3.8% of the 14,000 phospho-sites identified on 3727 proteins. MINCLE-dependent phosphorylation was observed for canonical players of CLR signaling (e.g. PLCg, PKCd), and was enriched for PKCd and GSK3 kinase motifs. MINCLE-dependent activation of the PI3K-AKT-GSK3 pathway contributed to inflammatory gene expression and required the PI3K regulatory subunit p85a. Unexpectedly, a substantial fraction of TDM-induced phosphorylation was MINCLE-independent, a finding paralleled by transcriptome data. Bioinformatics analysis of both datasets concurred in the requirement for MINCLE for innate immune response pathways and processes. In contrast, MINCLE-independent phosphorylation and transcriptome responses were linked to cell cycle regulation. Collectively, our global analyses show substantial reprogramming of macrophages by TDM and reveal a dichotomy of MINCLE-dependent and -independent signaling linked to distinct biological responses.

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DOI: 10.1002/eji.201970300

Identification of monoallelic *IRF2BP2* variants in a CVID cohort

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Background: IFN regulatory factor 2 binding protein 2 (IRF2BP2) was identified as an IRF-2-dependent transcriptional corepressor binding to the C-terminal repression domain of IRF2. IRF2BP2 also could bind to the C-terminus of nuclear factor of activated T cells (NFAT1) and act as a regulator of NFAT-responsive genes transcription. IRF2BP2 was described as an important transcriptional cofactor regulating various biological systems including cell cycle, apoptosis, immune responses, cell differentiation, inflammation and angiogenesis. More recently, it has been shown that *IRF2BP2* mutations could cause autosomal-dominant common variable immunodeficiency (CVID). CVID as a syndrome comprises a heterogeneous group of diseases, characterized by recurrent infections due to significant hypogammaglobulinemia.

Methods: Whole exome sequencing and targeted next-generation sequencing was performed with genomic DNA isolated from whole blood.

Results: In our study we identified four novel monoallelic *IRF2BP2* variants in five patients within a CVID cohort. Novel monoallelic missense mutations were detected in one male patient (c.352C>T; p.P118S) and two affected sisters from non-consanguineous parents (c.1645T>G; p.C549G). One patient was identified with a stop-gain mutation (c.1615_1616delinsT; p.K539Yfs*41), which is predicted to cause a truncation of protein length due to a premature termination of translation. Another patient harbored an in-frame deletion variant (c.293_295delAGC; p.Q94del).

Conclusions: We identified novel *IRF2BP2* mutations in a family with autosomal dominant CVID and in three sporadic CVID cases. Monoallelic *IRF2BP2* variants may cause monogenic CVID with different clinical manifestations and onset.

This study was supported by DZIF TTU 07.801 (German Centre for Infection Research) and MWK (Ministerium für Wissenschaft und Kultur), COALITION TP 11.

Key words: Common variable immunodeficiency, whole exome sequencing, next-generation sequencing, primary antibody deficiency, IRF2BP2

P213

Back signaling of HLA class I molecules and T/NK cell receptor ligands in epithelial cells reflects the rejection-specific microenvironment in renal allograft biopsies

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Question: During the last years, the diverse harmful mechanisms of donor-specific antibodies (DSA) have been studied towards endothelial cells. However, the role of kidney epithelial tubulus cells was less examined and may be underestimated despite their potential contact to DSA and infiltrating T and NK cells during antibody-mediated (ABMR) or T-cell-mediated rejection (TCMR) post kidney transplantation. Therefore, we investigated proximal tubular epithelial cells (PTEC) following stimulation via HLA class I and the ligands CD155 or CD166, which possess own signal-transducing capacities and, hence, may mediate back signaling after NK/T cell encounter or DSA ligation.

Results: Upon stimulation with αHLA, αCD166 or αCD155 mAb, PTEC secreted cytokines and chemokines i.e. IL-6, CXCL1,8,10, CCL-2 and sICAM1 (all $p<0.05$). This back signaling response was not suppressed by clinically approved immunosuppressive drugs or other conventional pathway inhibitors. Upon co-culture with T and NK cells, PTEC secreted chemokines such as CCL2, CXCL9, CXCL10 and the cytokine IL-6 as well as sICAM-1 as result of this contact-dependent back signaling. Simultaneously, modulation of the CD155 receptor CD226 and the CD166 receptor CD6 was observed in CD4+, CD8+ T cells as well as NK cells indicating direct ligand/receptor interactions. Furthermore this PTEC response was compared to the cytokine/chemokine microenvironment of BANFF-classified

DOI: 10.1002/eji.201970300

kidney biopsies. Rejection, especially ABMR, was associated with a distinct cytokine milieu, guided by significantly higher levels of chemokines (CXCL9, CXCL10, CCL5, all $p<0.05$). Histological analyses confirmed the expression of CD155 and CD166 in renal epithelial cells located at distal or proximal tubuli, respectively.

Conclusion: Our results indicate a contribution of PTEC back signaling to antibody-mediated rejection via chemokine release and contribute to a better understanding of the pathomechanisms involved in kidney allograft rejection.

P214

Filamin A phosphorylation at serine 2152 by the serine/threonine kinase Ndr2 controls TCR-induced LFA-1 activation in T cells

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Introduction: The integrin LFA-1 (CD11a/CD18) plays a critical role in the interaction of T cells with antigen presenting cells (APCs) to promote lymphocyte differentiation and proliferation. This integrin is present in either the inactive closed or the open active conformation. Three intracellular binding partners of LFA-1, i.e. Filamin A (FLNa), Kindlin-3 and Talin have been reported to regulate inside-out-signaling of LFA-1 in T cells.

Objectives: The current model of LFA-1 activation proposes that in non-activated T cells FLNa is bound to LFA-1 keeping the integrin in the closed conformation. Upon TCR-stimulation, FLNa dissociates from CD18 and Talin and Kindlin-3 are recruited to the plasma membrane and interact with LFA-1 to promote the open conformation. Thus, the dissociation of FLNa from LFA-1 appears to be critical step in this activation process. However, the molecular mechanism how the release of FLNa from CD18 is controlled is not very well understood.

Methods: To address the functional importance of Ndr2 for TCR-mediated LFA-1 activation, Jurkat T cells were transfected with suppression/re-expression plasmids that knock down endogenous Ndr2 and simultaneously re-express wild type or kinase dead mutant of this kinase to perform high affinity LFA-1 binding-, adhesion- and conjugation assays, Western blot analysis and immunoprecipitation studies. CD4+ T cells were isolated from WT and Ndr2-deficient mice to perform adhesion assays, Western blot analysis and to study the upregulation of CD69 upon TCR-stimulation.

Results: We identified FLNa as a substrate of Ndr2 kinase. We demonstrated that Ndr2 phosphorylates FLNa at serine 2152 (S2152) upon TCR-triggering. We showed that Ndr2-dependent phosphorylation of FLNa at S2152 releases the binding of this molecule from the closed conformation of LFA-1, thus allowing the TCR-mediated association of Talin and Kindlin-3 to the cytoplasmatic domain of CD18. Our data suggest that phosphorylation of FLNa at S2152 by Ndr2 is a critical step in TCR-mediated LFA-1 activation.

Conclusion: Ndr2-dependent phosphorylation of FLNa at S2152 seems to be the initial step of LFA-1 activation to switch from the closed to the open high affinity conformation of this integrin required for ligand binding (see Fig. 1).

DOI: 10.1002/eji.201970300

Figure 1

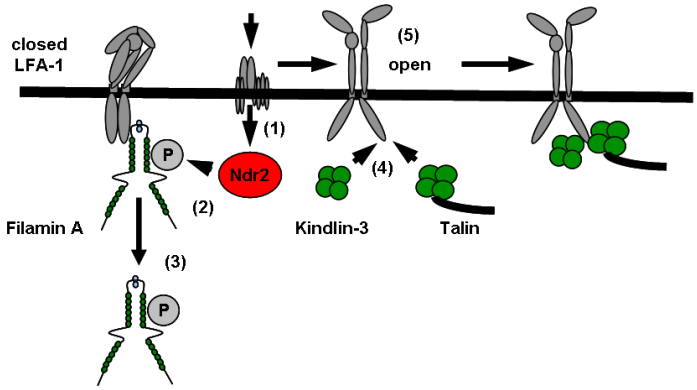

Figure 1. Model of Ndr2-controlled LFA-1 activation
TCR-induced activation of Ndr2 (step 1) promotes FLNa phosphorylation at S2152 (step 2) resulting in release of FLNa from LFA-1 (step 3). This in turn allows the association of Talin and Kindlin-3 with LFA-1 (step 4) thereby inducing full LFA-1 activation (step 5).

P215
Tumor-derived exosomes influence the activity of neutrophils in head-and-neck cancer.
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Introduction. Tumor-derived exosomes (TEX) can be responsible for the reprogramming of immune cells. We could previously show that neutrophils can influence tumor angiogenesis and regulate anti-tumor immune responses (presentation of tumor Ags or immune suppression). Accumulation of neutrophils in head-and-neck cancer (HNC) was shown to correlate with a poor prognosis. However, it is not entirely clear how tumorigenic activity of neutrophils is regulated. Possibly, TEX play a role in this process.

Objectives. We aimed to understand the role of TEX in the regulation of tumorigenic activity of neutrophils. Proteins associated with TEX should be identified to establish biomarkers that can facilitate patient diagnosis/prognosis, but also to identify molecules that could be targeted therapeutically.

Materials & methods. Exosomes were isolated and purified. Global proteomics analysis of exosomes was done to reveal their protein content. In addition, flow cytometry of exosomes was performed.For functional studies, PMNs were isolated from healthy donors and incubated with exosomes from HNC patients and healthy controls. The activity and survival of neutrophils was monitored. The material was taken for qPCR and western-blot analysis.

Results and discussion. We observed significant elevation of exosome content in HNC patient blood, compared to healthy individuals. Moreover, the amount of exosome associated proteins was higher in patients. Using flow cytometry, we confirmed the presence of surface markers involved in the regulation of immune responses, such as ICAM1 or PDL1, on exosomes. We expect that the interaction of such exosomes with neutrophils supports their apoptosis and inhibits their cross-presentation activity. This is in agreement with the results showing elevated apoptosis of neutrophils after their incubation with patient exosomes, as compared to healthy exosomes. Of note, we observed that after incubation of TEX with neutrophils, the majority of exosomes was internalized. This suggest that, tumor Ags that are presented by neutrophils could be delivered by exosomes.

Conclusion. Our preliminary studies show that TEX can modulate the functionality of neutrophils in cancer. More studies are needed to understand mechanisms that are involved in this process. Targeted inhibition of TEX-mediated stimulation of pro-tumor neutrophils might be a novel therapeutic approach in HNC.

P216
Exploring B cell receptor signaling by SILAC mass spectrometry - a global and comprehensive approach

DOI: 10.1002/eji.201970300

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Introduction: Signaling through the B cell receptor (BCR) is required for development and activation of B cells and is pivotal for humoral adaptive immunity. The BCR is composed of a membrane-bound immunoglobulin recognizing its antigen and an Igα/Igβ dimer that enables signal transmission. While developing B cells solely express a BCR of the IgM isotype, mature B cells co-express IgD. Although IgM and IgD can *in vivo* largely compensate for the loss of each other, their dual expression is highly conserved through evolution. Despite decades of research on BCR signaling, central questions - such as IgD's distinct funtion - remain elusive. It is thus vital to get off the beaten path and explore antigen receptor signaling using unbiased and global approaches.

Objectives: This project aims to establish mass spectrometry-based readout systems that enable comprehensive, global and unbiased analysis of BCR signaling. This new methodology is being applied to revisit the elusive function of IgD as well as the translocation of novel effector proteins to the nucleus as unsolved mysteries of BCR signal transduction.

Methods: To enable direct comparison of IgD and IgM, B cell lines were generated which either express an IgD or IgM BCR with identical affinity for the model antigen NIP. Global BCR-induced signal transduction was compared on the pYome and Ser/Thr GPome-level using SILAC mass spectrometry. SILAC MS was also utilized to discover novel BCR-induced protein translocation events. To this end, pure nuclear and cytosolic extracts of stimulated B cells were analysed, revealing the BCR-induced "translocatome".

Results: Key players of proximal and downstream signaling are phosphorylated to equal extents upon induction of the IgM and the IgD BCR. We confirmed these findings on the phosphoproteome level: While mapping a total of >2500 phosphosites, stimulation of IgM vs. IgD shows statistical correlation of >95%, further affirming the similarity of their response. Moreover, proteomic mapping identified several potential novel players in distal signal transduction which stably translocate to the nucleus upon BCR stimulation.

Conclusion: We are applying a novel and unbiased MS-based methodology to comprehensively investigate the BCR signaling cascade on the global genome-wide scale. This work contributes to our understanding of isotype-specific BCR signaling and offers new insights on the transcriptional regulation by novel nuclear effectors downstream of the BCR.

P217
Identification and characterization of single exosomes by high-resolution spectral flow cytometry

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Introduction: Exosomes are small lipid vesicles secreted from almost all cell types including infected and tumour cells. They carry proteins, RNA and DNA from their cell of origin which they can transfer to cells taking up exosomes. Due to this feature, exosomes have been identified as diagnostic parameter in many different disease entities.

Objectives: Due to their size of 30-150 nm, characterization of exosomes is restricted to bulk-analysis methods like Western blotting, ELISA or by bead-based clustering of exosomes followed by flow cytometry so far. Due to these restrictions single event information is lost, and different exosome populations cannot be distinguished. In this project we aim to analyse exosomes as single events therefore establishing a flow cytometry-based method for detection and characterization of single exosomes.

Materials & methods: Exosomes from cell-culture supernatant of tumour cells and blood obtained from healthy humans were stained using antibodies against the exosomal surface markers CD81, CD63, HLA-ABC and CD41 and analysed with a spectral flow cytometer with improved resolution characteristics. To verify the presence of exosomes we intend to perform electron microscopy and Western blotting of exosomes enriched by ultracentrifugation.

Results: High-resolution spectral flow cytometry detecting exosomes down to a size of 100 nm allowed to quantitatively detect marker expression on single exosomes. We could analyse single exosomes which showed a

DOI: 10.1002/eji.201970300

homogenous fluorescence intensity for individual markers, such as CD81 or MHC class I. However, in contrast to previous reports, the exosomes were negative for CD63 in our setting.

Conclusion: Here we reveal that high-resolution spectral flow cytometry has the capacity to resolve single exosomes thereby identifying distinct exosome populations that will be instrumental to improve their use in diagnosis of chronic inflammation and cancer.

P218

The role of canonical wnt signaling pathway in neonatal and adult human CD8+ T cells

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Introduction: Wnt signaling pathway is an important regulator of cell proliferation and differentiation. In CD8+ T cells, this pathway is activated during differentiation into memory, but not effector cells.

Question: Is the wnt signaling pathway important for cell fate (proliferation vs differentiation) in neonatal and adult CD8+ T cells?

Methods: We purified CD8+ T cells by negative selection. Cells were stimulated with wnt3a, Protein complexes associated with beta catenin were identified by proteomics. Chromatin immunoprecipitation, western blot and RT-qPCR were also used.

Results: We characterized the protein-complexes associated with beta-catenin in neonatal and adult CD8+ T cells after stimulating this pathway with wnt3A. Although 88% of the recruited proteins were conserved, the differentially recruited proteins established different complexes in adult and neonatal cells. In adult cells, proteins associated with beta-catenin were involved in cell signaling and immunological functions, whereas in neonatal cells, they were linked metabolic functions and proliferation. Wnt3a stimulation induced the expression of Wnt11 in adult cells and Wnt5a in neonatal cells, suggesting a putative connection with planar polarity and Wnt/Ca2+ non-canonical pathways, respectively. The transcriptional coactivators p300 and CBP were also differentially recruited by beta-catenin in neonatal and adult cells, suggesting the participation of the wnt/beta-catenin pathway in the higher self-renewal capacity of the neonatal cells and memory commitment in those of adults. In support to this idea, beta-catenin was more recruited to the promoters of proliferation and cell renewal genes in neonatal cells and in differentiation genes in the adult cells.

Conclusions: Our results suggest a role of the wnt/beta-catenin pathway as a cellular switch in CD8+ T Cells, controlling proliferation or differentiation.

P219

CD28 and associated class 1A PI3K regulates the glycolytic metabolic program associated to pro-inflammatory T cell responses in Multiple Sclerosis

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Introduction CD28 is a crucial costimulatory receptor necessary for full T cell activation. One important contribution of CD28 to T cell activation relies on its ability to regulate T cell metabolism by enhancing nutrient uptake, aerobic glycolysis and anabolic pathways. Indeed, CD28 binds class 1A PI3K that in turn recruits and activates the PDK1/Akt/mTOR pathway. We have recently found that CD28 stimulation strongly up-regulates the expression of cytokines related to the Th17 cell phenotype in relapsing-remitting multiple sclerosis (RRMS) patients.

DOI: 10.1002/eji.201970300

Objectives The aim was to characterize the role of CD28 and associated class 1A PI3K in the modulation of the metabolic programs regulating Th17 cell responses in RRMS.

Patients & methods 30 patients with a clinically defined MS according to the McDonald criteria and a clinically RRMS were enrolled from S. Camillo Hospital (Rome, Italy). 30 age-gender-matched healthy donor (HD) buffy coats from the blood bank of Sapienza University (Rome, Italy) with no previous history of neurological or autoimmune diseases were used as controls. CD4+ T cells isolated from the peripheral blood of HDs or RRMS patients were stimulated with agonistic anti-CD28 abs and changes in cellular metabolism (Seahorse technology), surface expression of activation markers and the glucose transporter 1 (Glut-1) (Flow cytometry) as well as the expression of pro-inflammatory cytokines and metabolic enzymes (RT PCR, ELISA, western blotting) were analysed.

Results CD28 stimulation up-regulated glycolysis without affecting oxidative phosphorylation in CD4+ cells from RRMS patients. The analysis of the major enzymes regulating the glycolytic pathway revealed that CD28 stimulation induced the increase of c-myc and the glucose transporter Glut1 in CD4+ T cells from RRMS. CD28-induced increase of glycolysis was also associated with the up-regulation of pro-inflammatory cytokines, most of which were related to the Th17 cell phenotype, as demonstrated by the strong inhibition exerted by the glycolysis inhibitor 2-deoxy-D-glucose. Finally, treatment of CD4+ T cells from RRMS patients with a class 1A PI3K inhibitor strongly impaired CD28-induced glycolysis, c-myc and Glut1 expressions, as well as the up-regulation of pro-inflammatory cytokines.

Conclusion Altogether these data strongly suggest a role of CD28 and associated class 1A PI3K in reprogramming the metabolic process that maintain/amplify the inflammatory phenotype of T cells in RRMS patience.

P220

HYPERGLYCAEMIC CONTROL OF HISTONE CODE IN METABOLIC INFLAMMATION

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Epigenetic mechanisms regulate development of diabetic vascular complications and metabolic memory. Histone code is an essential mechanism of epigenetic memory affected by diabetic conditions. Monocytes (Mo) and macrophages (MF) are key innate immune cells that control inflammatory reactions leading to vascular complications. However, the mechanisms of hyperglycemia-induced epigenetic changes in human macrophages are not known.

Using Affymetrix microarray profiling and RT-PCR we identified that hyperglycaemia induces elevated gene expression of IL-1β, CCR2, S100A9 and S100A12 in primary human macrophages. Association for activating and repressing histone marks with the promoters of these genes was analyzed by chromatin immunoprecipitation.

Hyperglycaemia induced the increased association of only activating H3K4me1, H3K4me3 but not repressive H3K9me3 and H3K27me3 histone marks with promoters of the selected genes in human M1 macrophages. Increased association of the activating histone marks correlated with induction of gene transcription. Application of inhibitors for histone methyltransferases showed that SET7/9 regulate expression levels of CCR2 and S100 proteins but not of IL-1β, whereas SMYD3 specifically regulates S100A12. An inhibitor of MLL methyltransferase core complex activity had no effect on the expression levels of IL-1β, CCR2, S100A9 and S100A12.

We concluded that hyperglycaemia induces upregulation of pro-inflammatory factors in M1 macrophages by activating histone code on the gene promoters while gene-specific involvement whereas SMYD3 and SET7/9 are involved in induction of the specific gene transcription. Mechanism of action of the distinct methyltransferases in hyperglycaemic conditions remains to be identified and will add an insight in the role of hyperglycemia-induced inflammatory processes that lead to vascular complications in diabetes.

P221

Mitochondrial metabolism influences plasma cell differentiation

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DOI: 10.1002/eji.201970300

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B cells encounter different metabolic demands during development, activation and terminal differentiation to plasma cells, but the specific contribution of mitochondrial metabolism is largely unknown. To define the function of mitochondrial metabolism during B cell activation and differentiation I use a mouse model, where CD23Cre drives the expression of a dominant negative mutant of the mitochondrial helicase Twinkle (DNTwinkle) and the reporter GFP in mature B cells. DNTwinkle expression leads to mitochondrial DNA (mtDNA) deletions in dividing cells and results selectively in impaired respiratory chain (RC) activity. Flow cytometry in non-immunized CD23CrexDNTwinkle mice did not reveal a difference in either total cell numbers or frequencies of mature B cell subsets. However, tracking GFP+ cells revealed that activated GFP-postive/DNTwinkle+ B cells have a great disadvantage in this endogenously competitive setting: While naïve follicular B cells (FOs) still consist of over 90% of recombined cells (GFP+), only 27% and 8% of cells in the plasma cell (PC) compartment in the spleen and bone marrow, respectively, were GFP+.These results suggest that proliferating cells pass a metabolic checkpoint that is indispensable for further PC differentiation. While survival of DNTwinkle-GFP expressing LPS blasts is not affected, extracellular flux analysis showed a decreased oxygen consumption rate (OCR), indicative of oxidative phosphorylation (OxPhos), and an increased extracellular acidification rate (ECAR), indicative of glycolysis. In support, DNTwinkle-GFP-postive LPS B cell blasts had higher lactate and lower glucose concentrations in their supernatants. DNTwinkle-GFP-positive cells, can still be activated and upregulate TACI. However, further differentiation, determined by upregulation of CD138, was strongly impaired. In accordance, western blot analysis revealed a reduction in the abundance of the plasma cell master transcription factors Blimp1 and Xbp1 but not IRF4. IgM expression as well as secretion was also strongly reduced. Our results demonstrate a critical role for the RC during plasma cell differentiation. One explanation could be that impaired respiration leads to altered mitochondrial ROS production or anaplerotic reactions, which influences heme synthesis or metabolic mediators of the citric cycle and thereby affecting Bach2 and Blimp1 expression. In summary, we show that mitochondrial metabolism is important for plasma cell differentiation.

P223

Hyperglycemia Enhances Inflammatory Responses to Titanium Material in Human Macrophages

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Patients with Diabetes Mellitus (DM) have elevated blood sugar levels and can suffer from consequences triggered by compromised immune responses. Amongst those consequences are the complications produced by metal implants. As a result of compromised biocompatibility properties, such as metal corrosion triggered by hyperglycemic environment, implant materials generate metal alloy debris, which are the implant wear off particles. The small alloy particles are gradually and constantly released from an implanted material throughout its entire life course. These microparticles and nanoparticles produce negative immune responses and inflammatory complications, which also may be different from initial acute inflammatory phase and rather correspond to chronic inflammation. Here we investigated the effects of hyperglycemic conditions on the inflammatory responses of primary human macrophages exposed to increasing concentrations TiO2 nanoparticles (NPs). A group of inflammatory factors have been assessed, however, the production of YKL-40(CHIT3L1) and CCL18, which are secreted by human primary macrophages in response to IFNgamma and IL-4, correspondingly was significantly altered in comparison to other inflammatory mediators. Furthermore, macrophage ROS levels have been assessed after their stimulation with TiO2 nanoparticle in normoglycemic and hyperglycemic environments.

P224

Lessons to learn from primary immunodeficiencies overlapping with atopic eczema

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DOI: 10.1002/eji.201970300

Several primary immunodeficiencies (PID) - such as hyper-IgE syndromes (HIES) - present next to recurrent infections with chronic eczema, and elevated serum IgE and show a significant clinical overlap with more common diseases like atopic eczema.

In contrast to most AE entities, PID are caused by monogenetic defects such as heterozygous mutations in the gene *STAT3* (STAT3-HIES) and homozygous mutations in *DOCK8* (DOCK8-HIES). Although identification of these underlying gene defects and corresponding molecular testing has improved diagnosis, there are still patients diagnosed after irreversible disease complications already have occurred.

Here we present challenges of identifying monogenetic gene defects in patients with hyper-IgE syndromes (HIES). In one family only the complex combination of clinical, immunologic, molecular, and bioinformatic diagnostic approaches confirmed a novel intronic splice site mutation causative for DOCK8-HIES. We show how somatic alterations and a skewed T cell phenotype leading to DOCK8 protein re-expression and an IL6-specific STAT3 signaling defect compromised molecular diagnosis of DOCK8-HIES caused by a novel intronic splice site mutation.

Taken together, solving the underlying disease mechanisms in PID is not only important for diagnosing patients early prior to irreversible disease complication have occurred. Novel insights in functional molecular disease pathology may lead to understanding more common afflictions such as atopic eczema.

P225

The aryl hydrocarbon receptor repressor (AhRR) regulates cell metabolism and diet-induced metabolic syndrome

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Introduction: The aryl hydrocarbon receptor (AhR) pathway regulates important functions in cellular detoxification, immune cell differentiation and intestinal homeostasis. AhR activation by environmental pollutants disrupts the endocrine hormone system, which affects lipid metabolism and causes liver pathology. The AhR repressor (AhRR) is mainly expressed in immune cells of barrier organs and controls AhR signaling in a

cell type specific manner. AhR-deficient mice were previously described to resist high fat diet (HFD)-induced metabolic syndrome. Here, we investigated the role of the AhRR in the context of global and myeloid cell-specific energy metabolism.

Marerial & Methods: Macrophages (Mφ) from WT and AhRR$^{-/-}$ mice were analyzed for their general metabolism in an Agilent Seahorse XFe96 Analyzer, for their lipid composition by mass spectrometry and for their gene expression by RNA sequencing. WT and AhRR$^{-/-}$ mice were fed a HFD for 14 weeks; weight gain, development of hepatosteatosis and signs of metabolic syndrome were assessed.

Results: Transcriptome and lipidome analyses revealed that expression of metabolism-associated genes and specific lipid classes was significantly altered in AhRR$^{-/-}$ compared to WT Mφ, suggesting that these cells may have defects in lipid and glucose metabolism. Seahorse analysis showed increased oxidative phosphorylation and overall energy consumption of AhRR$^{-/-}$ Mφ compared to WT controls. Both AhR$^{-/-}$ and AhRR$^{-/-}$ Mφ also showed higher dependency on glutaminolysis compared to WT Mφ which were more dependent on β-oxidation. Similarly, upon AhR activation WT Mφ shifted from β-oxidation towards glutaminolysis. When subjected to a HFD, both AhR$^{-/-}$ and AhRR$^{-/-}$ mice did not develop obesity. Compared to WT controls, HFD-fed AhRR$^{-/-}$ mice showed improved glucose tolerance, reduced serum levels of triglycerides and increased lipolysis in brown adipose tissue. Histological analysis of liver and adipose tissues proved ameliorated hepatosteatosis and reduced adipocyte hypertrophy in HFD-fed AhRR$^{-/-}$ mice.

DOI: 10.1002/eji.201970300

Conclusion: Thus, we show that AhRR, similarly to AhR, contributes to diet-induced obesity and associated pathology. This is underscored by transcriptional changes in genes essential for lipid and glucose metabolism and differential abundance of certain lipid classes in AhRR$^{-/-}$ Mφ compared to WT controls. Taken together, our data support that AhRR is a regulator of the cellular metabolism of Mφ and also affects systemic metabolism.

P226

N-acetylserotonin exerts neuroprotective effects enhancing IDO1 catalytic activity

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Introduction: Indoleamine 2,3-dioxygenase 1 (IDO1) is a Tryptophan (Trp) degrading enzyme which catalyzes the first and rate-limiting step in the kynurenine pathway, leading to Trp depletion and the production of a series of immunoregulatory molecules collectively known as kynurenines. Both effects are involved in the anti-inflammatory and immunoregulatory action of the enzyme by virtue of which it may represent a major therapeutic target in several autoimmune diseases, including multiple sclerosis (MS). The alternative degradation of Trp occurring along the serotonin pathway yields to N-acetylserotonin (NAS), a metabolite endowed with antioxidant and thus neuroprotective functions in the experimental mouse model of MS.

Objectives: The aim of the study was to investigate whether a correlation exists between NAS neuroprotective effects and IDO1 immunomodulatory activity.

Materials and methods: Conventional dendritic cells (cDCs; the main antigen presenting cells of the immune system) were stimulated with NAS to asses IDO1 expression and activity. WT and *Ido1*$^{-/-}$ mice were immunized with the myelin oligodendrocyte glycoprotein (MOG) peptide to induce the experimental autoimmune encephalomyelitis (EAE; an animal model of MS) and treated with NAS to evaluate the protective effect of this metabolite.

Results: We found that NAS does not modulate IDO1 expression at both transcript and protein levels, but significantly increases Kyn production in cDCs, suggesting that it acts as catalytic enhancer of IDO1. Moreover, by means of a delayed-type hypersensitivity assay we demonstrated that NAS confers an immunosuppressive phenotype on cDCs, an effect that requires IDO1 activity. We finally found that the protective property of NAS in EAE relies on IDO1 as it is lost in *Ido1*$^{-/-}$ mice.

Conclusion: Overall, our data prove that NAS is endowed with immunoregulatory properties acting as catalytic enhancer of IDO1 both *in vitro* and *in vivo*.

P228

The aryl hydrocarbon receptor and its's downstream target cytochrome P450 member CYP1B1 regulate allergic airway inflammation

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Background: The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor that is able to recognize xenobiotics as well as natural ligands such as tryptophan metabolites, dietary components and microbiota-derived factors. Functioning as an environmental sensor and transcription factor the AhR is important in the maintenance of homeostasis at mucosal surfaces. AhR activation induces the expression of cytochrome P450 1 (CYP1) enzymes which are able to metabolize various compounds including potential AhR ligands. Here we investigate the role of AhR and CYP1 family members in allergic airway inflammation.

Methods: In this study we used AhR- and severeal CYP1-deficient mice to examine their role in pollen- and house dust mite-induced allergic airway inflammation. Repetitive exposure with ragweed extract or house dust mite (HDM) via intranasal instillation was followed by assessment of bronchoalveolar cell counts, histology, cytokine release and specific antibody production. Bone marrow chimeras were generated to determine the contribution of hematopoietic versus non-hematopoietic cells. Cell type-specific expression of CYP1 enzymes was determined by quantitative RT-

DOI: 10.1002/eji.201970300

PCR and immunofluorescence microscopy from patients. Exposure of primary murine epithelial cells and human cell lines to allergens was used to reveal mechanistic insights.

Results: Exposure to HDM resulted in notable enhancement of total IgE and a heightened cellular infiltration of white blood cells, namely eosinophils, lymphocytes and macrophages, in the bronchoalveolar lavage of AhR- and CYP1B1-deficient mice. Interestingly, CYP1B1-deficient mice reconstituted with bone marrow of C57BL/6 mice phenocopied these results indicating a prominent role for CYP1B1 expressing non-hematopoietic cells. In line with these results RT-PCR and microscopy analysis of lung tissue showed a higher CYP1B1 expression in non-hematopoietic cells.

Conclusion: AhR-dependent CYP1B1 expression by non-hematopoietic cells - presumably epithelial cells - is necessary to prevent exaggerated allergic airway inflammation. Thus, this pathway may be involved in the protection from asthma and constitutes an attractive target for prevention or therapy.

P229

Influence of aging on calcium signals and cytotoxicity in murine CD8$^+$ T cells

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Introduction: Calcium (Ca^{2+}) is a crucial secondary messenger for proper T cell function. In addition to a whole range of cellular processes, it influences and regulates activation, proliferation, and migration as well as the T cell-mediated cytotoxicity. The ability of T cells to generate Ca^{2+} signals is diminished in elderly individuals, potentially contributing to the decrease in T cell functionality, declining immune competence and an increased risk for cancer in aging. The main pathway for Ca^{2+} to enter T cells is the store-operated Ca^{2+} entry (SOCE).

Objectives: The role of Orai channels and stromal-interaction molecule (STIM) proteins, as calcium sensors, in lymphocyte aging is completely unknown.

Materials and methods: To investigate, how STIM and Orai contribute to Ca^{2+} signal reductions in CD8$^+$ T cells from elderly mice, we performed flow cytometry, electrophysiology, and molecular biology experiments. Our main focus was the change accruing during healthy aging in murine CD8$^+$ T cells from an adult (3-6 month) and an elderly (18-24 month) age group. Furthermore, we compared their killing kinetic and efficiency using a time-resolved killing assay, investigated the Ca^{2+}-dependency of the process, and quantified the expression of relevant proteins involved in cytotoxicity.

Results: We were able to link the reduction in $Ca2^+$ signal parameters and a reduction in Ca^{2+} release-activated Ca^{2+} (CRAC) currents of (non-stimulated) CD8$^+$ T cells from elderly mice to a decrease in mRNA and protein levels of STIMs and Orais. Furthermore, we were able to show, that the reduced Ca^{2+} signals of stimulated sorted CD8$^+$ T cells from elderly mice occur in both of the most abundant CD8$^+$ T cell subtypes, central and effector memory. An upregulation of PMCA4 additionally contributed to faster Ca^{2+} extrusion. The cytotoxic T lymphocytes (CTLs) are key players in the adaptive immune response and several steps of the CTL killing machinery require or are modulated by Ca^{2+} itself. Therefore, we compared their cytotoxic killing kinetic and efficiency, investigated the Ca^{2+}-dependency of the process, and quantified the expression of relevant proteins involved in cytotoxicity. Strikingly, we found that CD8$^+$ T cells from elderly mice show altered cytotoxic ability with distinct kinetics for different tumor cells.

Conclusion: Taken together our data point out the crucial contribution of SOCE components to altered Ca^{2+} signals and distinct cytotoxicity in aged T cells.

P230

The Src-family kinase Lyn stimulates P-selectin mediated rolling of leukocytes during acute inflammation

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DOI: 10.1002/eji.201970300

Introduction: During acute inflammation leukocytes exit the microvasculature into inflamed tissue through a well-defined cascade of activation and adhesion steps. The first step consists in leukocyte rolling along the inflamed endothelium. This requires rapid mobilization of P-selectin stored in Weibel-Palade bodies to the surface of activated endothelial cells. Previous publications suggested a role of the Src-family kinase *Lyn* for P-selectin mobilization in platelets. Likewise, we observed abnormal leukocyte rolling of neutrophils in *Hck -/- Fgr -/- Lyn -/-* mice, which led us to hypothesize a role for endothelial derived *Lyn* in P-selectin mobilization and rolling during acute inflammation.

Objectives: To unravel the role of the Src-family kinase *Lyn* in P-selectin mediated neutrophil rolling in a model of acute inflammation in mice.

Materials & methods: Intravital microscopy of the mouse cremaster muscle was performed in *Lyn -/-, Lyn fl/fl Cadh5(PAC)-Cre (EC Lyn -/-)* and WT mice to compare leukocyte rolling properties between the different mouse strains. PSGL-1 surface levels on isolated *Lyn -/-, EC Lyn -/-* and *C57Bl6* neutrophils were analyzed by flow cytometry. In vitro flow chamber assays with immobilized recombinant P-selectin were performed to study rolling of *Lyn -/-* neutrophils. Confocal microscopy was conducted in cremaster muscle tissue sections to study P-selectin surface expression of activated endothelial cells.

Results: Intravital microscopy of the exteriorized mouse cremaster muscle demonstrated impaired P-selectin dependent leukocyte rolling in *Lyn -/-,* and *EC Lyn -/-* compared to control mice. In addition, we found increased rolling velocities and neutrophilia in the absence of *Lyn*. Immunofluorescence stainings indicated reduced P-selectin surface expression in postcapillary cremaste muscle venules in the absence of endothelial *Lyn*. Surprisingly, in vitro flow chamber experiments using isolated *Lyn -/-* neutrophils also showed decreased rolling on immobilized P-selectin compared to WT neutrophils. This was accompanied by modestly reduced PSGL-1 surface expression on *Lyn-/-* neutrophils.

Conclusion: Our findings identify *Lyn* as a modulator of P-selectin surface expression and rolling on endothelial cells. In addition, we also revealed a role of neutrophil expressed *Lyn* in mediating P-selectin dependent rolling possibly through regulating PSGL-1 surface expression.

P231

Regulation of Lck activity via a highly conserved cysteine residue within the kinase domain

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Introduction: Lck, a member of the Src family kinases, is crucial for T-cell activation and T-cell development. Regulation of the enzymatic activity of Lck occurs via phosphorylation of two conserved tyrosine residues: an activatory (Y394) and an inhibitory residue (Y505), respectively. Phosphorylation of Y505 by Csk results in the formation of an intramolecular interaction which in turn closes Lck. Dephosphorylation of Y505 by the phosphatase CD45 opens Lck. Subsequently, open Lck can auto- trans-phosphorylate Y394, thus resulting in an active enzyme.Recently, cysteine residues have gained much attention because they are also involved in the regulation of the activity of tyrosine kinases. We have shown that oxidation of C575 regulates the stability and function of Zap-70. This cysteine is highly conserved among tyrosine kinases and in Lck it is located at position 476.

Objective: The aim of this study is to investigate the function of C476 in the regulation of Lck activity.

Material and methods: To study the function of C476, we have generated an C476A Lck mutant. The C476A Lck mutant was expressed in an Lck-deficient Jurkat T-cell line (J.Lck) and its ability to reconstitute TCR signaling was evaluated biochemically. We have also assessed the conformation of the mutant Lck taking advantage of an Lck-biosensor and FLIM/FRET analyses.

Results: We have found that, conversely to the wild type molecule, the C476A Lck mutant is not phosphorylated on both Y394 and Y505. It is known that loss of phosphorylation on the two regulatory sites results in an open but not active enzyme (the so called "primed" Lck). To assess this further, we performed conformational studies which revealed that C476A Lck is indeed open. To test the activity of C476A Lck, we analyzed global tyrosine phosphorylation upon re-expression in J.Lck. The data indicate that C476A Lck does not reconstitute TCR signaling in J.Lck. The co-chaperone Cdc37 is involved in the regulation of the stability and activity of different kinases. To investigate whether Cdc37 is also involved in the regulation of the activity of the C476A Lck mutant, we conducted co-

DOI: 10.1002/eji.201970300

expression studies in J.Lck. Overexpression of Cdc37 partially rescues the phosphorylation of Y394 and global tyrosine phosphorylation in CD3-stimulated cells.

Conclusion: Collectively, our data suggest that C476 is a crucial residue involved in the regulation of Lck activity likely via an oxidation-dependent fashion.

P232

Role of SLy1 in p53-mediated DNA damage response (DDR) signaling pathways in NK cells

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SLy1 is found to be exclusively expressed in lymphoid organs and was identified as a X-chromosomally encoded SH3 protein that is specifically phosphorylated at serine 27 upon B and T cell receptor engagement. Moreover, inhibitor studies implied that this antigen receptor mediated phosphorylation is PI3 kinase and PKC dependent. These studies therefore defined SLy1 as a previously unrecognized target for antigen receptor signal transduction and suggested that it may play a role in adaptive immunity. Recently, we showed that in mature T lymphocytes SLy1 regulates Forkhead box protein O1 (Foxo1) shuttling after T cell receptor signaling to facilitate lymphocyte expansion upon *Listeria* infection. Lately, we demonstrated that in NK cells SLy1 provides ribosomal stability rather than signal transduction. SLy1-deficient NK cells are impaired in survival, activation, adhesion, and cytotoxicity. In the absence of SLy1 ribosomal instability affects the Mdm2-p53 pathway thereby leading to NK cell senescence and reduced NK-mediated lung tumor clearance. Interestingly, the NK cell defects in SLy1-deficient mice are reversible under inflammatory conditions and thus viral clearance is not impacted by SLy1-deficiency. Here, we elucidate the role of increased levels of p53 in SLy1-deficient NK cells and the impact on the DNA damage response-signaling pathways. Moreover, we deleted p53 specifically in NK cells and can restore the impaired functions of SLy1-deficient NK cells.

P233

Maintenance of bone marrow-resident memory T lymphocytes

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The current paradigm is that memory T lymphocytes, in the absence of antigen, are maintained over time by homeostatic proliferation, driven by cytokines like interleukin-7 (IL-7) and interleukin-15 (IL-15). Recently however, our group could show that bone marrow-resident memory T lymphocytes, which confer memory to systemic antigens, are not proliferating at all. Instead, they are resting individually in contact to bone marrow stromal cells. My hypothesis is that the memory T lymphocytes receive critical survival signals from the stromal cells, in particular signals activating the PI3K/AKT/FOXO pathway, preventing apoptosis induced by metabolic stress. To identify these signals and potential further survival signals from the bone marrow environment, I have set up a hypoxic in vitro co-culture system of ST2 stromal cells and memory T lymphocytes isolated ex vivo. In this co-culture system, survival of the memory T lymphocytes is indeed dependent on the presence of stromal cells, but not on IL-7 and IL-15. Signal transduction and apoptosis pathways involved are currently under investigation.

P234

Deficiency of the microprocessor component DGCR8 impairs macrophage growth and unleashes IFN-dependent gene expression in response to mycobacteria

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Mycobacterium tuberculosis subverts innate immune responses to survive and replicate in macrophages. Mycobacterial cell wall components are sensed by several C-type lectin receptors, including the receptor for the cord factor trehalose-dimycolate (TDM), Mincle. Evidence is emerging that miRNAs are critically involved in regulation of innate immune cells. Thus, MTB may exploit host miRNAs to establish its niche in macrophages. Here, we have employed conditional knockout mice for the microprocessor component DGCR8 to investigate the impact of miRNAs on the macrophage response to the mycobacteria. Deletion of DGCR8 during differentiation of macrophages from bone marrow progenitors significantly reduced the cell yield, but did not interfere with macrophage differentiation as determined by cell surface CD11b and F4/80 expression and phagocytosis capacity. DGCR8-deficient macrophages

DOI: 10.1002/eji.201970300

had severely reduced expression of several constitutive and TDM-inducible miRNAs, but showed an accumulation of primary miRNA transcripts. RNAseq analysis revealed a modest type I interferon (IFN) signature in resting DGCR8-deficient macrophages. Upon stimulation with TDM, large gene sets showed overshooting and prolonged induction in the absence of DGCR8. Hyper-induction of IFN-stimulated genes was associated with enhanced expression of IFNbeta and could be prevented to a large extent by blockade with anti-IFNalpha/beta antibodies. Together, our results reveal an essential role for DGCR8 in curbing IFN-biased macrophage activation by mycobacteria.

P235

CD14 Counterregulates Lipopolysaccharide-Induced Tumor Necrosis Factor-α Production in a Macrophage Subset

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Human macrophages (MΦ) form a heterogeneous cell population displaying multiple functions. Exposed to environmental stimuli such as GM-CSF or M-CSF, they can acquire pro- or anti-inflammatory properties, respectively. During the inflammatory response to bacterial lipopolysaccharides (LPS) macrophages play a central role. Considering the importance of CD14 in recognizing LPS, we studied the effect of anti-CD14 antibody mediated CD14 blockade on LPS-induced cytokine production, signal transduction and on the expression levels of CD14 and TLR4 in GM-MΦ and M-MΦ.

We found M-MΦ to express higher levels of both surface antigens and to produce more interferon (IFN)-β and interleukin-10 (IL-10), but less tumor necrosis factor (TNF)-α than GM-MΦ. Surprisingly, blockage of CD14 at high LPS concentrations increased the production of proinflammatory cytokines and decreased that of IFN-β in M-MΦ but not in GM-MΦ. We show that phosphorylation states of signaling molecules of the MyD88, TRIF and MAPK pathways are not altered in any way that would account for the cytokine overshoot reaction. However, CD14 blockage in M-MΦ decreased CD14 and TLR4 expression levels indicating that the loss of the surface molecules prevented LPS from initiating TRIF signaling. As TNF-α synthesis was even upregulated under these experimental conditions, we suggest that TRIF is normally involved in restricting LPS-induced TNF-α overproduction. Thus, surface CD14 plays a decisive role in the biological response by determining LPS-induced signaling.

P236

CD44 alternative splicing isoforms and trafficking properties of antigen-specific T cells are regulated by TLRs engagement on T cells.

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T cell trafficking is a highly regulated event in the immune response. Pathogen/pathogen receptors (PR) systems have a role in the control of trafficking of lymphocytes. We reported that mice differing for the a TLR2 polymorphic residue showed differences in the trafficking of T cells and in the distribution of lesions in the CNS during EAE (Piermattei, 2016).

This study about the role of TLR2 in the regulation of trafficking of T cells, focused on its ability to reshuffle the role of CD44 and the alternative splicing of its pre-mRNA. Specifically, in the SJL mice, the regulation of alternative splicing of CD44 pre-mRNA by TLR2 depended on the CD62low/CD62high status and on the simultaneous activation *in vitro* via CD3+/CD28+, and led to over-representation of mRNAs specific for isoforms v8-v10 and, more prominently, v9-v10. mRNA specific for isoform v9-v10 was upregulated via activation of TLR2/TLR6 and TLR2/TLR1; mRNA specific for isoform v8-v10 was upregulated only by TLR2/TLR1 dimerization. Ability of T cells to migrate through Matrigel (inhibited by CD44 in resting T cells) become CD44-dependent upon their stimulation via TLR2. On the other hand, in the C57/Bl6 mouse strain, only isoform v8-v10-specific mRNA was upregulated, and the failure to produce isoform v9-v10-specific mRNA was dependent on the polymorphism of TLR2 between SJL and B6 mice. Finally, the stimulation of T cells by LPS or CpG (that engage the other TLRs expressed by activated T cells) modified alternative splicing of CD44 pre-mRNA, with a pattern of CD44 isoforms-specific mRNAs that varied according to each stimulus.

DOI: 10.1002/eji.201970300

Thus, TLRs collectively represent a pathway through which pathogens or commensals of viral and bacterial origin modify trafficking of self- and allo-reactive antigen-activated T cells, by modulating the relative ratios of CD44 isoforms, leading to induction or inability to enter the CNS.

P237

The role of Vav family guanine nucleotide exchange factors and their substrates in B cell antigen receptor signaling

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Introduction: Binding of antigen to the B cell antigen receptor (BCR) initiates activation of intracellular signal transduction pathways that, together with co-stimulatory signals, can induce differentiation of the cells into antibody-producing plasma cells. The BCR-proximal signaling reactions involve a concerted interplay of cytosolic tyrosine kinases, adaptor proteins and lipid-modifying enzymes, which control the production of lipid-derived second messengers and a subsequent rise in the intracellular concentration of Ca2+ ions. Furthermore, published work and our own preliminary data indicate a role of Vav protein family members in that process. Vav family proteins are multi-domain guanine nucleotide exchange factors (GEFs) for small G proteins of the Rho/Rac family. Vav-deficient mice have a severe immunodeficiency that goes along with greatly reduced T and B cell numbers. The mechanisms that cause B cell-intrinsic malfunction in Vav-null mice remain however unknown.

Objective: Our aim is to understand the role of Vav family GEFs in BCR-induced signaling by investigating the biochemical effects that are executed by the various protein domains of distinct Vav family members.

Materials and methods: We investigated the role of Vav proteins in a human B cell model system that was made deficient for Vav1 by TALEN mutagenesis. BCR signaling was analyzed using various read-outs for activation of distinct signaling pathways as well as second messenger production.

Results: B cells lacking the expression of Vav1 show a disturbed Ca2+ mobilization. Reconstitution of the cells with catalytically inactive Vav does not restore BCR-induced Ca2+ mobilization whereas the wild type version of Vav1 is able to restore Ca2+ mobilization. Intriguingly, like the BCR-regulated protein tyrosine kinases and phospholipase C, Vav proteins need to be recruited directly to the BCR or its proximal signalosome to exert their function. Vav2, which is not able to rescue the loss of Vav1, can be turned into an active molecule if the inhibitory domain of the GEF activity, the acidic region, is deleted. This version of Vav2 as well as Vav3 are even more potent than Vav1 in supporting BCR-proximal signaling.

Conclusion: In conclusion, our results show that BCR-induced Ca2+ mobilization is not only controlled by protein tyrosine kinases and phospholipid-converting enzymes, but requires an additional class of enzymatic activity that is provided by Vav family GEFs.

P239

Phosphoproteome and secretome analysis reveal differences between TNF-induced apoptosis and necroptosis

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The tumor necrosis factor (TNF) is a pro-inflammatory cytokine and the major cytokine targeted by therapies in inflammatory diseases like Rheumatoid Arthritis and Psoriasis. The pro-inflammatory function of TNF not only relies on the induction of other cytokines but also on its ability to induce cell death, which can either be caspase dependent (apoptosis) or -independent (necroptosis). Despite extensive research on the TNF signaling pathway, signaling events that further differentiate apoptosis and necroptosis, as well as the physiological impact of apoptotic or necroptotic cells on surrounding cells, remain to be discovered.

Firstly, to identify novel signalling events we analysed the phosphoproteome of the lymphoma cell line U937 undergoing either apoptosis or necroptosis. Hereby, we used the recently published EasyPhos strategy to enrich for phosphorylated peptides, which were subsequently identified and quantified by mass spectrometry. We identified

DOI: 10.1002/eji.201970300

common and unique phosphorylation events of already known and novel players discriminating apoptotic and necroptotic cells.

Secondly we applied mass spectrometry-based proteomics to dissect protein release during apoptosis and necroptosis. We report hundreds of proteins released from human myeloid cells in time-course experiments. Both cell death types show reduced cytokine levels in supernatant. They also induce receptor shedding, but only apoptotic cells released nucleosome components. Conversely, necroptotic cells released lysosomal components by activating lysosomal exocytosis at early stages of necroptosis- induced membrane permeabilisation.

Overall, these findings add to a better global understanding of cellular processes during TNF-induced apoptosis and necroptosis and will contribute to the growing knowledge about their physiological consequences.

P240

Double-stranded RNA triggers cell death through an RLR-dependent priming and an RLR-independent effector phase

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Introduction: Cytoplasmic double-stranded RNA released by many viruses during infection is sensed by RIG-I-like receptors (RLR). Activation of RLR signaling ultimately leads to the induction of Type I interferons (IFN), proinflammatory cytokines and apoptosis. While IFN and cytokine induction through RLR signaling is well described, there is contradictory data on the mechanisms leading to cell death. Particularly the mechanisms of IFN induction have not been convincingly discriminated from cell death pathways.

Objectives: In the study presented, we elucidate the differential signaling mechanisms downstream of RLR activation leading to either cytokine secretion and/or cell death induction.

Methods: Human and murine knockout (KO) melanoma cell lines were established using CRISPR-Cas9 followed by analysis of cytokine and cell death induction upon 3P-RNA transfection using ELISA, Annexin V staining, viability and RNA degradation assays.

Results: IFN production and cell death were strongly dependent on intact RLR signaling. Surprisingly, co-culturing KO and wildtype cells or priming cells with IFN rescued the ability of RIG-I-, MAVS- and IRF3-deficient cell lines to undergo apoptosis in response to 3p-RNA. Affinity purification followed by mass spectrometry revealed 3p-RNA specific binding of oligoadenylate synthetase 1 (OAS1). Overexpression of OAS1 alone was sufficient to trigger apoptosis in RIG-I KO cells in response to 3p-RNA and cells deficient for RNAseL showed profoundly impaired ability to undergo cell death. Finally, we show, that the concerted action of translational arrest, triggered by RNAseL, and upregulation of NOXA by RIG-I is needed to rapidly deplete the anti-apoptotic BCL-2 family member MCL-1 and thereby induce intrinsic apoptosis in melanoma cells.

Conclusion: Our thorough analysis of RLR-induced signaling pathways using KO cell lines provides clear evidence, that cytokine release and cell death induction are two separable events downstream of cytoplasmic 3p-RNA recognition. While direct RLR signaling leads to transcriptional priming of the OAS/RNaseL pathway and mitochondrial priming via NOXA, the execution of cell death is dependent on translational arrest triggered by activity of the OAS/RNAseL system. This two-step mechanism consisting of priming and effector phase reminiscent of NLRP3 inflammasome activation appears to be a common mechanism in innate immunity allowing the cell to either cope or perish depending on the insult taken.

DOI: 10.1002/eji.201970300

Autoimmunity and chronic inflammation (P241-P285, P401, P403, P409)

P241
Ethanol consumption decreases autoantibody titers by blocking IL-21 secretion of Tfh cells during the development of arthritis

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Our understanding of autoimmune disorders has advanced with the identification of environmental risk factors promoting disease onset. Despite known adverse immune-related health effects byof alcohol consumption, epidemiological data identifyconsistently identifies alcohol consumption consistently as a protective factor foragainst the most common autoimmune inflammatory joint disease worldwide, rheumatoid arthritis (RA). The underlying mechanism for this tolerance-inducingdisease-protective effect of alcohol is unknown. Here, we explored the effects of alcohol, and its metabolite acetate, on T follicular helper (Tfh) cells as specialized providers for T cell help to which provide help to germinal center (GC) B cells and prevalent for in immunoglobulin (Ig) secretion and affinity maturation and secretion. *In vivo*, ethanol or acetate intake reduced Tfh cell numbers and Ig titers andleading to preventedameliorated disease onset in antibody dependent autoimmune animal models for RA and multiple sclerosis. *In vitro*, ethanol and more potently acetate specifically suppressed IL-21 secretion by Tfh cells. This effect mitigated B cell activation and autoantibody response leading to increased tolerance to RA.

P242
DETECTION OF MOG-ABS IN PATIENTS: COMPARISON OF MOG DISPLAYED IN TRANSFECTED CELLS AND LIPID COATED BEADS

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Introduction: Antibodies against Myelin Oligodendrocyte Glycoprotein (MOG) can be found in a proportion of patients with inflammatory CNS diseases. Currently, the most reliable detection of autoantibodies against MOG is a cell-based assay, in which the full-length version of MOG (FL-MOG) is expressed in transfected cells. Alternatively, autoantibodies are detected by an ELISA assay, in which only the external domain of MOG (ED-MOG) is used. However, for unknown reasons, this technique fails to recognize most of the MOG+ patients.

Materials: In this study, we systematically explore the underlying mechanism for the missing detection. We tested the influence of the fluid environment of the cell membrane on the antibody binding and detection. To create proximity to a membrane, we propose a new *in vitro* assay for MOG detection using silica beads coated with a supported lipid bilayer.

Results: The newly developed technique is able to detect the monoclonal antibody against MOG (8-18C5), but largely fails in the detection of autoantibodies in serum of MOG+ patients.

Conclusion: Then, we explored the differences in the structure between FL-MOG and ED-MOG that might cause the difference in detection. Here we present our latest progress: in contrast to cells expressing FL-MOG, also cells transfected with ED-MOG are less capable of detecting autoantibodies in the MOG+ patients. Strikingly, even highly MOG positive patients showed a strongly decreased detection rate. Leading us to believe that the intracellular part of MOG is essential for the proper antibody detection of its external domain. Making us investigating further in this direction.

DOI: 10.1002/eji.201970300

P243

Lipid metabolism and macrophage activation are broadly dysregulated in aspirin-exacerbated respiratory disease

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Background: Aspirin-exacerbated respiratory disease (AERD) represents a type 2 inflammatory disease which is driven by an aberrant metabolism of (the n-6 PUFA) arachidonic acid (AA). Macrophages are major producers of AA metabolites and subject to metabolic reprogramming, but they have been neglected in AERD.

Objective: We sought to define transcriptional and metabolic aberrations as well as macrophage activation profiles in AERD.

Methods: We performed RNA sequencing (RNAseq) analysis of nasal brushings and macrophages from AERD patients and healthy controls and compared metabolite and lipid mediator profiles in nasal lining fluid (NLF), sputum, plasma and cell culture supernatants by targeted metabolomics and lipidomics.

Results: Clinical parameters (SNOT-22, sniffing test and MALM score) were increased in AERD patients, whilst metabolic parameters such as BMI and plasma adipokines remained unchanged. RNAseq analysis identified genes regulating metabolism and macrophage function (APOE, HTR4, CD200R1, PTGS2, CD1 molecules, chemokines) as being differentially expressed in nasal tissue or macrophages from AERD patients. Targeted metabolomics and pathway analysis revealed aberrant production of multiple lipid metabolites (eicosanoids, sphingolipids and acylcarnitines) in the airways and/ or in plasma of AERD patients.

Conclusion: Our findings reveal a broadly dysregulated lipid metabolism and an aberrant macrophage activation as potential pathomechanisms of AERD. Restoring normal macrophage function and homeostatic lipid metabolism may aid to balance mediator profiles and reduce airway inflammation in AERD.

P244

Canonical and non-canonical functions of tyrosine kinase 2 (TYK2) during Concanavalin A-induced hepatitis

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Introduction: TYK2 belongs to the Janus kinase family of receptor-associated tyrosine kinases and is an integral part of signalling cascades utilized by many cytokines with important immune regulatory activities. TYK2 acts protective during microbial infections but also drives inflammatory and autoimmune diseases. TYK2 inhibitors are considered as promising therapeutic tools, although kinase-independent functions of TYK2 are incompletely understood.

Objectives: Aim of the study is to determine whether TYK2 and its canonical enzymatic activity contribute to the pathogenesis of Concanavalin A (ConA)-induced hepatitis, which is a T cell- and NKT cell-dependent experimental model of autoimmune hepatitis.

Materials & methods: Mice deficient for TYK2 (*Tyk2*$^{-/-}$) or expressing enzymatically inactive TYK2 (*Tyk2*^{K923E}) were treated with 20mg/kg ConA for 2h, 6h or 24h. Blood, liver and spleen were collected and analysed by FACS, qRT-PCR and immunohistochemistry. Liver damage was assessed by serum parameters (liver transaminases ALT and AST). 0,25ng/g IL-22-Fc or isotype control was administered 1h after ConA injection.

Results: We show that *Tyk2*$^{-/-}$ but not *Tyk2*^{K923E} mice have increased neutrophil infiltration and liver damage upon ConA treatment compared to *WT* mice. Increased sensitivity of *Tyk2*$^{-/-}$ mice correlated with impaired production of the hepatoprotective cytokine IL-22. Therapeutic administration of recombinant IL-22-Fc in *Tyk2*$^{-/-}$ mice reduced the levels

DOI: 10.1002/eji.201970300

of ALT and AST to those observed in *WT* and *Tyk2*^{K923E} mice. Surprisingly, *Tyk2*^{K923E} mice also had impaired production of IL-22. ConA-induced hepatocyte death is synergistically driven by IFNγ and TNFα. IFNγ production by T cells, NKT cells and innate lymphocytes was impaired in *Tyk2*$^{-/-}$ and *Tyk2*^{K923E} mice, whereas hepatic *Tnfa* mRNA levels were selectively reduced in *Tyk2*^{K923E} mice. We are currently investigating which cell types show genotype-dependent TNFα production and whether liver lymphocytes differ in regard to their cytotoxic activity.

Conclusion: Our study revealed an unanticipated protective function of TYK2 in autoimmune hepatitis and suggests that kinase-inactive TYK2 has gain-of-function activities.

This work is supported by the Austrian Science Fund (FWF, grants SFB-F6101, SFB-F6106, DK-W1212 and DOC 32-B28)

P245

The dopaminergic pathway – a potential new route to modulate B cells in rheumatoid arthritis

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Introduction & Objectives Studies revealed that the neurotransmitter dopamine (DA) contributes to local joint inflammation in rheumatoid arthritis (RA) and its pro-inflammatory role in systemic immune response was also demonstrated. Although limiting inflammation, current therapeutic approaches for RA are often accompanied by severe side effects. We aim to detect disease related-alterations of the dopaminergic system by investigating peripheral blood mononuclear cells (PBMCs) of healthy controls (HC) and RA patients and thereby to identify targets for directed modulation of RA.

Patients & Methods RA patients were recruited for a cross-sectional study and therefore heterogeneous in all parameters. PBMCs were isolated from whole blood and cryopreserved for batched analysis. Expression of DA receptors (DR) D1-5 was examined in PBMC subpopulations of HC (n=14-17) and RA patients (n=11-15) via flow cytometry. Expression data was correlated with age, gender and clinical features. To investigate the effect of DA especially for B cells *in vitro* we used DR agonists in T cell-dependent and -independent B cell stimulatory conditions. B cell maturation and proliferation was analyzed via FACS-staining and CFSE dye dilution after 6 days *in vitro*. Supernatants were stored for analysis of cytokine and immunoglobulin secretion.

Results DRD1-5 were expressed on all investigated leucocyte subpopulations, independent of health or disease. We discovered some gender-dependent differences in expression levels and especially a significantly increased expression of DRD1 in B cells of women affected by RA compared to HC (p=0,0007). The expression of DRD1 in B cells of female RA patients correlated with disease duration (P=0,0046) and functional impairment (p=0,008). Deeper phenotyping revealed that primarily naïve B cells were responsible for these findings. First results of *in vitro* experiments point to a role for DA in early B cell maturation. D1-like stimulation slightly increased cell proliferation and development, apparently independently of T cell help.

Conclusion Targeted modulation of overexpressed DRs in RA could inhibit B cell maturation and thus suppress pro-inflammatory effects of autoreactive B cells. More functional analyses are ongoing to define DA role in systemic immune response in RA.

P246

Keratinocytes costimulate naïve human T cells via CD2: A potential target to prevent the development of pro-inflammatory Th1 cells in the skin

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The interplay between keratinocytes and T cells plays an important role in the pathogenesis of chronic inflammatory skin diseases. During psoriasis, keratinocytes attract T cells by the release of chemokines while skin-infiltrating self-reactive T cells secrete proinflammatory cytokines (IFNγ, IL-17A), which cause the pathophysiological hallmarks of

DOI: 10.1002/eji.201970300

psoriasis, e.g. epidermal hyperplasia. Similarly, in chronic graft versus host disease, allogenic IFNγ-producing Th1/Tc1 and IL-17-producing Th17/Tc17 cells are attracted by keratinocyte-derived chemokines and accumulate in the skin. However, whether keratinocytes could act as non-professional antigen-presenting cells to activate naïve human T cells directly in the epidermis remained so far unknown.

We demonstrated that under pro-inflammatory conditions, primary human keratinocytes indeed activated naïve human T cells. This required cell contact formation and costimulatory signaling via CD58/CD2 and CD54/LFA-1. Naïve T cells, costimulated by keratinocytes, selectively differentiated into Th1 and Th17 cells. Especially keratinocyte-initiated Th1 differentiation was triggered by costimulatory signals through CD58/CD2. Gene expression analysis and subsequent phospho flow cytometry revealed a direct link between CD58/CD2 interaction, STAT1 activation and T cell specific IFNγ production. The latter one initiated the keratinocyte-dependent Th1 differentiation.

Costimulation of T cells by keratinocytes resulting in Th1 and Th17 differentiation provides a so far unknown explanation for the local enrichment of Th1 and Th17 cells in skin of patients with chronic inflammatory skin diseases. Consequently, local interference with T cell-keratinocyte interactions may open up novel strategies for the treatment of Th1 and Th17-driven skin diseases.

P247

Characterization of dendritic cells in primary sclerosing cholangitis

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Introduction: While the precise pathogenesis of primary sclerosing cholangitis (PSC) remains unknown, genetic associations suggest an important role of immune dysregulation. While T and NK cells have been described to show distinct phenotypes in PSC individuals, only few studies have investigated the role of dendritic cells (DCs). It has been proposed that pathogens in the bile of PSC individuals can activate DCs, inducing subsequent inflammation and recruitment of other immune cells.

Objectives: The aim of this study was to characterize peripheral blood and intrahepatic conventional dendritic cells (cDCs) and plasmacytoid dendritic cells (pDCs) from PSC patients and compare them to those of healthy control individuals, patients with primary biliary cirrhosis (PBC) and autoimmune hepatitis (AIH).

Patients & methods: Peripheral blood from healthy control individuals, patients with PSC, PBC and AIH, as well as liver samples from PSC patients undergoing liver transplantation and liver samples from liver resection surgeries, serving as non-PSC controls, were used for this study. Peripheral and intrahepatic lymphocytes were analyzed using multi-parameter flow cytometry. Plasma from PSC, PBC and AIH patients as well as healthy control individuals was analyzed for chemokine and cytokine levels. Cryosections from PSC livers and non-diseased control livers were used for fluorescence microscopy analysis.

Results: pDC frequencies showed a trend towards lower numbers in peripheral blood when comparing PSC patients and healthy control individuals. Furthermore, cDCs showed a trend towards higher numbers in the intrahepatic compartment of PSC patients compared to non-diseased controls. Peripheral blood pDCs from PSC patients expressed higher levels of CD86 compared to healthy control individuals, PBC or AIH patients. Multiplex bead analysis of cytokines showed significantly higher concentrations of CCL21, CXCL10 and TNF-α as well as significantly lower IL-12 levels in the plasma of PSC patients compared to healthy control individuals. Finally, we observed higher levels of CCL21 in PSC livers compared to non-diseased control livers.

Conclusions: Taken together, these data indicate an activation of DCs in patients with PSC, resulting in higher expression of chemokines, both in the peripheral blood and in the liver. DCs might therefore serve as an important driver of inflammation in PSC.

DOI: 10.1002/eji.201970300

P248

Restimulation of activated, circulating myelin-specific CD4 T cells in the liver prevents autoimmune neuroinflammation

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Introduction/Objectives: We have previously shown that ectopic expression of myelin basic protein (MBP) in the liver can prevent autoimmune encephalomyelitis (EAE); however, the mechanisms explaining how the liver can effectively protect distant tissues are not entirely clear.

Methods: To elucidate systemic effects of hepatic tolerance, we studied the fate of MBP-specific CD45.1 congenic tg4 T cells, which were adoptively transferred in abundance into CRP-MBP mice expressing MBP in the liver.

Results: BP-specific tg4 T cells were transferred into wildtype or CRP-MBP mice immunized to MBP. Transfer of tg4 T cells into wildtype mice led to an accelerated and aggravated EAE course, whereas CRP-MBP recipients remained resistant to EAE induction. However, the transferred tg4 T cells produced mild, transient hepatitis in CRP-MBP recipients, but not in wildtype mice, marked by transient liver infiltration and increased serum alanine transaminase levels. In CRP-MBP recipients, transferred tg4 T cells showed significant expansion and increased IFN-γ production, as compared to wildtype mice. CRP-MBP livers showed a five-fold increase in both, CXCL9 and CXCR3 expression, as compared to wildtype livers, providing a mechanism responsible for the observed hepatic retention of tg4 cells. The retained tg4 T cells in the livers of CRP-MBP mice showed a significant up-regulation of several co-inhibitory receptors, including Lag-3, PD-1, Tim-3, TIGIT and CTLA-4, and tSNE analysis confirmed their co-expression. Blockade of IFN-γ by in vivo administration of an anti-IFN-γ antibody reduced expression of CXCR3 and CXCL9 to levels seen in wildtype mice, and prevented hepatic retention of tg4 T cells. As a consequence, IFN-γ blockade also impaired immune tolerance to MBP in CRP-MBP mice, which developed mild EAE.

Conclusion: Our findings demonstrate that reactivation of circulating tg4 T cells in the liver induced the production of IFN-γ, concomitant up-regulation of CXCL9 and CXCR3 and subsequent retention of tg4 cells in the liver. Hepatic retention was associated with high expression of co-inhibitory receptors and complete resistance to EAE induction. Blockade of IFN-γ signals prevented this tolerogenic sequence and enabled EAE development.

P249

Deep immune profiling of human Peyer´s Patches in patients of inflammatory bowel diseases

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Introduction: One of the hallmarks of inflammatory bowel diseases is a dysregulation of the intestinal immune system. Although nutritional therapy is effective, little is known about its mechanism. The immune system is linked to food antigens through the Peyer´s patches (PP) in the ileum and PP seems to be essential for oral tolerance, Murine data indicate that food antigens induce an activation and subsequent apoptosis of the $CD4^+$ T-cells in the PP thus maintaining the healthy balance of the mucosal immune system.

Objectives: Human PP cells from the terminal ileum of IBD patients and healthy controls were analysed for their phenotype and fate.

Materials & methods: Human fresh PP cells were characterized between Crohn´s disease (CD), Ulcerative colitis (UC) patients and healthy controls. Apoptosis measurements and basic phenotyping were performed via flow cytometry. For a deeper analysis, PP cells were analysed via mass cytometry (CyTOF2 – Helios).

Results: PP $CD8^+$ T-cells with an effector memory phenotype expressing Tbet and Bcl2 were increased in CD and UC compared to healthy controls. Moreover, PP $CD4^+$ T-cells with an effector memory phenotype expressing Helios and Bcl2 were decreased in all IBD groups. Furthermore, PP $CD4^+$ T-cells of CD patients revealed a significantly reduced apoptotic rate compared to UC patients and healthy controls. This was accompanied by an increased

DOI: 10.1002/eji.201970300

expression of the survival marker Bcl-2. Further characterization identified a decrease of Helios$^+$FoxP3$^-$ in CD patients, described as diet-specific T-cells undergoing apoptosis.

Conclusion: In the healthy human gut, food-activated CD4$^+$ T-cells in PPs exhibited a pro-apoptotic phenotype characterized by the transcription factor Helios. In contrast, low Helios$^+$ FoxP3$^-$ expression and reduced apoptosis were observed in PP CD4$^+$ T cells of patients with IBD, suggesting that activation and subsequent death of food-reactive T cells is a hallmark of intestinal homeostasis.

P250

IL-17A/F mediates immune regulatory function of cholangiocytes in a mouse model of acute, T cell mediated cholangitis

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Introduction: Interleukin (IL)-17 has been associated with the pathogenesis of several autoimmune disorders. Increased numbers of Th 17 lymphocytes and IL-17A were observed in serum and livers of patients with autoimmune liver diseases, including primary sclerosing cholangitis. So far, the effect of IL-17 on cholangiocytes in an inflammatory environment is incompletely understood.

Objectives: We here investigated the role of IL-17A/F producing T cells on cholangitis severity and its specific effect on cholangiocytes using an inducible mouse model of acute cholangitis.

Materials & Methods: K14-OVAp recipient mice express an MHC I restricted ovalbumin peptide (SIINFEKL) on cholangiocytes. Acute cholangitis was induced by adoptive transfer of transgenic OVA-specific OT-1 CD8+ T cells or OT-1xIL-17A/F-/- CD8+ T cells to investigate the role of IL-17 in experimental cholangitis. Liver enzymes, histology, qPCR, cytokine expression and flow cytometry were used to assess liver inflammation.

Results: Adoptive transfer of antigen specific OT-1 CD8+ T cells led to portal inflammation in K14-OVAp recipient mice with the transferred cells localized around bile ducts. The lack of IL-17A/F in transferred OT-1 CD8+ T cells resulted in enhanced liver inflammation compared to the transfer of IL-17-competent OT-1 cells. Liver infiltrating antigen-specific OT-1xIL-17A/F-/- CD8+ T cells were highly activated, secreted large amounts of IFN-gamma and granzyme B and displayed increased proliferation. After contact with antigen-specific CD8+ T cells, activation of cholangiocytes and upregulation of inhibitory PD-L1 was observed. Lack of IL-17A/F in CD8+ T cells resulted in reduced expression of PD-L1 in cholangiocytes which was associated with uncontrolled expansion of cytotoxic CD8+ T cells.

Conclusion: We could here show that the lack of IL-17A/F in antigen specific CD8+ T cells induces a severe phenotype of experimental cholangitis. Our results indicate an important function of IL-17 in the control of T cell dependent cholangitis via upregulation of co-inhibitory molecules in cholangiocytes. Caution should be taken when targeting IL-17 for the treatment of cholangitis.

P251

Dissecting the functional role for homophilic and heterophilic CEACAM1-ligation in immune regulation

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Introduction.CEACAM1 (carcinoembryonic antigen-related cell adhesion molecule 1) is a homophilic and heterophilic adhesion molecule that acts as an immune co-receptor on leukocytes. Soluble CEACAM1 (sCC1) was originally discovered as a serum marker in human patients with obstructive and autoimmune liver disease. In murine autoimmune hepatitis (Concanavalin A-induced hepatitis), CEACAM1 promotes IL-2-dependent Treg induction and stability, and *Ceacam1*$^{-/-}$ mice exhibit hyperinflammation and persistence of liver injury. This immune-regulatory function requires tight regulation of CEACAM1 isoform expression; on CD4$^+$ T cells, CEACAM1-S, the activatory isoform with a short cytoplasmic domain, fosters cytokine production and Treg induction. Contrary, its inhibitory

DOI: 10.1002/eji.201970300

isoform with two ITIMs and a long cytoplasmic tail (CEACAM1-L) interacts with co-inhibitory immune receptors (e.g. TIM-3) and limits activation.

Objectives. The role of CEACAM1-ligation in hepatic immune regulation is unknown. The role of CEACAM1/sCC1 in co-cultures of $CD4^+$ T cells and antigen-presenting cells (dendritic cells, DCs) for T cell activation and Treg induction is investigated.

Materials & methods. In sera from human patients and mice, CEACAM1 was detected in Western Blots. Cocultures from bone-marrow derived, FACS-sorted DCs and MAC-sorted T cells from CEACAM1-deficient and WT mice were analyzed in FACS, and cytokines were quantified by multiplexing and ELISAs. Expression of *Ceacam1*isoforms was analyzed by semi-quantitative RT-PCR.

Results. CEACAM1 is detectable in sera of patients with advanced PSC, and in sera of WT mice. In cocultures of $CD4^+$ T cells and dendritic cells, expression of CEACAM1-S precedes up-regulation of CEACAM1-L in both cell types. sCC1 inhibits production of IL-2 by $CD4^+$ T cells and IL-12 by DCs, regardless of their CEACAM1 expression status. sCC1 strongly binds to activated $CD4^+CD25^+$ T cells which results in phosphorylation of STAT5 and upregulation of Foxp3.

Conclusion.CEACAM1 isoform expression is tightly regulated in activated immune cells. Homophilic ligation of CEACAM1 supports dampening of immune responses in vitro by reduction of IL-2 production and induction of Foxp3 in $CD4^+$ T cells. On DCs, sCEACAM1 binds to a heterophilic ligand, which leads to reduction of IL-12 production. This immunmodulatory function of (s)CEACAM1 will be further explored *in vivo*.

P252

The immunological role of adipose tissue in inflammatory bowel disease

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Introduction: Crohn´s disease (CD) is characterized by intestinal epithelial damage and subsequent translocation of bacteria from the intestinal lumen into the adjacent mesenteric fat, inducing fat hyperplasia as well as the recruitment of various immune cells. However, the role of adipose tissue in intestinal auto-immunity remains elusive.

Objectives: To better understand the role of mesenteric fat in the regulation of intestinal immune responses we compared the immune cell compositions of mesenteric fat in mouse models of intestinal inflammation and mesenteric fat of patients with Crohn"s disease, ulcerative colitis (UC) or colon rectal cancer (CRC) by mass cytometry.

Materials and method: Immune cells were isolated from mesenteric fat, gonadal fat, mesenteric lymph nodes and intestinal lamina propria of intestinal epithelial specific caspase-8 (*Casp8*$^{\Delta IEC}$) knockout mice or wild type littermates. Alternatively, immune cells were isolated from mesenteric adipose tissue collected from CD, UC, CRC patients undergoing disease-related surgery. Cells were analyzed by mass cytometry using a panel of 36 lineage and functional markers.

Results: Our data provide for the first time a comprehensive, comparative immune cell characterization of lamina propria, mesenteric lymph nodes, mesenteric fat and gonadal fat in *Casp8*$^{\Delta IEC}$-induced ileitis. $CD206^+$ macrophages were the most abundant myeloid cells found within adipose tissue and interestingly, we observed that solely $CD206^+$ macrophages infiltrating mesenteric fat of mice with ileitis displayed an up-regulation of some metabolic markers including CD38 and decrease of TNFα production. Moreover, mesenteric but not gonadal fat of *Casp8*$^{\Delta IEC}$ mice showed infiltration of $Ly6G^+$ cells. $CD206^+$ macrophages were abundant in mesenteric fat tissue of CD, UC, CRC patients while $CD8^+$ T cells were mainly enriched in adipose tissue collected from CD patients.

Conclusion: Our data suggest for the first time a dynamic immune-modulatory function of mesenteric fat in relation to the location and development of intestinal inflammation, highlighting the immunological contribution of adipose tissue to the decors of IBD.

DOI: 10.1002/eji.201970300

P253

The C5a/C5aR1 axis drives the development of autoimmune skin blistering disease through regulation of IgG auto-antibody production, IgG1 Fc glycosylation and cytokine production

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Introduction and Objectives: Epidermolysis bullosa acquisita (EBA) is a subepidermal blistering disease caused by auto-antibodies (aAbs) against collagen typ VII (Col7). Col7 is a major component of anchoring proteins, which are responsible for stability of the epidermal-dermal junction in the skin. In an antibody-transfer model of EBA, we previously showed that C5a receptor 1 (C5aR1)-deficient mice are protected from the development of skin blistering. This finding suggested a critical role of the C5a/C5aR1 in the effector phase of EBA.

Material and Methods: We immunized EBA-susceptible (B6.s), EBA-resistant (B6.j) wildtype (wt) mice and B6.s C5aR1$^{-/-}$ mice with vWFA2, the immunodominat region with Col7, and Titermax (1:1). Biweekly for eight weeks we scored the clinical symptoms of the mice and took blood samples for ROS analysis, Col7 epitope mapping and cytokine assays. We purified Col7-specific serum aAbs and quantified the different IgG subclasses. Finally, we analyzed the IgG Fc-glycosylation pattern as an addition measure of pro- or anti-inflammatory properties of IgG aAbs.

Results: We saw first clinical symptoms in B6.s mice between weeks 4 -6 after immunization, whereas B6.s C5aR1$^{-/-}$ mice were completely protected from EBA development up to week 12. In the serum of B6.s C5aR1$^{-/-}$, we found significantly lower levels of Col7-specific, complement-activating IgG2b and IgG2c aAbs and a significantly higher concentration of IgG1 aAbs. Additionally we analyzed the Fc glycosylation of IgG1 aAbs at week 12. We found a low frequency of pro-inflammatory agalactosylated IgG1 aAbs in sera from B6.s C5aR1$^{-/-}$ mice. In contrast, the frequency of anti-inflammatory, highly sialylated and or galactosylated IgG1 was high in sera from B6.s C5aR1$^{-/-}$ but not from B6.s mice. Also, IL-6, IL-12 and IL-17a serum cyokine concentrations in B6.s wt mice were significantly higher than those in B6.s C5aR1$^{-/-}$ mice at weeks 6 and 8. Of note, IL-10 serum levels in B6.s C5aR1$^{-/-}$ mice were significantly higher than those in B6.s wt mice.

Conclusions: Our findings point towards a less pro-inflammatory environment in B6.s C5aR1$^{-/-}$ mice and identify the activation of the C5a/C5aR1 axis as an important immune mechanism driving EBA.

P254

Newly generated metal complex has immunosuppressive functions by inducing TGFß expression

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The aryl hydrocarbon receptor (AHR) functions in various biological processes in a ligand-dependent manner. Upon ligand binding the receptor translocates into the nucleus and acts as a transcription factor. We could show that a newly generated gold metal compound 3 (MC3) binds to the aryl hydrocarbon receptor (AHR). Binding was 100-fold stronger compared to the AHR ligand 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD).

To investigate the in vitro immunosuppressive function of MC3, primary murine WT CD4$^+$ T cells were isolated and treated over night with MC3 followed by stimulation with CD3/CD28. Treatment with MC3 showed an upregulation of CYP1A1 and TGFβ1, TGFβ2 and TGFβ3 in comparison to mock solution using real time PCR. In addition, flow cytometry analysis showed a 2-3 fold higher CD4$^+$CD25$^+$foxP3$^+$Treg frequency upon MC3 treatment in comparison to mock treated CD4$^+$ T cells. Furthermore, the increased Treg frequency could be reversed by using AHR antagonists or TGFß inhibitors in vitro.

DOI: 10.1002/eji.201970300

The effect of MC3 was additionally investigated using an autoimmune-mouse model. Scurfy mice lack functional Treg and show activation of autoreactive T cells which leads to T-cell mediated inflammation of several organs including liver, lung and skin. On average scurfy mice die due to multi-organ failure at day 24 of life. When treated with MC3, scurfy mice showed a prolonged life expectancy up to 70 days. Furthermore, we found after 24 days treatment a reduction in the frequencies of activation markers (CD69, CD25) on $CD4^+$ pre-gated T cells in comparison to mock-treated mice. RtPCR data show that MC3 treated mice (WT and scurfy) have higher *TGFβ1* expression in liver, spleen and thymus in comparison to mock treated mice. Similar results could be shown by *TGFβ1* in-situ staining of thymus and liver.

In summary, the new gold compound MC3 functions as AHR agonist which induces increased TGFβ1 expression and thereby might lead to Treg induction. Furthermore MC3 has an immunosuppressive effect on T cells in an autoimmune model and increases the life expectancy of scurfy mice.

P255

Impaired degradation of phagocytosed nuclear material contributes to inflammation in C1q-deficient mice

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C1q-deficiency is strongly associated with the development of systemic lupus erythematosus (SLE) in humans and lupus-like autoimmunity in mice. Often, macrophages (MPs) show a reduced ability to engulf apoptotic cells in SLE patients. In agreement with this, it has been shown that C1q-KO mice show impaired engulfment of dead cells. However, the precise mechanisms of how this leads to the development of SLE is unclear. Using apoptotic cells with fluorescently-labeled H2B histones, we could show that C1q-KO peritoneal macrophages (pMPs) exhibit a delayed degradation of nuclear material derived from phagocytosed apoptotic cells. We found a significant reduction of lysosomal DNASE2A in C1q-KO splenic enriched MPs and pMPs which is necessary for digesting the engulfed apoptotic nuclear material. As a consequence, C1q-KO MPs show elevated levels of factors involved in the STING-pathway, which is activated by recognition of cytosolic DNA. We also found a significant reduction of RAB5 in C1q-KO MPs which is necessary for maintaining the endo-lysosomal system. In addition, enlarged lysosomal compartments were seen in C1q-KO MPs which is an indication of dysfunctional lysosomes. In order to restore the degradation of phagocytosed nuclear material, we coated apoptotic cells with a DNASE2A fused to the apoptotic-cell binding protein MFGE8, to deliver DNASE2A directly to the lysosomes containing DNA of phagocytosed apoptotic cells. This not only restored DNA-degradation in C1q-KO MPs, but also lead to reduced production of inflammatory cytokines upon phagocytosis. Hence, we postulate that C1q-deficiency leads to accumulation of non-degraded DNA in phagocytic cells triggering production of pro-inflammatory cytokines via the STING pathway. In addition, reduced DNASE2A expression could be a consequence of the disrupted endo-lysosomal system seen in C1q-KO MPs.

P256

Functional role of the Tec kinase ITK in the pathogenesis of inflammatory bowel disease

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Introduction The tyrosine-protein kinase ITK (interleukin-2-inducible T-cell kinase) is a member of the Tec family kinases and an important component of the TCR signaling pathway. ITK is required for activation of T-cells and promotes Th2 plus Th9 differentiation. Crohn"s disease (CD) and ulcerative colitis (UC) are chronic inflammatory disorders of the intestine and are considered as clinical prototypes of inflammatory bowel disease. In contrast to CD patients the mucosal T-cell cytokine profile in UC is characterized by the production of Th2 and Th9 cytokines. Moreover, the immunosuppressive drug Cyclosporin A (CsA) shows a protective effect in UC patients rather than CD patients. However, the molecular mechanism of action is unknown. In experimental colitis model ITK-deficient mice (ITKKO) show a protected phenotype after acute oxazolone treatment.

Objectives Our goal is to get new insights into the functional role of ITK controlling the resolution of inflammation in experimental colitis. Furthermore, we want to elucidate the molecular mechanism of action for CsA. We hypothesize that CsA interacts with ITK via the immunophilin CypA in UC patients.

DOI: 10.1002/eji.201970300

Patients & Methods ITK and CypA expression in patients with inactive or active UC or CD was measured by qPCR analysis. Acute and chronic oxazolone mediated colitis was induced in ITKKO mice and controls. Disease activity was measured by means of histological and endoscopic score of inflammation activity. IL6-mini-circle-vector was administrated by hydrodynamic injection technique. Lamina propria mononuclear cells (LPMC) were isolated and the rate of apoptosis induction after treatment with CsA was assessed via flow cytometric analysis. Cytokine concentration (IL6, IL9, IL13, IL17A, TNFa) was assessed using ELISA.

Results Patients with active UC show a higher expression of ITK compared to patients with inactive UC .The expression rate of ITK is antagonistic to the expression of CypA. In oxazolone colitis model control mice treated with CsA show a significant induction of apoptosis in LPMCs and a reduction of IL6 expression. Administration of an IL6-mini-circle-vector restored the inflamed phenotype in ITKKO mice with and without CsA treatment.

Conclusion Our results indicate that CypA might be the link for CsA to inhibit ITK function. The restoration of the inflamed phenotype after administration of an IL6-mini-circle-vector in ITKKO mice treated with CsA underscores our hypothesis that CsA works via ITK.

P258

MBP85-99 specific TCR repertoire in DR2+ patients affected by multiple sclerosis

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T and B cells play a prominent role in the pathogenesis of Multiple Sclerosis (MS) and in particular peripherally activated T cells specific for myelin antigens become able to cross the blood-brain barrier, causing damage and recruitment of other immune cells. The T cell mediated self-reactivity against myelin is strongly linked to HLA; specifically in HLA-DR2+ subjects, an immunodominant T-cell epitope exists within MBP residues 85-99. Immunoscope analysis (TRBV-TRBJ spectratyping) of the repertoire of T cells specific for MBP85-99 revealed the usage of a limited number TCRs. The analysis was performed on the blood and cerebrospinal fluids (CSF) samples of 30 untreated DR2+ patients and 7 healthy DR2+ subjects, allowing to identify 2 TCR rearrangements shared by more than 50% of MS DR2+ patients during the active phase of the disease. the immunophenotyping analysis revealed that one of the two shared TCRs was exclusively used by IL-17 secreting cells and that its presence was down-regulated during remission after IFNβ therapy, that also reduced the overall circulating MBP specific TCR repertoire. Moreover, it was possible to detect MBP specific TCRs in patients" CSF during a relapse but not during a remission phase. Twenty-five % of the investigated TCR rearrangements were used by IFN-g secreting cells.

To date there are no available biomarkers for response to therapy and therapy efficacy is measured on the absence of clinical and radiological activity during follow-up.

The identification of a limited number of TCRs involved in the recognition of MBP85-99 in complex with HLA-DR2 specifically associated with disease can lead to development of tools aimed at measuring their number in the blood of MS patients as potential biomarkers of disease.

P259

Type 3 Innate Lymphoid Cells in models of psoriasis and psoriatic arthritis

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Introduction: Psoriasis (PsO) and psoriatic arthritis (PsA) are two types of chronic inflammatory diseases that share a similar cytokines profile. About 30% of PsO patients also develop a joint involvement, but the underlying mechanism is still unclear. Innate lymphoid cells (ILC) and specifically the type 3 ILCs (ILC3s) have raised increasing interest as possible player in the pathogenesis of both diseases, as they produce the pathological key cytokine IL-17A.

DOI: 10.1002/eji.201970300

Objective: We addressed the contribution of ILC3s to the pathogenesis of PsO and PsA in murine *in vivo* models.

Methods: PsO was induced by the repeated local application of imiquimod (IMQ) on the skin of hind paws. PsA was induced through the systemic IL-23 overexpression (IL-23OE) using a minicircle vector. PASI (Psoriasis Area Severity Index) score was used to assess the skin lesions and epithelial thickness was measured through histomorphometric analysis of skin. Joint inflammation was assessed through magnetic resonance imaging (MRI) and haematoxylin and eosin (H&E) staining of the ankle area. Peripheral blood was obtained and flow cytometry analysis was performed to address circulating ILC3s.

Results: Systemic IL-23OE induced psoriatic-like skin lesions at different areas of the mouse body compared to the limited inflammatory effect induced by IMQ. Simultaneous application of IMQ and IL-23OE induced a stronger skin phenotype in mice as compared to IL-23OE or IMQ treatment alone. MRI and histomorphometric analyses revealed strong musculoskeletal inflammation after 3 weeks in the IL-23OE model as compare to control mice and to IMQ treated mice. Of note, mice who received IMQ in addition to IL-23OE showed significantly less joint inflammation. IL-23OE resulted in a significant increase of circulating ILC and specifically of ILC3 compared to controls. Interestingly, simultaneous application of IMQ in addition to IL-23OE resulted into a significant decrease of circulating ILC3 compared to IL-23OE alone.

Conclusion: We showed that IMQ aggravated the PsO phenotype, but is able to ameliorate the arthritic involvement of PsA in mice. This phenotype correlated with the circulating numbers of ILC3s. ILC3s were increased in IL-23OE mice, suggesting that these cells can be mobilised and recruited upon cytokine stimulation. By contrast, IMQ interfered with the systemic effect of IL-23, limiting joint inflammation and reducing circulating number of ILC3.

P260
Innate lymphocytes (ILCs) respond to crystal-deposition and promote local inflammation and fibrosis

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Crystal-induced inflammation is a common manifestation of a variety of genetic and acquired metabolic disturbances as well as drug or toxin induced tissue damage. Here, DCs or macrophages sense the crystals, activate the NLRP3-inflammasome and produce cytokines like IL18 and IL1b. These, together with other cytokines such as IL23, promote local inflammation and induce the development of kidney fibrosis.

Our data indicated that a Rorγt+ and RAG-independent population play an important role in the development of kidney fibrosis. Based on this we aim to understand the mechanism by which ILCs sense and respond to crystal deposition and influence the development kidney fibrosis

Using a mouse model of crystal-induced nephritis by feeding an adenine-enriched chow we analysed the cytokines profile of Rorγt+ and Rorγt- ILCs. We detected different effects of IL18 and IL1β on ILC3s by analysing the cytokine production and cell proliferation.

IL18 and IL1β are produced in crystal nephropathies by resident DCs and macrophages. ILCs can sense this inflammatory environment and respond to these stimuli by releasing pro-inflammatory cytokines, such as IL17 and IFNγ, which promote the development of kidney fibrosis.

Our data suggests that the pro-inflammatory environment promoted by crystal deposition has an effect on ILCs, which defines the cytokine production suggesting plasticity within ILCs groups.

As a consequence, ILCs can be considered as early responders in a context of crystal nephropathies and might serve as a key player in the development of kidney fibrosis.

DOI: 10.1002/eji.201970300

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Tissue-destructive type 17 immunity is controlled by keratinocyte-derived de novo glucocorticoids in the skin

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The skin represents the outermost epithelial barrier of the body and controls immunity and tolerance while being constantly challenged with stress signals from the external and internal environment. *De novo* glucocorticoids (GC) synthesized in the skin are proposed to maintain the skin immune homeostasis and barrier competency through the regulatory and anti-inflammatory action of the glucocorticoid receptor present in various types of stromal and immune cells within the skin. To which extend cutaneous *de novo* GC regulate skin-resident immune cells is still not clear due to the lack of experimental models. To specifically investigate how skin-derived *de novo* GC may regulate the local skin immune system *in vivo*, we employed an inducible mouse model with Cre recombinase-mediated ablation of the relevant Cyp11b1 enzyme in keratinocytes. We here show that conditional deletion of this key enzyme in keratinocytes abrogates the *de novo* GC synthesis in the skin and observed that knockout (KO) mice deficient in keratinocyte-specific GC synthesis display spontaneous skin activation and increased numbers of activated CD40+ migratory dendritic cells (DC) in skin draining lymph nodes (dLN). Accordingly, cytokine expression such as IL-12, TGF-ß, IL-23, IL-6, IL-17 and $CD4^+$ and $CD8^+$ T cell memory subsets in the dLN were elevated whereas expression of IL-10 and $Foxp3^+$ Treg cells were decreased indicating that dLN are spontaneously primed and T cell differentiation is dominantly driven towards type 1/17. Ultimately, KO mice develop a spontaneous skin inflammation with epidermal hyperplasia and inflammatory immune cell infiltration. We conclude from our results that local GC production in the skin regulates the skin immune system through migratory DC. If disrupted, DCs are licensed to favorably trigger Th1/Th17 immune responses whereas induction of regulatory T cell differentiation is impaired, leaving the skin to be more prone against tissue-destructive immune responses. Our results highlight the potency of skin-derived GC in suppressing destructive immune responses and the induction of tolerance and further consider the relevance of endogenous skin GC in inflammatory and autoimmune skin disorders.

P262

Phenotypic and functional study of T regulatory cells and natural killer cells and their interplay with micronutrients and markers of disease severity in newly diagnosed Graves" disease patients

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Question: Graves" Disease (GD) is the most common cause of hyperthyroidism in iodine-sufficient areas. Autoantibodies directed to the TSH-receptor (TRAb) represent the ultimate cause. In the literature the results on the implication of both T regulatory (Treg) and natural killer (NK) cells are incomplete. Treg cells are key cell mediators inhibiting autoimmune disorders, whereas NK cells, in particular the regulatory CD56bright NK cell subset is increasingly seen as having regulatory features, and it has been suggested that they could play a role in controlling T cell responses and maintaining homeostasis. The potential role of micronutrients is on study. This case-control study aimed to evaluate the interplay between clinical markers of disease severity, micronutrients, NK and Treg cells in newly diagnosed GD.

Methods: Blood samples from consecutive newly diagnosed GD were collected. Healthy blood donors, matched for sex and age, served as control group. Several distinct fluorophore-conjugated mAbs were used for Flow Cytometry, i.e. anti-CD45, CD4, CD8, CD25, CD3, CD127 and anti-CD3, CD56, CD16, NKGD2, NKG2A, CD49d, CD69, CD161, for Treg cells and for NK cells, respectively. Furthermore, blood samples were collected to measure thyroid hormones (FT4, FT3), TSH, thyroid autoantibodies (TRAb, AbTPO), selenium (SE) and vitamin D (VITD) levels in GD. Thyroid volume was established by ultrasonography.

Results: Twenty-eight patients (women 22/28; mean age 44±11 years) and 22 controls (women 12/22; mean age 43.5 ±11 years) were enrolled. Treg cells were not decreased in GD compared to controls. Functional studies are ongoing. On the contrary, the percentage of CD56dim NK cell subset was reduced in GD compared to controls. Accordingly, the percentage of CD56bright NK in GD was almost doubled compared to donors. In GD, serum negative correlations were found between SE (mean values 92±13mcg/l), FT4, FT3 and TRAb levels. The relationship between

DOI: 10.1002/eji.201970300

VITD, TRAb and percentage of Treg/CD4+, initially demonstrated, was not confirmed after samples enlargement. Treg/CD4+ percentage was slightly inversely related both to FT4 and FT3 and significantly inversely related to TRAb levels.

Conclusions: Although preliminary, our results suggest a correlation between low SE status, immune regulatory cells and GD severity. Ongoing functional tests on T regulatory cells and NK cells will aim to clarify their role in the clinical manifestations of the disease.

P264

Diet-induced obesity influence collagen-induced arthritis in mice

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Introduction: At present, there are only a few reports concerning the effects of obesity on collagen-induced arthritis (CIA) in mice and rheumatoid arthritis in patients. Studies conducted by our team demonstrated that diet-induced obesity (DIO) aggravates T-cell mediated immune response such as contact hypersensitivity in mice. Unexpectedly, our preliminary data employing CIA model showed that male mice with DIO develop less severe disease.

Objectives: The purpose of this work is to determine the influence of obesity on collagen-induced arthritis**.**

Materials & Methods: To study the influence of obesity on CIA, DBA/1 mice were fed high-fat diet (HFD) or normal diet for 4 or 8 weeks prior to CIA induction.

Results: Our data show that male but not female mice fed HFD for 4 or 8 weeks develop less severe disease (clinical score). Decreased disease severity in male mice correlated with reduced myeloperoxidase (MPO) activity in joint tissue. Moreover, reduced concentration of anti-COLL II IgG2a correlated with decreased disease severity in HFD-fed male mice. Observed reduction of anti-COLL II IgG2a in male HFD-fed mice is in line with decreased percentage of $CD19^+$ $B220^+$ B cells in the spleen when compared to female mice fed HFD. Further flow cytometry analysis of lymphoid organs showed increased percentage of $CD4^+IL10^+$ lymphocytes in the spleen of HFD-fed male mice that developed less severe disease. This was not observed in female mice with DIO.

Conclusion: In summary, our data show that DIO alleviates CIA in male but not female mice suggesting that this phenomenon is determined by gender.

This work was supported by grant N43/DBS/000005 to MS.

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Nucleic acid recognition through specific receptors aggravates ANCA-associated vasculitis in the lung

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Introduction: ANCA associated vasculitides (AAV) are a group of life-threatening autoimmune diseases that lead to inflammation in the vasculature. Vessel inflammation frequently occurs in the lung and kidneys, causing not only pulmonary hemorrhages but also necrotizing glomerular nephritis. It is generally believed that microbial stimuli drive the generation of autoantibodies against the anti-microbial proteins myeloperoxidase (MPO) and proteinase 3 (PR3) which are predominantly stored in granules of neutrophils. Upon activation, neutrophils release highly immunogenic DNA complexed with MPO or PR3, followed by binding of the respective autoantibodies.

Objectives: In this study we wanted to investigate the underlying cellular and molecular mechanisms provoking pulmonary AAV.

DOI: 10.1002/eji.201970300

Materials & methods: Here, we present a novel and robust mouse model for anti-MPO-associated pulmonary vasculitis. It results from the combinatorial administration of immunological danger signals into the lung followed by systemic injection of anti-MPO Ig. Our model recapitulates the major clinical hallmarks of the human disease, such as severe lung pathology along with impaired lung function.

Results: We found that the absence of the stimulator of interferon genes (STING)-dependent signaling pathway protected mice from pulmonary AAV. Immune cell infiltration into the lung as well as weight loss were lower than in wild-type control mice, indicating a crucial role for DNA recognition in our model. Consistent with this, we observed that deficiency for the nucleic acid sensor cyclic GMP-AMP synthase (cGAS) attenuated the disease as well.

Conclusion: We suggest that the mechanistic basis for STING activation involves the release of highly immunogenic MPO-decorated DNA derived from neutrophils and the subsequent activation of accessory cells by immune complexes following anti-MPO binding.

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Hepatic interleukin-33 signalling induces an alternative pathway to counteract immune-mediated hepatitis

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Introduction: Autoimmune hepatitis (AIH) is a chronic inflammatory disease that is due loss of tolerance towards hepatic self-antigens. AIH is mainly driven by autoreactive $CD4^+$ Th1 cells that continuously express pro-inflammatory thereby causing ongoing necroinflammation in the liver. The alarmin interleukin (IL-) 33 is expressed in epithelial and endothelial cells with barrier function and in hepatocytes. Following necrosis, IL-33 activates $ST2^+$ immune cells, such as type 2 innate lymphoid cells (ILC2s) and a subset of regulatory T cells (Tregs). IL-33 was shown to counteract immune-mediated hepatitis by a yet unknown mechanism.

Question: Activated ILC2s and Tregs express the EGF-like molecule Amphiregulin (AREG) that mediates regeneration of wounded liver tissue and enhances the suppressive function of Tregs. Hence, it was investigated if IL-33-responsive cells regulate hepatic inflammation in an AREG-dependent manner.

Materials and Methods: Immune-mediated hepatitis was induced by treating C57BL/6 (WT), $ST2^{-/-}$ and $AREG^{-/-}$ mice with Concanavalin (Con) A. Additionally, a subset of mice was treated with IL-33 on three consecutive days prior to Con A-application. Hepatic immune cells were analysed by flow cytometry and unbiased tSNE-clustering. Cytokines were determined by qRT-PCR and multiplex-assays.

Results: WT mice developed severe hepatitis in response to Con A as demonstrated by necrotic lesions and significantly elevated serum ALT values. In contrast, hepatitis was suppressed in mice that received IL-33 prior to Con A. While Th1-associated cytokines were reduced, Th2-cytokines were significantly increased in sera of IL-33-treated mice. ILC2s and $ST2^+$ Tregs were increased in response to either Con A or IL-33, yet the highest frequencies were found in response to IL-33. Additionally, ILC2s expressed AREG upon IL-33-treatment. Hepatic macrophages were increased upon Con A or IL-33. Yet, Con A induced a $Ly6c^{hi}$ $CCR2^+$ M1 phenotype, while IL-33 promoted a $Ly6c^{lo}$ $CX3CR1^+$ M2 phenotype. Immune-mediated hepatitis was exacerbated in $AREG^{-/-}$ and $ST2^{-/-}$ mice. Interestingly, Treg-frequencies were significantly increased in the knockout animals compared to WT mice, suggesting $ST2^+$ Tregs and AREG were required as regulators of hepatitis. Indeed, adoptive transfer of IL-33-elicited Tregs protected WT mice from immune-mediated hepatitis.

Conclusion: IL-33-signalling in the liver activates a network of immune-modulatory cells to impair liver inflammation.

P267

Circulating mitochondrial DAMPs in Multiple Sclerosis patients and their effects on microglial cells

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DOI: 10.1002/eji.201970300

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Introduction: In multiple sclerosis (MS), neurodegeneration can be closely associated with inflammation, but the initial stimulus (or stimuli) that triggers and maintains inflammation, and also the mechanisms causing microglia activation are still a matter of debate. The importance of molecules containing the so-called damage-associated molecular patterns (DAMPs) was first recognized through the observation of the role of the chromatin protein HMGB1, but less attention has been paid to mitochondria-derived DAMPs (MTDs).

Objectives: This project investigates the role of MTDs, particularly mtDNA, in inflammation and neurodegeneration during MS progression, and their importance in the mechanisms at the basis of these phenomena.

Materials & methods: We could quantified mtDNA, HMGB1 and the pro-inflammatory cytokines TNF-α, IL-1β, IL-6, IL-8 and IFN-γ in plasma from 73 untreated progressive MS patients [38 with Secondary Progressive (SP) and 35 with Primary Progressive (PP) MS] and 42 healthy subjects (CTR), and correlated these data with demographic/clinical parameters. We also characterized the effects of mtDNA, N-formyl peptides (fMLP) and cardiolipin (CL) on microglia activation *in vitro* using a human microglial cell line (HMC3 cells).

Results: We found that plasma level of mtDNA was higher in SPMS than in PPMS. In PPMS, TNF-α and IL-8 were higher than in CTR and SPMS, while all progressive MS patients had significantly more IL-6 and IL-1β than CTR. In PPMS, plasma levels of HMGB1 correlated with disease duration, and those of TNF-α with MS Severity Score. Considering microglia activation, at the transcriptional level ICAM1 expression decreased in HMC3 cells following mtDNA stimulation but increased following fMLP stimulation, while ERK2 and SPHK1 levels were down-regulated after CL treatment. Concerning activation markers and the production of reactive oxygen species (ROS), the percentage of HLA-DR+ HMC3 cells did not change upon stimulation, but intracellular ROS production increased after mtDNA and CL treatment, without effects on cell viability.

Conclusion: In progressive MS patients, increased plasma levels of mtDNA and pro-inflammatory cytokines likely contribute to the systemic inflammatory status of these patients. Moreover, the fact that mtDNA and CL stimulate ROS production and activate human microglia likely suggests that MTDs can contribute to neuroinflammation.

P268
HLA-C*06:02 drives a CD8$^+$ T-cell mediated autoimmune response against melanocytes by autoantigen presentation in psoriasis

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Introduction Psoriasis vulgaris is a common T-cell mediated inflammatory skin disease with a suspected autoimmune pathogenesis. HLA-C*06:02 is the main psoriasis risk gene, contributing up to 50% of disease risk. Epidermal CD8$^+$ T cells are essential for psoriasis development.

Objectives To investigate functional implications of HLA-C*06:02 and mechanisms of lesional T-cell activation in psoriasis.

Materials & methods We reconstituted T-cell receptors (TCRs) from epidermal CD8+ T-cell clones of HLA-C*06:02-positive psoriasis patients in a reporter T-hybridoma cell line and analysed their specificity by target cell and peptide library screening.

Results By the reactivity of a paradigmatic Vα3S1/Vβ13S1 TCR we identify melanocytes as skin-specific target cells of an HLA-C*06:02-restricted psoriatic T-cell response. Following peptide library screening to define the conserved amino acid motif of peptide ligands recognized by the Vα3S1/Vβ13S1 TCR peptide motif, we identified ADAMTS-like protein 5 (ADAMTSL5) as an HLA-C*06:02-presented melanocytic autoantigen. Consistent with the Vα3S1/Vβ13S1-TCR reactivity, we observed that numerous CD8+ T cells in psoriasis lesions show signs of activation at the contact site with melanocytes, the epidermal cells expressing ADAMTSL5. Furthermore, ADAMTSL5 peptide stimulation

DOI: 10.1002/eji.201970300

induced the psoriasis signature cytokine, IL-17A in $CD8^+$ T cells from psoriasis patients only, supporting a role as psoriatic autoantigen.

Conclusion This unbiased analysis of a TCR obtained directly from tissue-infiltrating $CD8^+$ T cells reveals that in psoriasis HLA-C*06:02 directs an autoimmune response against melanocytes through autoantigen presentation. These results suggest a pathogenic principle for HLA-class I-associated immune-mediated inflammatory diseases, where the disease-specific HLA-class I molecule drives a $CD8^+$ T-cell mediated autoimmune response against a specific cell type of the organ affected by the inflammation.

P270

The minor allelic variant G at rs75862629 in the ERAPs intergenic region is protective for AS in Sardinia and correlates with a lower expression of HLA-B*2709.

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Introduction: The association of Ankylosing Spondylitis (AS) with HLA-B27 has been know since many decades. However, there is a strong evidence that other genes are also involved. Indeed only 1-5% of HLA-B27 positive individuals develop AS. In particular, the Endoplasmic Reticulum aminopeptidases (ERAP1 and ERAP2), two ubiquitous, zinc-dependent multifunctional enzymes involved in peptide trimming before HLA class I presentation, play a role in several HLA-class I-mediated diseases, including AS. How much is due to an aberrant processing of the antigenic peptides is not clear. Neither it is known to what extent the two ERAP genes work in concert or independently from each other in conferring susceptibility/protection to AS.

Objectives: A SNP in the ERAP2 promoter (rs75862629) coordinates the transcription of both ERAP genes. We investigated whether this SNP associates with AS and whether it affects the expression of the two major HLA-B27 alleles present in Sardinia, the AS-associated B*2705 and the non-AS-associated B*2709.

Patients and Methods: All patients and controls came from different areas of Sardinia and were representative of the population distribution. Four SNPs in the ERAP region were genotyped in HLA-B*2705 positive patients with AS (n=145), B27 positive healthy subjects (n=126) and B27 negative controls (n=250) and allele and haplotype frequencies determined. The expression of ERAP1 and ERAP2 mRNAs in 36 HLA-B27 positive B-Lymphoblastoid cell lines (B-LCLs) was measured by qPCR. To search for a nuclear factor binding the DNA sequence including rs75862629 we performed electrophoretic mobility shift assay (EMSA). The expression of HLA-B27 molecules related to SNP at rs75862629 was checked by flow cytometry.

Results: The frequency of the minor allele G at rs75862629 is significantly increased in B27 healthy individuals both B*2705 and B*2709 compared to B*2705 positive patients with AS and B27 negative controls. The observed decrease in the ERAP2 concomitant with a higher ERAP1 expression can be explained by the lack of binding of a transcription factor as suggest by EMSA. This is correlated with a different cell surface expression of the HLA-B*2705 and HLA-B*2709 molecules.

Conclusions: The expression of ERAP1 and ERAP2 can be simultaneously modulating by SNP rs75862629 that provides protection from AS in HLA-B27 positive subjects in Sardinia. This has a functional impact on the HLA-B27 expression and probably on the disease onset.

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Network-based analysis reveals signatures of IgG autoantibodies targeting G protein-coupled receptors in healthy and diseases

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Background: Functional antibodies (ab) against G protein-coupled receptors (GPCR) were found in various diseases and partially in healthy individuals. Their role in physiology and in pathophysiology remains to be identified.

DOI: 10.1002/eji.201970300

Methods: Sera from 952 patients with different rheumatic diseases were analyzed for the presence of ten different anti-GPCR ab by ELISAs. For deeper ab phenotyping, ab levels against 30 different autoantigens including GPCR, growth factors, and growth factor receptors were studied in sera from 491 healthy controls (HC), 84 patients with systemic sclerosis, 91 with Alzheimer's disease, and 207 with ovarian cancer by ELISAs. Spearman's rank correlation coefficients, hierarchical cluster analyses, network mapping and target interactions using STRING and gene ontology (GO) analyses were performed as well as transwell migration assays. In addition, in vtro studies and animal experiments were performed to identify the pathogenic role of one GPCR ab.

Results: Specific signatures were identified in different autoimmune diseases and in HC. Ab correlations and clusters of correlations were identified in HC, which were specifically affected by age, sex, and autoimmune and non-autoimmune diseases. Network mapping and GO analysis of ab targets displayed multiple associations and a central role of the endothelin receptor type-A (ETAR) in cell migration and chemoattraction. Indeed, the ab were chemoattractive for immune cells, which could be ameliorated by ETAR blockers. In addition, the antibodies induce cytokines and adhesion molecules in immune and resident tissue cells. Immunization of C56/Bl6 mice with hAT1R as well as transfer of Anti-AT1R ab indicate a contribution of these ab in SSc features such as interstitial lung disease and skin fibrosis.

Conclusions: Our data indicate a functional ab network, which is affected by age, sex, and diseases. The ab could affect receptor functions and could guide immune cells or their targets via chemoattraction to organs. Since the immune system is involved in nearly all diseases, anti-GPCR ab may represent novel so far underrecognized effector mechanisms.

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Wheat consumption triggers immune activation in patients with Familial Mediterranean Fever

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Question: Familial Mediterranean Fever (FMF) is an autoinflammatory disease, mainly affecting people living in the Mediterranean area. It is an autosomal recessive inherited disease, characterized by unprovoked inflammation caused by defects in innate immunity (1). In our clinical practice, we observed a high frequency of self-reported Non-Celiac Wheat Sensitivity (NCWS), a condition in which the patients report symptoms eating wheat or other gluten-containing foods (2), in subjects who received a definitive diagnosis of FMF.

Objectives: A) To define the frequency of FMF diagnosis and the association between FMF and NCWS. B) To evaluate the clinical manifestations and the innate immunity responses, before and after double-blind placebo-controlled (DBPC) wheat challenge (WC), in subjects suffering from both FMF and NCWS.

Methods: Six females affected by FMF and NCWS underwent to DBPC wheat challenge. Twelve NCWS females and eight healthy subjects were selected as controls (HC). Before and after DBPC WC the following parameters were evaluated: clinical symptoms by FMF-specific score (AIDAI), serum soluble CD14 (sCD14) and the percentage of CD14+leukocytes producing IL-1band TNF-a, both in PBMC and in rectal biopsies.

Results: AIDAI score significantly increased in FMF patients during the 2-weeks DBPC WC, sCD14 values did not differ in FMF before and after the challenge, but were higher in FMF patients than in HC. In PBMC of FMF patients, the percentage of both CD14+/IL-1b+ and CD14+/TNF-a+leukocytes significantly increased after DBPC WC. In the rectal biopsies of FMF patients we did not find significant differences between the percentage of CD14+/IL-1b+and CD14+/TNF-a+leukocytes before and after BDPC WC, but in both conditions the values were higher than in HC. Symptomatic NCWS controls showed a cytokine profile close to FMF patients.

DOI: 10.1002/eji.201970300

Conclusions: Self-reported NCWS can hide a FMF diagnosis and wheat ingestion can exacerbate FMF crisis associated to innate immunity activation; in these subgroup of FMF patients a wheat-free diet could contribute to the treatment.

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NFAT proteins are essential for thymic selection processes.

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Introduction: The NFAT family of transcription factors, activated by the Ca2+/calcineurin (CN) signals downstream of T-cell receptor (TCR), are key regulators of several T-cell functions. Thymocytes and T cells express the family members NFATc1 – c3. To ensure a functional, but not self-reactive TCR repertoire, thymocytes undergo positive and negative selection processes, partly dependent on the signaling strength of the individual TCR.

Objectives: Several NFAT single or double-deficient as well as CN subunit B-deleted mice have been generated. However, the effects on T-cell development (and function) were different. The apparent discrepancy to the reported absolute requirement for CN in positive selection could be due to a redundancy among NFAT family members. Likewise, other CN-signaling pathways could be involved, e.g. NF-κB and / or MAPK.

Materials & methods: To test this, we have generated a T cell-specific *Nfat*-triple knockout mouse (TKO) by crossing *Nfatc2*-/- with *Nfatc1*fl/fl, *Nfatc3*fl/fl and *Cd4cre*. The phenotype of the TKO mice has been macroscopically monitored and all lymphoid and non-lymphoid tissues were collected for histochemical studies. *Ex vivo* analyses of cells from secondary lymphoid organs were collected for flow cytometry staining with different multi-marker panels.

Results: NFAT TKO mice showed growth retardation and weight loss associated with age-related multiple organ inflammatory leukocyte infiltration, lymphadenopathy and splenomegaly. The overall number of T cells in secondary lymphoid organs was reduced compared to wild type mice, with a strongly tilted CD4/CD8 T-cell ratio towards CD8+ T cells and an impaired functional regulatory/conventional T-cell ratio. Interestingly, those alterations were found in the thymus of TKO suggesting an altered selection process during thymocytes development upon the combined loss of NFATc1–c3. In fact, the transitional and post-positive selection subpopulations were clearly underrepresented. Furthermore, TKO mice exhibited a high percentage of mature but TCR– thymocytes, pointing to a perturbed maturation process.

Conclusion: We hypothesize that the lack of all expressed NFAT proteins during positive selection mimics too low affinity/avidity of the TCR, leading to few, but falsely selected T cells and thus an autoimmune-like syndrome.

P274

T helper 1 response is correlated with widespread pain, fatigue, sleeping disorders and the quality of life in patients with fibromyalgia and is modulated by hyperbaric oxygen therapy.

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Objective: Hyperbaric oxygen therapy (HBOT) has been used as treatment for different clinical conditions, including Fibromyalgia (FM), modulating brain activity, ameliorates chronic pain and modifies the ratio of immune cells. Clinical studies have provided evidence that FM is associated with immune system dysregulation. In the present study we have characterized immune cells in the peripheral blood of FM patients and evaluated the effect of HBOT on immune system and on global life style, with the aim of better understanding the pathogenesis of the disease.

Patients & Methods: Patients with primary FM and controls were treated with HBOT. Physical, emotional and social assessment, quality of sleep, tender points, intensity score, WPI and symptom severity were evaluated before and

DOI: 10.1002/eji.201970300

after HBOT. Furthermore, a characterization of CD4 T lymphocytes and their cytokine production was performed by flow cytometry. The expression of TNF-a, IFN-g, IL-17, IL-9 and IL-22 was also assessed by RT-PCR. Finally, the serum levels of serotonin were evaluated by ELISA.

Results: FM patients show a Th1 signature characterized by increased expression of the Th1-related cytokines TNF-a and IFN-g. Moreover, this proinflammatory status was dramatically modified during HBOT, which contributes to a global improvement of the quality of life, pain perception and cognitive capacities.

Conclusion: Our results highlighted for the first time the participation of immune system in the pathogenesis of FM and the impact of HBOT treatment, with particular regard to the changes on proinflammatory cytokines production by CD4 T cells subsets.

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P275

Interferon-γ promotes CD8 T cell and NK cell cytotoxicity and fibrosis in *Mdr2*$^{-/-}$ mice

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Introduction Primary sclerosing cholangitis (PSC) is an idiopathic, chronic cholestatic liver disorder characterized by biliary inflammation and fibrosis. Increased numbers of IFNγ$^+$ lymphocytes and IFNγ-induced chemokines have been detected in liver tissue and plasma of PSC patients.

Objectives: Aim of this study is to analyze the role of IFNγ as well as IFNγ-producing cell populations in the immune pathogenesis of PSC.

Materials & Methods: Liver tissue samples were collected from PSC patients (n=6) undergoing liver transplantation and from control patients (n=6) undergoing resection of metastases. Blood samples were obtained from PSC patients and healthy controls (n=8-9). For mouse studies multidrug resistance protein 2 knockout (*Mdr2*$^{-/-}$) mice were used, an established mouse model resembling PSC. The contribution of lymphocytes to liver pathology was assessed in *Mdr2*$^{-/-}$ *x Rag1*$^{-/-}$ mice and following depletion of CD90.2$^+$ and NKp46$^+$ cells in *Mdr2*$^{-/-}$ mice. The role of IFNγ for liver pathology of PSC was determined in *Mdr2*$^{-/-}$ *x IFNγ*$^{-/-}$ mice and following anti-IFNγ antibody treatment. Liver inflammation and fibrosis were determined by histological analyses. Immune cell composition was analyzed by flow cytometry.

Results: PSC patients showed increased IFNγ serum levels and elevated numbers of hepatic CD56^{bright} NK cells. In *Mdr2*$^{-/-}$ mice the hepatic CD8$^+$ T cells and NK cells were the primary source of IFNγ. Depletion of CD90.2$^+$ cells, which are mainly T cells, reduced the production of IFNγ, NK cell cytotoxicity and overall liver injury. Similar results were obtained in *Mdr2*$^{-/-}$*x Rag1*$^{-/-}$ mice. Depletion of NKp46$^+$ NK cells resulted in reduced CD8$^+$ T cell cytotoxicity, IFNγ production and liver fibrosis. Complete absence of IFNγ in *Mdr2*$^{-/-}$*x IFNγ*$^{-/-}$ mice led to reduced frequencies of NK cells and CD8$^+$ T cells expressing the cytotoxic effector molecules granzyme B or TRAIL. Additionally *Mdr2*$^{-/-}$*x IFNγ*$^{-/-}$ mice

DOI: 10.1002/eji.201970300

exhibited reduced liver fibrosis in comparison to *Mdr2*$^{-/-}$ mice. Furthermore, antibody-dependent neutralization of IFNγ confirmed the anti-fibrotic effect of IFNγ in *Mdr2*$^{-/-}$ mice.

Conclusion: IFNγ changed the phenotype of hepatic CD8^{+} T cells and NK cells towards increased cytotoxicity and had profibrotic properties in *Mdr2*$^{-/-}$ mice. So far no adequate treatment for PSC is available, since the underlying mechanisms leading to PSC are unknown. Focusing on IFNγ and its role in the immunopathogenesis of PSC might help to develop novel treatment options.

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T follicular helper (-like) memory cells in lung tissue

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Introduction: Local inflammation in non-lymphoid tissue frequently results in the recruitment of antigen-specific T and B cells. The typical findings in human autoimmune diseases are diffuse, unstructured lymphocytic infiltrates. We could recently show in a murine lung inflammation model that active T/B cooperation can also take place in such FDC-negative, unorganized infiltrates (Vu Van et al., 2016). Here, a CXCR5/Bcl-6 negative but T follicular helper- (Tfh-) like cell population, which has the ability to produce substantial amounts of IL-21, promotes the differentiation of germinal center-like B cells. Even after full resolution of inflammation, antigen-specific lymphocytes persist in residual lung infiltrates.

Objectives: We aim to further define both phenotype and biological function of lung-resident Tfh-like memory cells.

Material & Methods: As a general model for T/B interaction in inflamed tissue, we developed a murine lung inflammation model, which allows us to track antigen-specific T and B cells simultaneously in lung-draining lymph nodes and the inflamed lung tissue.

Results: After full resolution of inflammation and in the absence of antigen, residual infiltrates in the lung contained remarkable numbers of antigen-specific, resting memory CD4+ T cells. In contrast to the acute inflammation phase, where activated antigen-specific T cells were almost exclusively found in lung tissue, lung airways (BAL), and lung-draining lymph node, memory T cells now spread to additional organs like spleen and bone marrow. However, the majority of antigen-specific T cells still remained in the lung, organized in cluster-like structures in close contact with antigen-specific B cells. Compared to classical memory T cells from spleen, lung memory T cells are characterized by the absence of the classical Tfh memory cell markers CXCR5 and folate receptor-4 and also by a high expression of CD69. Despite the lack of classical Tfh memory markers, memory T cells from lung tissue were equally effective to produce IL-21 upon restimulation and to provide fast help to tissue-resident B cells.

Conclusion: Tfh-like cells generated in the tissue remain at the original site of antigen entry as tissue-resident memory T cells to provide prompt help for tissue B cells upon antigen recall, whereas Tfh cells generated in the draining lymph node distribute to other organs to confer systemic protection.

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STRUCTURAL ORGANIZATION OF T AND B CELL INFILTRATES IN THE INFLAMED LUNG

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Introduction: Lymphocytic infiltrates in the lung are not only present in classical interstitial lung diseases like sarcoidosis but also in many autoimmune diseases, for instance as an extra-articular manifestation of rheumatoid arthritis. Immunohistological investigations of our group revealed that the inflamed lung is predominated by unstructured leukocytic aggregates without distinct T and B cell zones and follicular dendritic cell (FDC) networks. Despite the lack of any germinal center (GC)-like structures, B cells resembling a classical GC phenotype and T cells exhibiting follicular helper-like properties could be found, raising the question how active T/B cell cooperation can take place in these diffuse infiltrates.

Objectives: This study not only aimed to identify chemokine/chemokine receptor pairs being essential for the recruitment and accumulation of cells within the lung infiltrates, but also to characterize accessory cells that could take over the function of FDC and present native antigen to the tissue-residing B cells.

DOI: 10.1002/eji.201970300

Materials & Methods: To analyze cell interactions *in vivo*, a murine lung inflammation model, based on the adoptive transfer of antigen-specific T and B cells, was used. Systematic histological analyses of lung tissue and lung-draining lymph node (Ln) were performed and complemented by flow cytometry.

Results: Several chemokine receptors were found to be differentially expressed on B cells from the lung versus Ln. Whereas CXCR5 was downregulated, the expression of CXCR3 and CXCR4 was increased in lung B cells compared to Ln B cells. The presence of CXCL10- and CXCL12-producing cells within the infiltrates suggests an important role of both the CXCR3-CXCL10 and CXCR4-CXCL12 axis in the effective recruitment of leukocytes into sites of inflammation. With the help of a fluorescent antigen conjugate different antigen-bearing cells could be detected in the lung. Intriguingly, the majority of these cells were found to be distributed within the lung parenchyma, adjacent to the infiltrates instead of being accumulated within them. Since both macrophages and dendritic cells were identified as antigen-capturing cells and due to their high expression of Fc receptors, they might replace the function of FDC.

Conclusion: The presented data give a more detailed insight into immune cell interactions in non-lymphoid tissue and hence, raise important considerations for the development and refinement of therapies against immunopathological disorders.

P278

Effects of diet induced obesity on intestinal inflammation in a DSS colitis model

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Obesity has emerged as a serious disease especially in industrial countries, which is characterized by an abnormal or excessive fat accumulation. It is associated with insulin resistance, an elevated systemic inflammation and probably intestinal inflammation. However, the effect of obesity in intestinal inflammation is still not well described. To study the effects of obesity in the DSS induced colitis model male C57BL/6NCrl mice were fed a high fat diet (60%; HFD) or a matched low fat diet (10% LFD) for 10-11 weeks. After 8 weeks the animals were treated with 1% DSS via the drinking water for 5 days to induce colitis. Our results reveal that after 10 weeks the HFD fed control animals developed obesity. No histological differences between LFD and HFD fed animals could be observed, but high fat feeding increased the expression of TNFα, IL10 and RALDH2 in the mesenteric lymph nodes (mLN). Furthermore, only animals fed the LFD showed significant decrease in weight after DSS treatment. Moreover, DSS treatment induced an inflammation in the colon independent of the diet, but the inflammation was more pronounced in the colon of LFD mice. Furthermore, only the expression of TNFα was increased after high fat feeding in the DSS treated group. In summary, diet induced obesity leads to an impaired immune response, with a decreased inflammation in the colon and alterations of the gene expression in the mLN of HFD mice in the DSS induced colitis model. These findings indicate an impact of obesity on intestinal inflammation.

P279

Immune response of preterm macrophages with a focus on sustained inflammation in chronic lung diseases

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Preterm infants are highly susceptible to sustained lung inflammation which may be triggered by exposure to multiple environmental cues such as infections and supplemental oxygen. The underlying mechanisms are still poorly understood. Here we hypothesize that dysregulated macrophage activation is a key feature leading to lung inflammation in preterm infants.

To address this hypothesis we aimed to characterize age-dependent differences in immune responses of monocyte-derived macrophages from preterm infants compared to term infants and adults. To get a better understanding for lung immunity, we developed an *ex-vivo* double-hit model (different O2 concentrations and subsequent LPS) to mimic key lung exposure factors.

DOI: 10.1002/eji.201970300

We collected cord blood samples of preterm infants (n=14) and term infants (n=19) as well as peripheral blood from healthy adults (n=17). We compared surface marker, cytokine release, transcriptome pathway profiles and T cell polarization of preterm macrophages with term and adult macrophages after double-hit exposure.

Preterm macrophages demonstrated increased interferon and chemokine signaling and decreased respiratory electron transport chain gene expression determined by mRNA sequencing and pathway profiling compared to term macrophages. Additionally, increased basal TLR4 surface expression and enhanced cytokine release were detected in preterm macrophages which led to a Th17 rather than a Th1 polarization in naïve T cells. Term and adult macrophages did not show major differences in their immune response.

After a double-hit with 65% O2 and subsequent LPS, preterm macrophages showed increased expression of HLA-DR on the surface and amplified cytokine release compared to LPS stimulation alone. These released cytokines led to a downregulation of Treg polarization. Furthermore, chemokine signaling pathways were upregulated, whereas respiratory electron transport chain was downregulated after the double-hit with 65% O2 and subsequent LPS compared to LPS stimulation alone.

Preterm macrophage responses to key lung exposure factors suggest their involvement in an excessive inflammation due to age dependent differences possibly inducing a Th17/Treg imbalance in the developing lung.

P280

Dimethyl fumarate targets IL-17-producing CD8$^+$ T cells to limit autoimmunity

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Introduction: Dimethyl fumarate (DMF) is approved for treatment of relapsing remitting multiple sclerosis (RRMS), a chronic inflammatory disease of the central nervous system (CNS) that is mediated by autoreactive T cells. The enrichment of IL-17-producing CD8+ T (Tc17) cells in cerebrospinal fluid of MS patients and their co-pathogenic function during experimental autoimmune encephalomyelitis (EAE) point to their contribution to CNS autoimmunity in men and mice. So far, a direct impact of DMF on CD8+ T cells is unknown.

Objectives: We sought to determine the impact of the mechanistically elusive drug DMF on type 17 T cells and examine the underlying mechanisms leading to amelioration of MS.

Material and Methods: Analysis of human and murine Tc17 cells and quantification of ROS levels were determined by flow cytometry. To examine changes on transcriptome, RNA-Seq and bioinformatical analysis (GSEA, PCA) were performed. Chromatin immunoprecipitation was conducted to evaluate histone modifications.

Results : We revealed that DMF upregulated reactive oxygen species (ROS) in Tc17 and Th17 cells by glutathione depletion, leading to IL-17 suppression preferentially in Tc17 cells. Accordingly, IL-17 production of CD8$^+$ but not CD4$^+$ T cells was reduced in DMF-treated MS patients and DMF abrogated Tc17 cell pathogenicity in EAE. Accumulated ROS altered the ratio of RORgt to T-bet and shifted Tc17 cells towards a CTL signature by enhancing PI3K-AKT and STAT5 pathways along with altered histone modifications at the *Il17* locus. AKT deactivated FOXO1, leading to the upregulation of T-bet, which in turn suppressed IL-17.

Conclusion : Our findings provide mechanistic insights into the DMF-mediated selective modulation of Tc17 cell fate by upregulated ROS and IL-2 signalling with relevance for IL-17-driven pathologies.

DOI: 10.1002/eji.201970300

P282

CXCL13 elevation in early kidney allograft rejection in mice and men

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Background: Early recognition of allograft rejection after kidney transplantation (ktx) is relevant in order to initiate the optimal treatment as quick as possible. In the context of delayed graft function (DGF) renal function impairment measured by serum creatinine elevation is missing. Further, biomarkers are of high medical interest to identify ongoing rejection and activation of the immune system as early as possible. CXCL13 is a biomarker candidate which is a potent chemoattractant for B-cells and has been investigated in autoimmune diseases previously. Here, we investigated patient blood samples longitudinally after ktx for CXCL13 expression. Furthermore, we characterized CXCL13 expression in blood and tissue samples of mice with mixed T-cell and antibody mediated allograft rejection (TCMR-AMR).

Methods: Patients scheduled for ktx were enrolled in a prospective clinical study and had blood sampling prior to ktx, at day 1, 3 and 7 after ktx. CXCL13 expression was measured by ELISA. For allogenic ktx Balb/C (H-2d) donor kidneys were transplanted into a completely mismatched C57Bl/6 (H-2b) male recipients and control isogenic ktx was performed with C57Bl/6 donors and recipients. Blood samples were drawn weekly and flow-cross match was performed with BalbC splenocytes. At the designated endpoints at 2, 3 and 6 weeks after ktx work up of the renal tissue was done. CXCL13 mRNA was analysed in the renal tissue and blood samples were investigated by CXCL13 ELISA. Flow cytometry was performed to characterize infiltrating leukocyte subsets of the graft, the blood, the spleen and the regional lymph nodes at 10 days after ktx.

Results: Patients with initial function had stable CXCL13 levels. In contrast, two patients with early TCMR had >5 fold CXCL13 elevation within the first week after ktx. Biopsies revealed TCMR with B-cell infiltrates in both patients.

In the mouse model CXCL13 levels increased over time and correlated with enhanced CXCL13 mRNA levels in the allograft and with acute rejection and severe interstitial inflammation with CD3 positive cellular infiltrates and perivascular B-cell rich clusters. By flow cytometry enhanced plasma cell infiltrates in the allograft were measured.

Conclusion: Systemic CXCL13 elevation in patients after ktx is a promising biomarker for B-cell activation and needs further clinical evaluation in a larger patient cohort.

P284

Roquin and Regnase-1 cooperatively determine cell fate decisions in T cells

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Introduction: The RNA-binding proteins Regnase-1 and the paralogues Roquin-1 and Roquin-2 are critical regulators of activation and differentiation of T cells. They bind to similar cis-elements in the 3´-UTR of immune-related mRNAs and induce mRNA degradation and translational silencing of a shared set of mRNA targets. TCR activation triggers cleavage of Roquin as well as Regnase-1 via the MALT1 paracaspase, thereby releasing target mRNAs from repression. Importantly, mice with either a T cell-specific deletion of Roquin-1/2 or Regnase-1 or mice with a single point mutation in Roquin-1 M199R similarly develop severe autoimmune or auto-inflammatory diseases. Nevertheless, a functional relationship between Roquin and Regnase proteins has not been formally established.

Objectives: We analyze whether Roquin and Regnase-1 cooperate in the regulation of target mRNAs, T cell fate decisions as well as prevention of autoimmunity, and ask how to place the Roquin-1 M199R mutation into this context.

DOI: 10.1002/eji.201970300

Materials & Methods: We analyze inducible and T cell-specific deletion of individual and combined alleles of Roquin-1/2 (DKO), Regnase-1 (KO) or all three genes (TKO) by flow cytometry and global mRNA sequencing.

Results: Our comparison of the phenotype of DKO, KO and TKO mice or T cells unravels a high similarity in respect to immune cell dysregulation. By comparing inducible gene ablation in CD4 T cells we identify the major mode of gene regulation by Roquin and Regnase-1 as cooperativity. Zc3h12a mRNA, encoding for Regnase-1, is one of the strongest cooperative targets, which we used in a mutagenesis screen to define the protein surface of Roquin-1 that participates in cooperative suppression. In this assay we identify M199R as well as several new mutations, a part of them being in close vicinity to M199R, that display a similar impairment in cooperative suppression. These findings suggest that cooperative regulation of targets and T cell fate decisions depend on Regnase-1 interaction with a bi-partite binding in Roquin, which we currently evaluate.

Conclusion: Our data delineate cooperative post-transcriptional gene regulation by Roquin and Regnase proteins as a molecular mechanism in the prevention of autoimmunity.

P285

Autoreactive CD8⁺ t effector cells escape treg control in rheumatoid arthritis

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Introduction: Rheumatoid arthritis (RA) is one of the most common autoimmune diseases, characterized by chronic inflammation. We previously demonstrated that the frequency of autoreactive CD8⁺ T cells specific to apoptotic-epitopes (AE), (derived from self protein such as vimentin, actin cytoplasmic 1, or non-muscle myosin heavy chain 9), was higher in peripheral blood of patients responders (Rs) to anti-tumor necrosis factor (TNF)-α therapy than in non-responders (NRs), before the start of treatment (Citro *et al.* 2014).

Objectives: In this scenario, we investigated more in depth the role of AE-specific CD8⁺ T cells in RA immunopathology, studying the different mechanisms occurring in Rs and NRs. We also characterized regulatory T cells (Tregs), which play an essential role in maintaining peripheral self-tolerance.

Materials & Methods: We characterized phenotype and functions of AE-specific CD8⁺ T cells and Tregs by multiparametric flow-cytometry analysis. Various *in vitro* assays were performed to provide the mechanistic basis of correlations between Tregs and AE-specific CD8⁺ T cells *in vivo*. Gene expression profile of sorted Naïve (N) and effector (EM+EMRA) AE-specific CD8⁺ T cells was investigated by Nanostring technology. We also performed IHC and multiplex IF analysis of inflamed synovial tissues from RA patients, to observe *in situ* behaviour of studied populations.

Results: In Rs, AE-specific CD8⁺ T cells, despite conserving a conventional N phenotype, produced high levels of TNF-α and showed a gene expression signature close to effector cells (partially-activated, pa). These paCD8⁺ TN cells directly correlated with both disease activity score and Tregs *in vivo,* which controlled the differantiation and proliferation *in vitro* of the former.

On the other hand, in NRs AE-specific CD8⁺ T cells showed effector phenotype, gene expression profile, and inversely correlated with Tregs that were not able to suppress the proliferation of AE-specific CD8⁺ Teffector cells which in turn killed Tregs indirectly in an NKG2D-dependent bystander fashion *in vitro*.

Lastly, multipex IF analysis of inflamed synovial tissues showed that a remarkable number of CD8⁺ T cells expressed granzyme-B and co-localized with Tregs, some of which were in an apoptotic state, validating our *in vitro* results.

DOI: 10.1002/eji.201970300

Conclusion: These data provide evidence of a previously undescribed role of such mechanisms in the progression and therapy of RA.

P401

Combined single-cell transcriptome and immune cell repertoire sequencing reveal insight into the role of artery tertiary lymphoid organs (ATLOs) in Atherosclerosis

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Introduction: ATLOs emerge in the connective tissue coat, i.e. the adventitia, of human and mouse atherosclerotic arteries. Murine ATLOs have been shown to protect from disease progression suggesting that they may be a future target for immune cell-directed therapies.

Objectives: To understand atherosclerosis immunity, it is mandatory to identify the phenotypes and location of leukocytes within the arterial wall. For this purpose, we compared immune cells of atherosclerotic plaques and ATLOs vs aorta-draining renal lymph nodes (rLNs) using single-cell transcriptome and immune repertoire sequencing.

Methods: Using droplet-based single-cell technology, we combined transcriptome, TCR repertoire, and BCR repertoire sequencing at the resolution of single cells.

Results: We identified 19 distinct immune cell subsets from a total of 9647 cells. ATLOs showed distinct T cells, B cells, and myeloid cells subsets when compared to LNs and plaques. Clonally expanded T cells were limited to memory T cell subsets and highly expressed CCL4 and CCL5. IL-10 B cells and plasma cells showed higher frequencies of clonal expansion when compared to other B cell subsets. ATLO and plaque cells showed diverse myeloid cell subsets including monocyte-derived DCs, plasmacytoid DCs, TREM2high macrophages, and tissue-resident macrophages. Cell cycle-related genes revealed less proliferating cells in atherosclerotic plaques and ATLOs vs rLNs. Myeloid cells had considerably higher glucose transport- and glycolysis-related transcripts whereas lymphocytes showed higher tricarboxylic acid cycle and oxidative phosphorylation-related transcripts. The inflammatory atherosclerosis environment triggers higher levels of glucose transport- and glycolysis-related genes in lymphocytes.

Conclusion: These data yield new insights into innate and adaptive atherosclerosis immunity by providing the first blueprint for cellularity, clonality, proliferation, and metabolic activities of distinct leukocytes subsets.

See next pages for Figure 1 & Figure 2

Figure 1

DOI: 10.1002/eji.201970300

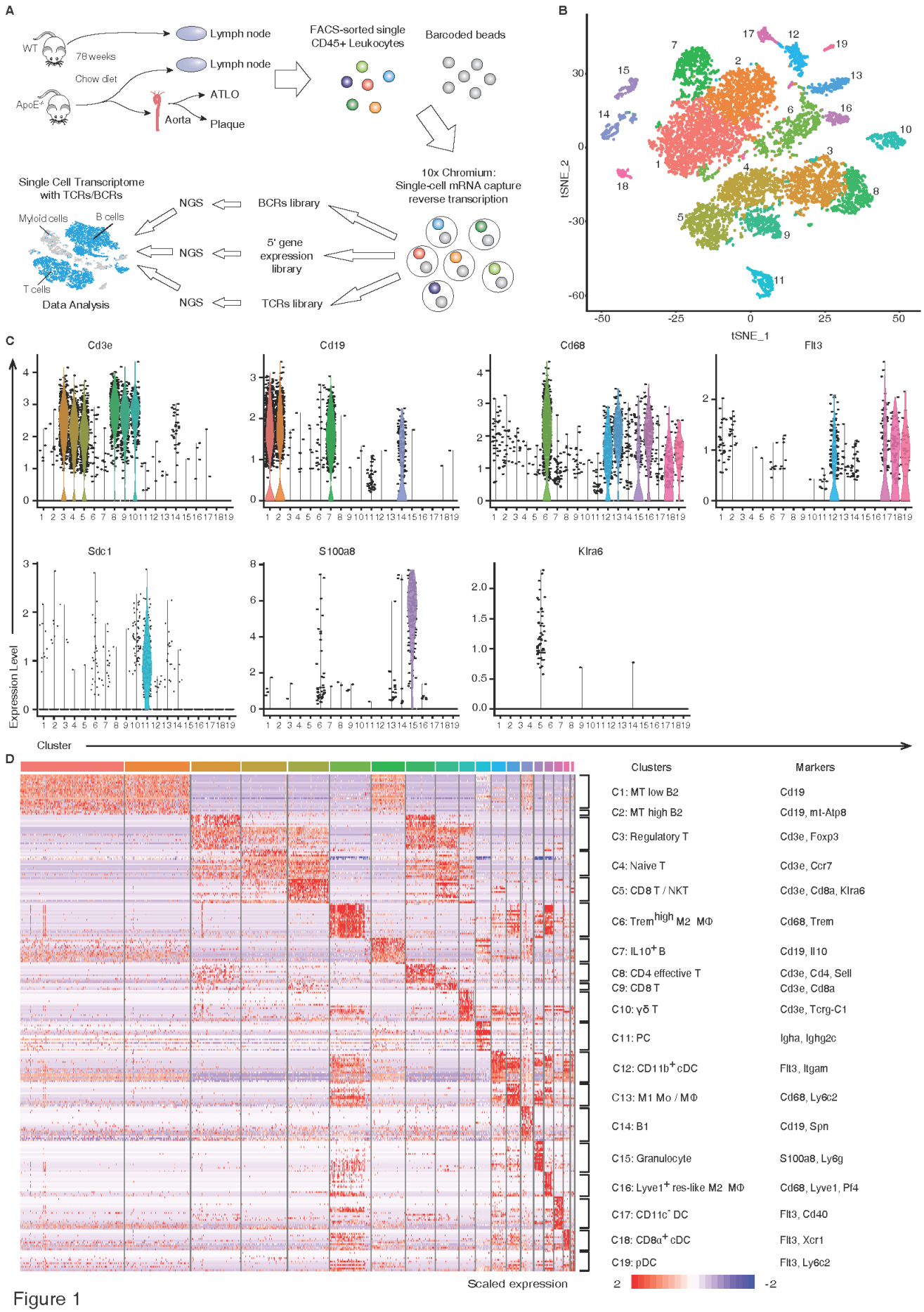

Figure 1

DOI: 10.1002/eji.201970300

Figure 2

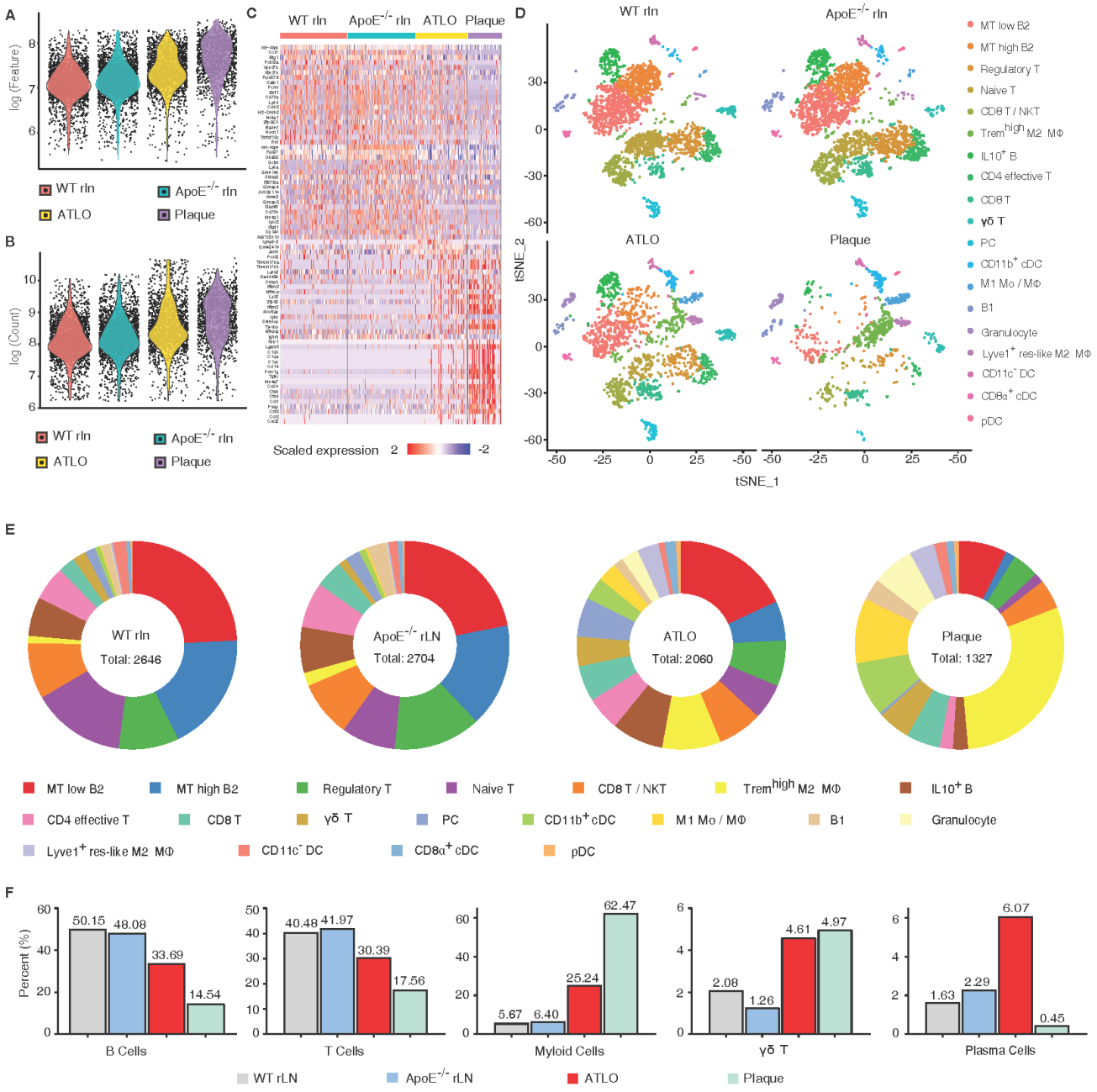

Figure 2

P403

Mass cytometry combined with computational data mining reveals a multifactorial immune cell signature of active rheumatoid arthritis

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Innate and adaptive immune mechanisms drive the pathogenesis of rheumatoid arthritis (RA) and are targets of approved therapies. However, not all patients can be appropriately treated, which defines the need for additional therapeutic concepts combined with personalized treatment. A systematic assessment of immune cell dysregulation in

DOI: 10.1002/eji.201970300

the patients` blood that may provide insight into common and individual immune pathology features of active RA and reveal treatment options.

We here employed 43-dimensional mass cytometry to deeply profile PBMC in 34 patients with active RA vs. 31 age/gender-matched controls, permitting the automated identification of 60 global PBMC, 80 T cell, and 50 B cell populations by a nested FlowSOM clustering approach.

Active RA was characterized by diminished frequencies of MAIT and γδ T cell, as well as IgA and IgM memory B cell clusters, while the frequency of classical CD14highCD16lowmonocytes was significantly increased (MWU test, BH-adjusted p-values, $p<0.05$). While MAIT and gd T cells frequencies were inversely correlated with serum CRP ($r=-0.55$ and -0.56, $p<0.001$), IgA memory B cells inversely correlated with DAS28 values ($r=-0.34$, $p=0.04$), suggesting that at least some components of the RA signature are associated with disease activity. Notably, the vast majority of differentially abundant T and B cell clusters were memory or effector cells, underpinning the impact of antigen-dependent lymphocyte differentiation for the immunological fingerprint of active RA. Furthermore, computational data mining by Citrus and CellCNN consistently revealed significantly lower detection of the inflammatory chemokine receptor CXCR3 in RA patients across different T, B,and NK cell subsets.

In this study, we established a multi-component immune cell fingerprint of active RA featuring aberrations of innate and adaptive immune cells. This immune cell reference map of RA will serve for comparison with data from other autoimmune diseases and longitudinal profiling of patients during therapy.

P409

TNF hampers intestinal tissue repair in colitis by restricting IL-22 bioavailability

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Successful treatment of chronic inflammatory diseases integrates both cessation of inflammation and induction of adequate tissue repair processes. One of the examples of such therapy is Tumor Necrosis Factor (TNF) inhibition in IBD patients. However, molecular mechanisms of intestinal repair upon TNF blockade during IBD remain not understood. Here, by the usage of human TNF Knock-in mice (hTNFKI) in a model of adoptive T cell transfer, we revealed that TNF interferes with tissue repair program via induction of soluble natural antagonist of IL-22 (IL-22Ra2; IL-22BP) in the colon and abrogates IL-22, STAT3-mediated mucosal repair during colitis. Pharmacological T-TNF blockade reduced IL-22BP expression in the colon leading to the increased IL-22 levels, colonic epithelial cell proliferation and restoration of colonic epithelium functions. Thus, our data revealed the mechanism of how anti-TNF therapy induces mucosal healing and provides novel potential targets for IBD treatment in humans.

This work was funded by the DFG (NE1466/2-1).

Neuroimmunology (P286-P297)

P286

Investigating the role of cytokine-producing B cell in initiation and regulation of EAE

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Introduction: Multiple sclerosis (MS) is a an autoimmune, inflammatory disease in which the immune system attacks the myelin sheath of the central nervous system (CNS) leading to tissue inflammation, massive demyelination, axonal damage and loss. Although MS is a quite common disease affecting almost 2.5 million people worldwide, MS pathogenesis is still not fully understood. Originally, Th1 and Th17 cells were thought to be the main drivers of CNS tissue inflammation and demyelination in MS and its mouse model experimental autoimmune encephalomyelitis

DOI: 10.1002/eji.201970300

(EAE). However, increasing evidence suggests also a role of B cells in disease pathogenesis. Clinical data indicate that B cells contribute to disease initiation and progression through antigen-presentation and cytokine production, possibly by promoting differentiation of specific pathogenic T cell subsets such as Th1 or Th17 cells.

Objectives: The aim of this study is to investigate how cytokine production by antigen-specific B cells can shape the T cell response in EAE.

Materials and methods: B cell phenotype and cytokine production in C57BL/6 and SJL WT, IgHMOG, 2D2xIgHMOG and TCR1640 mice were analyzed by flow cytometry, qPCR and ELISA. B cells were expanded using the iGB culture system and manipulated by retroviral transduction.

Results: We have found that B cells specific for the CNS antigen MOG produce more pro-inflammatory and less anti-inflammatory cytokines indicating a pathogenic potential of these B cells. Furthermore, we have established an *in vitro* culture system for the retroviral transduction of B cells allowing for both the overexpression and knock-down of different cytokines in B cells.

Conclusion: Using our retroviral transduction system in combination with different EAE mouse models, we will be able to investigate whether antigen-specific B cells can support or suppress differentiation of pathogenic T cell subsets via cytokine production. This will shed light on the relevance of antigen-presentation and cytokine production by antigen-specific B cells for disease initiation and progression and help to understand the relationship between B cells and different T cell subsets in EAE and MS.

P287

Denervation causes popliteal lymph node expansion and an adaptive immune response

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Introduction: Loss of neural tone and induction of neural traumatic responses concurrently occur during peripheral nerve injuries but their specific influences on regional immune responses remain unclear.

Objectives: The aim of the present study was to understand the effect of peripheral neural injury on the immune system.

Materials and methods: Denervation surgeries were conducted and downstream popliteal lymph nodes (popLNs) and draining areas were examined using immunofluorescence, quantitative PCR, ELISA and flow-cytometry techniques.

Results: Unilateral sciatic denervation of the popLN and its draining area caused ipsilateral paw thickening and dramatic nodal expansion. However, neural surgeries not directly affecting the popLN, such as resection of the femoral nerve or individual sciatic nervous branches below the popliteal fossa did not induce nodal expansion. Sciatic denervation enhanced expression of *Cxcl12, Cxcl13, Il1a, Il1b, Il6, Il10, Il17a* and *Il17f* levels in denervated popLNs and paws. Adoptively transferred cells exhibited increased recruitment to denervated popLNs, indicating that homing was responsible for the initial increase in LN cellularity. This was followed by enhanced proliferation of B cells and germinal center formation and increased circulating immunoglobulin G levels. Blockade of antigen presentation as well as surgical isolation of popLNs from afferent lymphatic drainage ablated denervation-induced nodal expansion

Conclusion: Our data provide insights into the development of the specific immune response caused by peripheral nerve trauma.

DOI: 10.1002/eji.201970300

P288

$G\alpha_s$-coupled receptor agonists suppress, whereas sleep promotes integrin activation on antigen-specific T cells in humans

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Effector T-cell responses require the formation of cell-to-cell contacts between T cells and their target cells, e.g., virus-infected cells. These contacts are termed cytotoxic immunological synapses. The formation of efficient immunological synapses depends on the T-cell receptor (TCR)-mediated activation of β_2-integrins. $G\alpha_s$-coupled receptor agonists are known to have an immunosuppressive role, but their impact on TCR-mediated integrin activation and formation of immunological synapses are unknown. Using multimers of peptide major histocompatibility complex molecules (pMHC) and ICAM-1 – the ligand of β_2-integrins – we show that the $G\alpha_s$-coupled receptor agonists catecholamines, prostaglandins, and adenosine potently inhibit integrin activation on human cytomegalovirus- and Epstein-Barr virus-specific $CD8^+$ T cells in a dose-dependent manner, starting at physiological concentrations. In contrast, sleep, a natural condition of low levels of $G\alpha_s$-coupled receptor agonists, upregulates integrin activation compared to nocturnal wakefulness. This effect was dependent on the suppression of $G\alpha_s$-coupled-receptor signaling. These findings provide a potential mechanism underlying some of the immune-supportive effects of sleep on a new, so far unrecognized, level, namely the formation of immunological synapses. Our findings also suggest that the formation of immunological synapses is suppressed in several pathological conditions associated with increased levels of $G\alpha_s$-coupled receptor agonists, such as cancer, stress, and hypoxia.

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Towards exclusively local therapy of glioblastoma - Engineering IL-12Fc with superior tissue retention and minimal systemic exposure after CNS administration

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Interleukin (IL)-12 is a pro-inflammatory cytokine and has a powerful anti-tumor effect on various solid tumors - including glioblastoma (GB) - in preclinical models. We and others have shown that local, intratumoral application of IL-12 overrides the prevailing local immunosuppression and unleashes a striking anti-tumor response. Local instead of systemic application of IL-12 decreases systemic exposure and the fusion to an immunoglobulin heavy chain constant region ("Fc tag") should increase tissue retention of IL-12. However, export of IL-12Fc into the blood via the neonatal Fc receptor (FcRn) could nevertheless lead to systemic exposure. Subsequent systemic recycling via FcRn would lead to gradually increasing and eventually toxic serum concentrations. With clinical studies currently testing local expression of IL-12 in brain tumors, concerns on IL-12 systemic toxicity – so potent that it led to casualties in early clinical trials – resurface.

We analysed the effect of the Fc-tag on IL-12 tissue retention and evaluated whether FcRn also is involved in brain export and systemic recycling of IL-12Fc upon local delivery. Human or murine IL-12Fc was delivered in GB-bearing or naïve wt or FcRn-humanized mice (mFcRn-/- hFcRn tg) continuously via mini osmotic pumps or as bolus via convection-enhanced delivery (CED). Brain and blood concentration levels were assessed via ELISA. FcRn binding of hIL-12Fc was abrogated via site directed mutagenesis. FcRn affinity of IL-12Fc mutants was assessed via ELISA and surface plasmon resonance.

We observed much higher tissue retention of IL-12Fc compared to unmodified IL-12, but also an FcRn-dependent gradual increase of IL-12Fc serum levels. Testing a battery of amino acid substitutions at the FcRn interaction

DOI: 10.1002/eji.201970300

interface, we discovered unique mutations that largely abolish brain export and systemic accumulation while preserving IL-12Fc functionality, leading to a 100-fold higher brain to blood ratio than unmodified IL-12.

Achieving high local concentrations at low to absent systemic exposure is an important prerequisite for a large therapeutic window for local treatment of neurologic diseases. The discovered novel Fc-modifications present a promising platform for reducing systemic leakage of Fc-containing therapeutics including antibodies in the context of continuous or intermittent CNS delivery beyond brain cancer therapy. We currently test the impact on efficacy of exclusively local treatment in vivo.

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Siponimod (BAF312) Treatment in the MCAO Stroke Mouse Model.

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Question - Stroke remains the second leading cause of deaths worldwide, and therapeutic options are still limited to intravenous tissue-type plasminogen activator and endovascular thrombectomy. These approved therapies only target at reperfusion by opening the blocked vessel. A variety of poststroke immunologic changes lead to complications such as the increased risk of bacterial infections, for example, pneumonia, that favor a bad outcome and increase 30-day mortality. Thus, neuroprotective or immunomodulatory therapies after stroke seem a promising therapeutic option to reduce complications. The interaction of lymphocytes with endothelial cells and platelets, termed thromboinflammation, fosters microvascular dysfunction and secondary infarct growth. Siponimod is an S1PR (sphingosine-1-phosphate receptor) modulator, which blocks the egress of lymphocytes from lymphoid organs and has demonstrated beneficial effects in multiple sclerosis treatment. We investigated the effect of treatment with siponimod on stroke outcome in a mouse model of cerebral ischemia.

Methods - Transient middle cerebral artery occlusion was induced in middle-aged wild-type mice. Animals were either treated with siponimod (3 mg/kg; intraperitoneal) or vehicle for 6 days. Stroke outcome was assessed by magnetic resonance imaging (spleen volume: prestroke, day 3, and day 7; infarct volume: days 1, 3, and 7) and behavioral tests (prestroke, day 2, and day 6). Immune cells of the peripheral blood and brain-infiltrating cells ipsilateral and contralateral were analyzed by VETScan and by flow cytometry.

Results - Siponimod significantly induced lymphopenia on day 7 after transient middle cerebral artery occlusion and reduced T-lymphocyte accumulation in the central nervous system. No effect was detected for lesion size.

Conclusions - For siponimod administered at 3 mg/kg in transient middle cerebral artery occlusion mouse model, our findings do not provide preclinical evidence for the use of S1PR1/5 modulators as neuroprotectant in stroke therapy.

P291

Specialized pro-resolving lipid mediators are differentially altered in multiple sclerosis and attenuate peripheral inflammation and blood-brain barrier dysfunction.

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Chronic neuro-inflammation is a key pathological hallmark of multiple sclerosis (MS) which suggests an impairment of the resolution of inflammation, a process orchestrated by a novel class of endogenous molecules known as specialized pro-resolving lipid mediators (SPMs), which avoids collateral tissue damage derived from uncontrolled inflammation. Here, through targeted-metabololipidomics in peripheral blood of MS patients, we revealed that each disease form was associated with distinct lipid mediator profiles that significantly correlated with disease severity. In particular, acute and progressive MS was associated with high eicosanoids levels, whereas the majority of SPMs were

DOI: 10.1002/eji.201970300

significantly reduced or below limits of detection and correlated with disease progression. Furthermore, we found impaired expression of several SPM biosynthetic enzymes and receptors in blood-derived leukocytes of MS patients. Mechanistically, differential synthesis of SPMs like LXA4, LXB4, RvD1 and PD1 reduced monocyte activation and cytokine production in MS patients and inhibited inflammation-induced blood-brain barrier dysfunction and monocyte transendothelial migration. Altogether, these findings reveal peripheral defects in the resolution pathway in MS, suggesting SPMs as novel diagnostic biomarkers and potentially safe therapeutics.

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Protection from EAE in DOCK8 mutant mice occurs despite increased Th17 cell frequencies in the periphery

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Mutation of Dedicator of cytokinesis 8 (DOCK8) has previously been reported to provide resistance to the Th17 cell dependant experimental autoimmune encephalomyelitis (EAE) in mice. Contrary to expectation, we observed an elevation of Th17 cells in two different DOCK8 mutant mouse strains in the steady state. This was specific for Th17 cells with no change in Th1 or Th2 cell populations. In vitro T helper cell differentiation assays revealed that the elevated Th17 cell population was not due to a T cell intrinsic differentiation bias. Challenging these mutant mice in the EAE model, we confirmed a resistance to this autoimmune disease with Th17 cells remaining elevated systemically while cellular infiltration in the central nervous system (CNS) was reduced. Infiltrating T cells lost the bias towards Th17 cells indicating a relative reduction of Th17 cells in the CNS and a Th17 cell specific migration disadvantage. Adoptive transfers of Th1 and Th17 cells in EAE-affected mice further supported the Th17 cell-specific migration defect, however, DOCK8-deficient Th17 cells expressed normal Th17 cell-specific CCR6 levels and migrated towards chemokine gradients in transwell assays. This study shows that resistance to EAE in DOCK8 mutant mice is achieved despite a systemic Th17 bias.

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Further evidence for a role of B cells in Multiple Sclerosis – a musical approach

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The pathogenesis of multiple sclerosis (MS) is still unknown. However, it is clear that the activation of the immune system plays a critical role. We applied the recently developed GeneExpressionMusicAlgorithm (GEMusicA) for the characterization of immune cell signatures in blood and brain samples from patients with MS. Based on publicly available gene expression data sets (Gene Expression Omnibus (GEO) data set GSE93776) we identified probe sets which can discriminate between different immune cell populations (monocyte subpopulations, T cell populations, B cell populations, NK cells, NKT cells and neutrophils). These probe sets were used for GEMusicA analysis of publicly available gene expression data from blood samples from patients with MS and controls (GEO data set GSE59085 and GEO data set GSE21942), and samples from different MS plaque types and normal brain (GEO data set GSE38010]). Besides individual differences in the gene expression profile, chronic MS plaques as well as chronic active MS plaques showed a distinct T-cell signature in comparison to normal controls, whereas the signature of myeloid cells was reduced. Surprisingly, the active MS plaque type showed a similarly reduced myeloid signature but did not show a T-cell signature. Instead, a distinctive B-cell signature was present. This B-cell signature was also more frequently detectable in the blood of patients with MS than in controls ($p < 0.0005$ - 0.01; two-sided t test). Our analyses suggest that GEMusicA can be used to characterize immune cell signatures in MS. Furthermore, our data suggest that B-cells and the balance between B-cells and myeloid cells play a central role in MS. Further investigations are necessary to interpret these cell signatures functionally.

DOI: 10.1002/eji.201970300

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Investigating the interaction of Th17 cells and B cells in initiation and progression of EAE

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Introduction: Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS). Both Th1 and Th17 cells contribute to disease pathogenesis in MS and its animal model experimental autoimmune encephalomyelitis (EAE). Compelling evidence also suggests a pivotal role of B cells in CNS autoimmunity. However, it is only poorly understood how T cells and B cells cooperate to induce disease. Meningeal ectopic lymphoid follicle-like structures (eLFs) in MS and EAE have been hypothesized to fuel chronic inflammation directly in the CNS.

Objectives: The aim of this study is to investigate the unique relationship of Th17 cells and B cells in initiation and progression of EAE, and to analyze whether and how Th17 cells and B cells interact with each other in eLFs.

Materials & methods: To differentiate between Th1- and Th17-driven aspects of disease, we established adoptive transfer EAE. Naïve myelin oligodendrocyte glycoprotein (MOG)-specific CD4+ T cells are *in vitro* differentiated with polarizing cytokines into Th1 or Th17 cells and transferred into wild-type recipient mice. To visualize the interaction of Th17 cells and B cells in eLFs, both cell types can be labeled with genetically-encoded protein activation sensors which will allow us to study their interactions and possible subsequent activation events in real-time using intravital microscopy of the CNS.

Results: We found that both Th1 and Th17 cells induced EAE with similar severity, and maintained their original cytokine profile also in the inflamed CNS. Importantly, large lymphocytic aggregates were found in association with meninges of brain and spinal cord primarily in Th17 recipients. These follicles contained clusters of T cells and B cells, with fractions of both being positive for the proliferation marker Ki67, and are thus reminiscent of follicle-like structures that have been described in MS patients.

Conclusion: We hypothesize that Th17 cells are uniquely equipped to recruit B cells to the CNS and to interact with them in meningeal eLFs, thereby fuelling chronic inflammation directly in the CNS. Unraveling the special relationship of Th17 cells and B cells in disease pathogenesis may provide targets for therapeutic intervention in MS.

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Serum levels of soluble CD23 are elevated in primary chronic progressive multiple sclerosis

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Question: The soluble form of CD23 is released from macrophages and B lymphocytes which are key players in the pathogenesis of multiple sclerosis. An imbalance of the cytokine network, including TNF-alpha and IFN-gamma, is suggested to play an important role in the immunopathogenesis of this disease. Recently, sCD23 has been shown to regulate the production of TNF-alpha and IFN-gamma in inflammatory diseases. Therefore, we asked whether sCD23 release is dysregulated in multiple sclerosis.

Methods: Serum levels of sCD23 were determined in 70 patients with multiple sclerosis and 25 healthy donors by solid-phase ELISA.

Results: Serum-levels of sCD23 were significantly (p=0.034, Mann-Whitney-U-test) higher in patients (n=23) with primary chronic progressive multiple sclerosis [PCP-MS](mean value: 2.1 U/ml, 25th and 75th percentile: 1.44 and 2.18) in comparison to healthy donors (n=25, Mean value: 1.2 U/ml, 25th and 75th percentile: 1.15 and 1.86). Serum levels were not elevated in relapsing-remitting multiple sclerosis (n=47) and not different between 10 patients with acute exacerbation or remission.

Conclusion: These results show an upregulation of sCD23 in PCP-MS. This indicates a possible involvement of sCD23 in cytokine dysregulation in PCP-MS.

DOI: 10.1002/eji.201970300

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Decrease of disease score in in vivo model of multiple sclerosis targeting S100B

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S100B, a small-calcium binding protein, acts as an intracellular regulator and through extracellular signaling. It has been demonstrated a direct correlation between increased amount of astrocytic S100B and its role in demyelination and neuroinflammatory processes upon demyelination in *ex vivo* demyelinating model.

The aim of the study is to investigate the possible role of pentamidine isethionate, a small molecule with antiprotozoal activity, able to target S100B, in the modulation of clinical outcome of RR-EAE mouse model.

Experimental Autoimmune Encephalomyelitis (EAE) was induced in female SJL wild-type mice and four groups were formed: EAE with vehicle (saline), EAE treated with pentamidine, Control mice treated with pentamidine and untreated control group; the pentamidine dosage was, 4mg/kg, intraperithoneally. Weight and clinical score of the animals were evaluated each day post injection. Following sacrifice, brains were collected and analyzed for the expression of myelin and neuroinflammation by immunohistochemistry and qRealTime PCR. We observe that pentamidine-treated mice display a faster decrease in disease score during the remission.

Thus, these preliminary results indicate that S100B is involved in MS pathology and that its inhibition may be a new therapeutic strategy not only to reduce damage but, hopefully, to fasten the recovery.

Disclosure**:** Funded by FISM pilot Grant 2018.

Dendritic and myeloid cells (P298-P327)

P298

Nanomaterials affect oxidative phosphorylation and phagocytosis in alveolar macrophages

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Introduction: Nanomaterials (NMs) are widely distributed in products of daily use as for example medicine products and cosmetics and have been shown to induce immunotoxicity and immune perturbation. Since novel NMs are developed continuously, risk assessment requires a mode of action (MoA) based grouping strategy.

Objectives: To facilitate mechanism-based risk assessment, we applied an untargeted proteomics approach, which reveals information about affected pathways and adverse effects on the immune system, thus giving insights into NMs MoAs. For this purpose, alveolar macrophages were used as model system since the lung is considered the primary entry portal for airborne NMs and alveolar macrophages are particularly involved in particle clearance as well as inflammation and disease upon NM exposure.

DOI: 10.1002/eji.201970300

Materials & Methods: For the untargeted proteomics approach, alveolar macrophages were treated with various doses of NMs for 24 h, followed by cell lysis, protein extraction and tandem mass spectrometry, resulting in abundance data for 3011 proteins. Next, enrichment analyses were conducted for those proteins that showed significantly altered abundances upon NM treatment. Additionally, a p-value independent approach called Weighted Gene Correlation Network Analysis (WGCNA) was applied to unravel novel key drivers involved in immunotoxicity.

Results: The MoA analysis allowed distinguishing NMs with minor or no effects from those that modified the function of alveolar macrophages and led to an increase of phagocytosis and oxidative phosphorylation. Furthermore, the observed immunological alterations were linked to physico-chemical properties as surface area and zeta potential and the identification of key drivers highlighted proteins that are connected to the affected pathways (e.g. Sod2, Lamp2). Importantly, these showed dose-dependent effects, thus rendering them valuable biomarker candidates to assess immune perturbations induced by NMs.

Conclusions: Taken together, the conducted proteomics approach revealed insights into NMs MoAs in alveolar macrophages and unraveled highly relevant physico-chemical properties and key drivers that might serve as biomarkers to predict immunotoxicity in future.

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Role of BATF on development of plasmacytoid dendritic cells

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Background: We found that the transcription factor (TF) BATF (Basic leucine zipper transcription factor, ATF-like) is highly expressed in interferon (IFN) β-producing plasmacytoid dendritic cells (pDCs). While the functions of BATF for T helper cell subset differentiation and B cell class switching have been well described, no biological role of BATF in pDCs has been shown so far.

Objectives: Characterizing the role of BATF in pDC development.

Materials & methods: pDCs from bone marrow (BM)-derived Flt3-L cultures of *Batf*$^{+/+}$ or *Batf*$^{-/-}$ mice were characterized by FACS. Next generation sequencing (NGS) was used to assess the impact of *Batf* on global gene expression in naïve and CpG stimulated pDCs. The expression of selected genes was confirmed with quantitative RT-PCR in sorted cells from BM-derived Flt3-L cultures. BATF ChIP-seq revealed direct binding of BATF in the promoter and intron regions of genes. ATAC-Seq was used to characterize the chromatin landscape of pDCs in the presence and absence of BATF.

Results: We found that pDCs are significantly increased in numbers in BM-derived Flt3-L cultures from *Batf*$^{-/-}$ as compared to *Batf*$^{+/+}$ animals. Interestingly, RNA-Seq revealed that out of the 581 TFs that are expressed in pDCs 26% are expressed in a *Batf*-dependent manner. ChIP-Seq suggests that 72% of the *Batf*-dependent TFs are directly regulated in their expression by binding of BATF to promoter and or intron regions of the respective gene. Further, BATF acts as a pioneering factor to regulate chromatin accessibility of about 8500 DNA regions in pDCs, resulting in modulation of the chromatin structure of about 28% of all TFs in pDCs. RNA-Seq showed that pDC specific TFs such as E2-2 and Irf8 are significantly increased in expression in naïve *Batf*-deficient vs. wildtype pDCs, indicating a possible role for BATF in pDC differentiation. At the same time, cDC specific TFs such as Id2 and Nfil3 were significantly reduced in *Batf*-deficient pDCs. These results were confirmed with RT-PCR in sorted cells from BM-derived Flt3-L cultures. Thus, BATF seems to regulate pDC specific TFs as a direct DNA binding repressor while cDC specific TFs are regulated by the pioneering function of BATF.

Conclusion: Our results propose a functional involvement of BATF in pDC differentiation by regulating the expression and chromatin accessibility of key transcription factors in pDC development.

DOI: 10.1002/eji.201970300

P300

CD49a expression identifies a specific subset intrahepatic macrophages in humans

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Introduction: Monocytes and macrophages play central roles in hepatotropic infections, inflammatory reactions and the initiation of immune responses. In human peripheral blood, monocytes (pbMOs) are classified in $CD14^{hi}CD16^{lo}$ cells, $CD14^{int\text{-}hi}CD16^{hi}$ cells and $CD14^{lo}CD16^{hi}$ cells. Human intrahepatic macrophages constitute more than 80% of all tissue macrophages of the human body. While studies on liver-resident macrophages in mice have established their central role in regulating liver inflammation in different disease settings, their phenotype and function are still not well-described in the context of human liver samples.

Objective: The main objective of this study was to characterize intrahepatic human macrophages (ihMφ) and compare their phenotypical and functional patterns to conventional pbMOs from matched human donors.

Materials and Methods: We obtained matched liver tissue and peripheral blood samples from individuals undergoing liver transplant surgery or surgical tumor-free liver tissue resection due to liver metastases. Intrahepatic leukocytes (IHLs) and peripheral blood mononuclear cells (PBMCs) were isolated using protocols established in our laboratory. Freshly recovered cells were stained with antibodies directed against surface markers, fixed and measured using a BD LSR Fortessa flow cytometer. Donor-matched IHLs and PBMCs were stimulated 16h with LPS or CL097 and cytokine responses were measured by flow cytometry. Unstimulated cells were used as baseline control. Immunofluorescence staining of CD68, MARCO, CD49a, VSIG4 and Hoechst was performed on frozen human liver biopsies.

Results: When comparing paired ihMφand pbMOs, the proportion of $CD14^{int\text{-}hi}CD16^{int}$ was significantly increased and the proportion of $CD14^{hi}CD16^{lo}$ decreased in ihMφ. Moreover, ihMφ contained a variety of cell subsets with differential expression of CD49a, a liver-resident marker of NK cells, and VSIG4 and/or MARCO, two newly described human tissue Mφ markers. On a phenotypical and functional level, $CD49a^{+}$ ihMφ contained a higher proportion of activated and mature cells (CD80, CD83, CD86 and CD69) with a higher baseline proportion of cells expressing IL-10, TNF-α and IL-12 and a lower additional *in vitro* stimulation response, compared to $CD49a^{-}$ ihMφ and pbMOs.

Conclusion: Taken together, we characterized expression of CD49a, VSIG4 and MARCO in human ihMφ and showed that $CD49a^{+}$ ihMφ, $CD49a^{-}$ ihMφ and pbMOs are three phenotypically and functionally different cell subsets.

P302

Cholinergic inhibition of ATP-induced interleukin-1β release by novel endogenous agonists that trigger metabotropic functions at nicotinic acetylcholine receptors

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DOI: 10.1002/eji.201970300

Introduction: The pro-inflammatory cytokine interleukin-1β (IL-1β) is important for host defense against infections. High systemic IL-1β levels, however, contribute to the pathogenesis of numerous life-threatening inflammatory diseases like the systemic inflammatory response syndrome. Therefore, mechanisms controlling IL-1β release are of considerable clinical interest. Recently, we identified a cholinergic mechanism that inhibits the ATP-associated release of IL-1 β by human monocytes via nicotinic acetylcholine receptors (nAChRs) composed of α7, α9 and/or α10 subunits. Interestingly, we discovered novel nAChR agonists that efficiently and metabotropically inhibit monocytic IL-1β release without interfering with neuronal nAChR functions.

Methods: Human monocytic U937 cells were primed with lipopolysaccharide. Thereafter, the cells were stimulated with ATP in the presence and absence of potential nAChR agonists. ATP-induced IL-1β release was measured in cell culture supernatants via ELISA. A panel of nAChR antagonists was used to test for involvement of nAChRs. Electrophysiological measurements were performed to monitor ion-currents in U937 cells (whole-cell patch-clamp) and *Xenopus laevis* oocytes (two-electrode voltage-clamp) that heterologously overexpressed human CHRNA7, CHRNA9 or CHRNA9/CHRNA10.

Results: Phosphocholine (PC) as well as molecules bearing a PC-headgroup (e.g. lysophosphatidylcholine, glycerophosphocholine, dipalmitoylphosphatidylcholine, PC-laden C-reactive protein) were identified to function as novel endogenous nAChR agonists. These molecules efficiently inhibit ATP-induced IL-1β release by eliciting metabotropic but not ionotropic activity on monocytic nAChRs containing subunits α7, α9 and/or α10. Interestingly, using *Xenopus laevis* oocytes as an experimental model for neuronal nAChRs, these agonists did not elicit ion-currents at heterologously expressed human nAChRs, and thus functionally resemble nAChR silent agonists.

Conclusions: We identified anti-inflammatory properties of several novel, endogenous nAChR agonists that elicit metabotropic functions in monocytic cells to inhibit IL-1β release without inducing canonical ionotropic receptor functions of excitable cells. These nAChR silent agonist-like functions open a promising opportunity for the development of therapies against excessive inflammation involving IL-1β.

P303

House dust mite drives proinflammatory eicosanoid reprogramming and macrophage effector functions.

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Background: Eicosanoid lipid mediators play key roles in type 2 immune responses, for example in allergy and asthma. Macrophages represent major producers of eicosanoids and they are key effector cells of type 2 immunity.

Objective: We aimed to comprehensively track eicosanoid profiles during type 2 immune responses to house dust mite (HDM) or helminth infection and to identify mechanisms and functions of eicosanoid reprogramming in human macrophages.

Methods: We established an LC-MS/MS workflow for the quantification of 52 oxylipins to analyze mediator profiles in human monocyte-derived macrophages (MDM) stimulated with HDM and during allergic airway inflammation (AAI) or nematode infection in mice. Expression of eicosanoid enzymes was studied by qPCR and western blot and cytokine production was assessed by multiplex assays.

Results: Short (24 h) exposure of alveolar-like MDM (aMDM) to HDM suppressed 5-LOX expression and product formation, while triggering prostanoid (thromboxane and prostaglandin D_2 and E_2) production. This eicosanoid reprogramming was p38-dependent, but dectin-2-independent. HDM also induced proinflammatory cytokine production, but reduced granulocyte recruitment by aMDM. In contrast, high levels of cysteinyl leukotrienes (cysLTs) and 12-/15-LOX metabolites were produced in the airways during AAI or nematode infection in mice.

Conclusion: Our findings show that a short exposure to allergens as well as ongoing type 2 immune responses are characterized by a fundamental reprogramming of the lipid mediator metabolism with macrophages representing

DOI: 10.1002/eji.201970300

particularly plastic responder cells. Targeting mediator reprogramming in airway macrophages may represent a viable approach to prevent pathogenic lipid mediator profiles in allergy or asthma.

P304

Macrophage/dendritic cell - *Taenia solium* cyst interaction: understanding the regulation and pathogenesis in neurocysticercosis

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Introduction: Neurocysticercosis, the larval stage of the pork tapeworm *Taenia solium* in the human central nervous system, is a food-borne neglected tropical disease and the most common cause of epilepsy in endemic regions. The immune response in affected individuals and associated clinical presentations are diverse involving complex type 1/2 immune responses and strongly depends on the stage of larval cyst, which alters innate immune cell reactivity to prevent potentially harmful responses in the brain. However, up to now, macrophage and dendritic cells" responses to the different cyst components remain unknown.

Objective: The present work aims to investigate the effects of different *Taenia solium* cyst-related products on macrophage and dendritic cells.

Materials And Methods: *Taenia solium* cyst antigen lysate (CLys), cyst vesicular fluid (CVF) and culture supernatant (CSN) were prepared from cysts collected from an infected pig and cultured with peripheral blood mononuclear cells (PBMC) from healthy German volunteers as well as murine alveolar macrophages (AM) and bone-marrow derived dendritic cells (BMDDC). The effect of the lysates was investigated by flow cytometry analysis of Th subsets (Th1/Th2/Th17/Treg) and TNFα, IL-6, TGF-ß and IL-10 cytokine secretion. Underlying mechanistic aspects were explored using mass spectrometry, enzymatic digestion and IL-10R deficient mice.

Results: In contrast to CVF, which is proinflammatory and induced significant levels of TNFα and IL-6, CLys and CSN induced IL-10 in AM and BMDDC, which was significantly reduced after enzymatic digestion of cyst products. In periphery, significant induction of peripheral Tregs ($CD4^+CD25^{high}FoxP3^+IL\text{-}10^+$) was observed in presence of CLys only. However, IL-10R deficiency did not completely impair Treg induction. Unexpectedly, both CVF and CLys were not prominent Th2 inducers as revealed by decreased expression of IL-5 and GATA-3. In addition, differences were observed in protein composition of cyst products as identified by mass spectrometry.

Conclusion: Our findings suggest that whereas the vesicular fluid, contained within viable cyst, is rather proinflammatory, lysate from whole cysts may modulate macrophage, DC and subsequently peripheral immune responses by inducing Tregs and anti-inflammatory cytokines to favor a suppressive environment and parasite establishment. Current work focuses on identification of active molecules and the underlying cellular networks in the brain.

P305

Upregulation of CD177 on neutrophil granulocytes upon inflammation enables highly specific tracking by non-invasive ^{19}F MRI *in vivo*

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Neutrophil granulocytes are crucial for the development and progression of inflammatory diseases. One major subpopulations is characterized by expression of CD177. However, little is known about the role of $CD177^+$ neutrophils for inflammatory diseases. The aim of the present study was to target perfluorocarbon nanoemulsions (PFCs) to CD177 enabling the subsequent visualization of neutrophil granulocytes by ^{19}F magnetic resonance imaging (^{19}F MRI).

DOI: 10.1002/eji.201970300

Peptides against CD177 [1] were coupled to maleimide-PFCs (NGP-PFCs) on the particle surface. Binding studies were performed by flow cytometry and ^{19}F MRI using human neutrophils from healthy volunteers and patients after myocardial infarction. Murine neutrophils were obtained from blood of C57BL/6 mice or isolated from LPS-doted matrigel-plugs [2]. MR-experiments were performed at a 9.4 T Bruker AVANCEIII Wide Bore NMR spectrometer and datasets were acquired using a 25 mm birdcage resonator tuneable to ^{1}H and ^{19}F.

Binding studies with NGP-PFCs revealed specific labelling of neutrophil granulocytes. Importantly, binding and internalization of NGP-PFCs by neutrophils was strongly upregulated in patients suffering from myocardial infarction or after ex vivo LPS stimulation. Increased NGP-PFC binding was also found in murine neutrophils isolated from subcutaneous matrigel/LPS inflammatory hot spots.

In this study, we show that neutrophil granulocytes can be specifically labelled with PFCs by targeting the cell surface receptor CD177. CD177 is expressed on all neutrophils in mice but only on a subpopulation of human neutrophils. CD177 is strongly upregulated under inflammatory conditions in mice and men. This enables the tracking of $CD177^{+}$ neutrophils by ^{19}F MRI to further investigate their role in inflammatory diseases.

Figure 1: A+B) Human (left) and murine (right) neutrophils were incubated with NGP-PFCs and the uptake determined by flow cytometry. **C)** ^{19}F MRI of human neutrophils were incubated with hNGP-PFCs, separated by density centrifugation and analyzed by 1H/^{19}F MRI.

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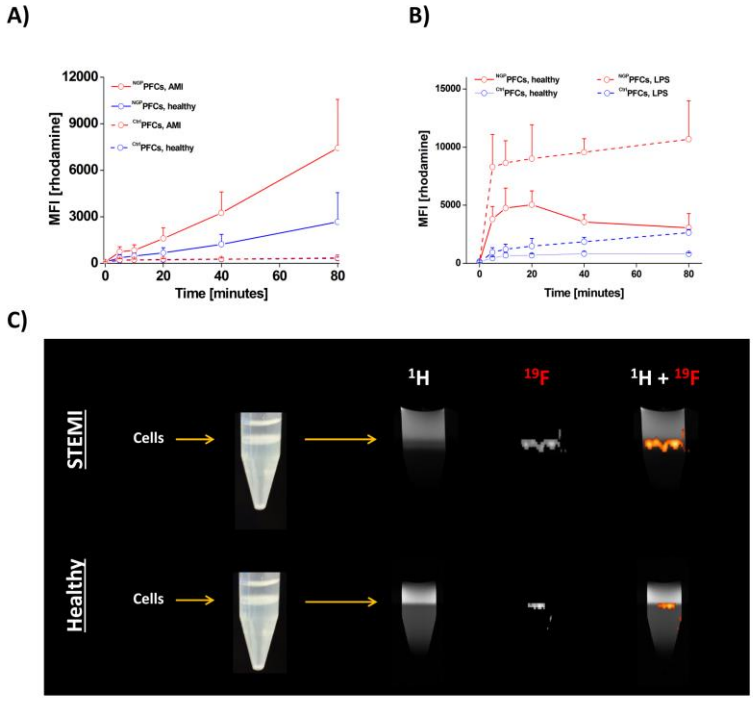
Figure 1



DOI: 10.1002/eji.201970300

P309

The kidney contains ontogenically distinct mononuclear phagocyte subtypes throughout development that differ in their inflammatory properties

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Introduction. Mononuclear phagocytes (MPs) include macrophages, monocytes and dendritic cells (DCs). They are vital players in immune defense and organ homeostasis. In the kidney the origin of MPs has been highly debated because of the large phenotypic overlap of macrophages and DCs in this tissue. Namely, the adult kidney contains the main subsets of cDCs, cDC1 and cDC2, as well as two cell populations that express typical macrophage markers, CD64 and F4/80, but arise from DC progenitors, as was shown by *Clec9a*-expression history.

Objectives. In the present study we aimed to characterize renal MP development and function throughout life.

Materials & Methods. We use mouse models to fate map MPs of distinct origin. By using single cell technologies and transcriptomic analysis we aim to define cell type-specific markers and transcriptional signatures. The functional properties of MPs are analyzed *in vitro*. We further use Histo-Cytometry to define the precise localization of the MP subsets in the kidney.

Results. We demonstrate that these populations are phenotypically, functionally and transcriptionally distinct from each other. We further reveal that the kidney MP compartment exhibits a unique age-dependent developmental heterogeneity. Kidneys from newborn mice contain a prominent population of yolk sac-derived F4/80^{hi}CD11b^{low} macrophages that express TIM-4 and MERTK, but lack MHCII expression. We show that these macrophages are replaced within a few weeks after birth by phenotypically similar cells that can be distinguished by MHCII expression and a relative lack of TIM-4 and MERTK. Fate mapping further suggests that MHCII^{+}F4/80^{hi} cells in themselves exhibit age-dependent developmental heterogeneity and in adult mice have a low homeostatic turnover. We show that upon acute renal inflammation F4/80^{hi} cells that lack MHCII, reappear in the kidney as the result of a phenotypic switch of the resident MHCII^{+}F4/80^{hi} population.

Conclusions. We demonstrate that distinct developmental programs contribute to renal MPs throughout life.

P311

Defining the cellular mediators of CARD9 signaling in Colitis-associated-Cancer

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Introduction: In chronic inflammatory bowel disease (IBD) inflammation is the key driver for development of colitis associated cancer (CAC). CARD9 mediates inflammatory signals in myeloid cells and contributes to intestinal homeostasis. Genetic polymorphisms in Card9 are associated with human IBD. Different studies addressed the role of CARD9 in CAC by subjecting *Card9-/-* mice to an AOM-DSS model with seemingly conflicting results. In our hands *Card9-/-* mice develop smaller and less dysplastic tumours (Bergmann et al., EJI, 2017), whereas others show increased tumour incidence (Wang et al., Immunity 2018; Malik et al., Immunity, 2018). Since Card9 deficiency alters the intestinal microbiome (Lamas, NatMed, 2016) these differences are likely explained by differences in housing of the animals. These studies demonstrate a role for CARD9 in AOM-DSS, but the cell type in which CARD9 mediates inflammatory signals has not been defined.

Objectives: We aim to define the cellular basis of CARD9 function during inflammation and oncogenesis.

DOI: 10.1002/eji.201970300

Materials & methods: Acute colitis (DSS) and CAC (AOM-DSS) model, Flow Cytometry, Histology, 16s RNA and single cell RNA Sequencing (scRNA seq), Fate mapping of myeloid cell subsets

Results: Card9-/- mice show more severe disease during the first DSS cycle during AOM-DSS colitis. We therefore studied the composition of myeloid cells in the colon WT and Card9-/- mice in steady state and during acute colitis. To minimize the effects of the microbiota we cohoused *Card9-/-* and WT mice prior to DSS treatment. We found no obvious differences in the myeloid cell compartment of WT and Card9-/- mice, indicating that Card9 does not influence myeloid cell differentiation, but rather leads to altered inflammatory signals. Next, we will perform scRNA seq to identify the cellular signalling pathways that are altered in *Card9-/-* mice.

Conclusion: *Card9-/-* mice show decreased severity of colorectal cancer. We find that Card9 deficiency does not lead to alterations in the composition of myeloid cells. Future experiment will define which signalling pathways are altered in the absence of CARD9 during AOM DSS and in what cell type.

P312

Plasmacytoid dendritic cell differentiation is distinct from the myeloid lineage and occurs from Ly6D+ early lymphoid progenitors

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The dendritic cell (DC) family currently comprises conventional dendritic cells (cDCs) and plasmacytoid dendritic cells (pDCs). However, pDCs and cDCs exhibit distinct origins, phenotypes and functions, questioning whether or not pDCs belong to the DC lineage. Here, we combined single cell transcriptomic analysis with mass cytometry, *in vitro* and *in vivo* assays, to show that the priming of mouse hematopoietic progenitor cells towards the pDC lineage occurs at the common lymphoid progenitor (CLP) stage, indicative of an early divergence of the pDC and cDC lineages. We found transcriptional signatures of pre-pDCs within the CLP and CD115- CDP (common DC progenitors) populations, and identified Ly6D, IL-7Rα, CD81, and CD2 as key markers of pDC differentiation, distinguishing pre-pDCs from pre-cDCs. Thus, pDCs developed in the bone marrow from a Ly6DhiCD2hi lymphoid progenitor cell and differentiated independently of the myeloid cDC lineage.

P313

Adventitial macrophages invade the atherosclerotic plaque

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Introduction: Macrophages populate the vasculature in health and disease. They confer critical homeostatic and protective functions on the one hand, and propagate inflammation and disease progression on the other. According to the prevailing paradigm, resident tissue macrophages of primitive origin are typically supplemented by monocyte derived macrophages in inflamed tissues. A majority of macrophages in the healthy aorta in mice express the chemokine receptor CX3CR1 and reside in the adventitia. During atherogenesis, macrophages accumulate in the nascent plaque in the intima. It is unclear, whether adventitial macrophages infiltrate the intima during disease.

Objectives: In order to distinguish resident and monocyte-derived macrophages in the aorta in health and disease, we established an atherosclerotic reporter mouse model taking advantage of the selective CX3CR1 expression by monocytes and vascular macrophages.

Materials & methods: We analyzed the aortas of LDLR-/- CX3CR1-GFP mice fed a chow (healthy) and a high cholesterol diet (atherosclerosis). CX3CR1 CD68 expressing cells were identified in the adventitia, media and intima. By crossing LDLR-/-, CX3CR1-CreER and R26tomato mice, we generated a tamoxifen-inducible reporter model. Following tamoxifen injection, CX3CR1-expressing monocytes and tissue resident macrophages were labeled tomato

DOI: 10.1002/eji.201970300

red. Within 7 weeks, tomato expressing monocytes had been replaced by unlabeled upstream progenitors, while long-lived, self-sustaining resident CX3CR1+ macrophages remained tomato+.

Results: Tomato+ macrophages identified in the intimal plaque constituted up to 20% of CD68+ cells and derived from adventitial macrophages that were labeled prior to disease onset.

Conclusion: Previously, macrophages in the plaque were reported to derive from infiltrating monocytes, proliferating lesional macrophages and smooth muscle cells through transdifferentiation. We identified yet another origin of plaque macrophages, adventitial resident macrophages of largely prenatal origin, that may contribute distinctly to disease progression.

P314

Inhibition of ATP-induced monocytic IL-1β release by endogenous alpha1-antitrypsin is not affected by cardiopulmonary bypass surgery

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Introduction: Increased levels of the pro-inflammatory cytokine interleukin-1β (IL-1β) in patients after cardiopulmonary bypass surgery (CPBS) are associated with the development of systemic inflammatory response syndrome. We demonstrated before that the anti-protease alpha1-antitrypsin (AAT) inhibits ATP-induced IL-1β release from human monocytes. Here, we examined if patients acquire a functional deficiency in AAT during CPBS.

Methods: AAT from the plasma of healthy donors and CPBS patients was purified by affinity chromatography. U937 cells were primed with lipopolysaccharide and stimulated with ATP for 30 minutes in the presence or absence of AAT. The concentration of IL-1β in cell culture supernatants was measured by ELISA. Anti-protease activity of AAT was investigated by complex formation with neutrophil elastase (NE).

Results: Healthy donor and patient-derived AAT inhibit ATP-induced IL-1β release from monocytic cells. Furthermore, the capacity of AAT (i) to inhibit IL-1β release and (ii) to form complexes with NE is unimpaired throughout CPBS. Therefore, we conclude that AAT is not inactivated during CPBS.

Conclusions: Healthy donor and patient-derived AAT have the potential to prevent trauma-associated, IL-1β-mediated systemic inflammation. This activity of AAT and its anti-protease function are not inactivated during CPBS.

P315

Characterization of monocyte subsets during the course of healthy pregnancy.

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Pregnancy uniquely challenges the maternal immune system to guarantee immunological tolerance towards the semi-allograft foetus while simultaneously maintaining effective antimicrobial defense. Augmented innate immune responses contribute to this balance. Innate immune activation is triggered by a combination of factors, including maternal pregnancy hormones and contact of immune cells with the placenta and their encounter with soluble placental products. Monocytes as part of the innate arm of the immune system play a central role in the inflammatory response. The three different monocyte populations (classical, intermediate and non-classical) have been described to have distinct phenotypic and functional characteristics, appearing to be sequential developmental stages in monocyte differentiation with smoothly shifting surface expression of their defining CD molecules. Deviations from physiological

DOI: 10.1002/eji.201970300

monocyte function and composition have regularly been observed in various types of infectious and (auto-) inflammatory conditions. Particularly the intermediate subset often exhibited an increase in frequency as well as proinflammatory function. Since monocytes play an essential role for successful trophoblast implantation and remodelling, they represent a relevant field of research in pregnancy. Using multiparameter flow cytometry we characterized monocytes from pregnant women recruited to the PRINCE cohort, a population-based prospective birth cohort study in Hamburg. Pregnant women were followed throughout the course of pregnancy and blood samples were analysed at predefined time points. In addition, pregnancy-associated hormones and soluble activation markers were measured in serum. Principle component analysis revealed an association of the expression levels of several markers especially on the intermediate subtype with healthy pregnancy. In general, classical and intermediate monocytes hereby positively correlated. Furthermore, hCG significantly associated with the expression levels of CD116, CD11b and CCR2 on monocyte subsets. Phenotypic changes were accompanied with changes in the polyfunctionality of monocytes upon TLR4 stimulation. In conclusion, our data describe the phenotypic and functional changes of monocyte subsets in healthy pregnant women and elaborate a selective contribution of different monocyte subsets and their activation and maturation status to healthy pregnancy.

P316

Antisense oligonucleotides as a new approach to inhibiting the NLRP3 inflammasome

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NLR family pyrin domain containing 3 (NLRP3) is part of the proteolytic NLRP3 inflammasome complex that is required for maturation of IL-1β and IL-18 from their pro-forms and additionally for induction of pyroptotic cell death. Being involved in a broad range of inflammatory disorders like CAPS (cryopyrin associated periodic syndromes), chronic kidney disease, Alzheimer or liver fibrosis, the NLRP3 inflammasome is a highly attractive therapeutic target. However, direct targeting of NLRP3 by antibodies is hampered by its intracellular localization while small molecule-based approaches lack specificity, resulting in toxic side effects. These limitations can be overcome by using LNA modified antisense oligonucleotides (ASOs) directed against NLRP3. This type of ASO is internalized by cells without need of a transfection reagent, binds to its target (pre) mRNA and causes RNase H mediated (pre) mRNA cleavage and subsequent degradation.

We screened over 100 either mouse- or human-NLRP3 specific ASOs in relevant cell lines such as, Raw 264.7, THP-1 and U-87MG in order to identify the most potent candidates. The half maximal inhibitory concentration (IC50) of the best human- or mouse-specific ASOs was in the nano- or low micromolar range, respectively. We further demonstrated that NLRP3 mRNA expression could be reduced by 80% to 90% in macrophages that were differentiated from human monocytes or murine bone marrow cells in presence of NLRP3 specific ASOs. Consequently, inflammasome-mediated IL-1β cleavage after LPS + ATP stimulation of murine macrophages was inhibited by NLRP3 specific ASOs. Finally, sub cutaneous injection of healthy mice with NLRP3 specific ASOs resulted in over 50% reduction of NLRP3 mRNA in the kidney, liver and lymph nodes, while the control oligonucleotide had no effect.

Taken together, NLRP3-specific ASOs were identified that induce potent target knockdown *in vitro* and *in vivo*. While mouse-NLRP3 specific ASOs will be a valuable tool to demonstrate proof-of-concept in relevant pre-clinical models, the human-specific candidates might provide a new therapeutic modality to treat inflammsome-mediated diseases.

P317

Immunostimulatory functions of adoptively transferred MDSCs in experimental blunt chest trauma

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Question: Myeloid-derived suppressor cells (MDSCs) expand during inflammation and exhibit immunomodulatory functions on innate and adaptive immunity. However, their impact on trauma-induced immune responses, characterized by an early pro-inflammatory phase and dysregulated adaptive immunity involving lymphocyte apoptosis, exhaustion and unresponsiveness is less clear. Therefore, we adoptively transferred in vitro-generated MDSCs shortly before experimental blunt chest trauma (TxT).

DOI: 10.1002/eji.201970300

Methods: To generate MDSCs, bone marrow from femur and tibia were cultured with granulocyte-macrophage colony-stimulating factor (GM-CSF) for 4 days. $2x10^7$ MDSCs were adoptively transferred into tail veins of B6 mice 1h before TxT. C57BL/6 mice underwent a blast wave to induce TxT. Cytokine and chemokine secretion were analyzed in bronchoalveolar lavage (BAL) fluid and blood serum samples. Homing of MDSCs and number of splenocytes were determined. T cell activation status and their expression of Th1/2-associated cytokines were analyzed. Additionally, the proliferative capacity of T cells was determined in vitro as well as in vivo.

Results: GM-CSF-induced MDSCs express arginase-1, iNOS and HO-1 and suppressed allogeneic-induced T cell proliferation in vitro. At 6h up to 7d after TxT, adoptively transferred MDSCs preferentially homed into spleen and liver, but were undetectable in the injured lung, although pro-inflammatory mediators (IL-6, G-CSF and MCP-1) increased in the BAL early after trauma-induction. Surprisingly, MDSC treatment strongly increased splenocyte numbers, however, without altering the percentage of splenic leukocyte populations. 7 days after TxT, T cells of MDSC-treated TxT mice exhibited an activated phenotype characterized by expression of activation markers and elevated proliferative capacity in vitro, which was not accompanied by up-regulated exhaustion markers or unresponsiveness towards in vitro activation. Most importantly, also T cell expansion after staphylococcal enterotoxin (SEB) stimulation in vivo was unchanged between MDSC-treated or untreated mice. After MDSC transfer, T cells preferentially exhibited a Th1 phenotype, a prerequisite to circumvent post-traumatic infectious complications.

Conclusions: Our findings reveal a totally unexpected immunostimulatory role of adoptively transferred MDSCs in TxT and might offer options to interfere with post-traumatic malfunction of the adaptive immune response.

P318

Role of short-chain fatty acids in dendritic cell function

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Accumulating evidence suggests microbial metabolites present in the gut lumen can have a strong influence on host immune responses. Short-chain fatty acids (SCFAs), such as butyrate, propionate and acetate, are the main end products of the fermentation of undigested complex carbohydrates by commensal bacteria in the colon. Butyrate is known to be an important energy source for intestinal epithelial cells and together with propionate, to promote the differentiation of colonic Treg cells. SCFAs have also been shown to modulate $CD103^+$ dendritic cells (DCs) and thus induce the differentiation of Treg cells. However, the molecular mechanisms that underlie the effects of SCFAs on DCs and how they affect their interaction with Treg cells have not been thoroughly investigated so far. SCFAs can act as histone deacetylase inhibitors or bind and signal through G-protein-coupled receptors (GPCRs). There are three known GPCRs, which can bind SCFAs: Free Fatty Acid Receptor 2 (Ffar2/Gpr43), Ffar3/Gpr41 and Hca2/Gpr109a. We first decided to test the expression pattern of these receptors in different DC populations by RT-PCR. GM-CSF-derived DCs and *in vitro*-generated $CD103^+$ DCs both expressed high levels of Gpr109a and Ffar2 but not Ffar3. Ffar2 was also previously shown to be expressed on colonic myeloid and Treg cells and to be directly involved in the induction of Treg cells. However, further reports on the involvement of this receptor in the mechanisms of SCFAs-induced tolerance have been contradictory. One reason for this, could be that the mode of action of SCFAs and signalling via Ffar2 has only been studied using complete receptor knockouts. In order to target specific cell populations for Ffar2 deletion, we generated mice with loxP-flanked Ffar2 alleles and crossed them to CD11c-Cre mice. Preliminary results show that the numbers of DCs are not affected in Ffar2fl/fl x CD11c-Cre mice, suggesting this receptor is not essential for the development of the different populations of DCs. To further test the role of Ffar2 in DC function, we used the experimental autoimmune encephalomyelitis mouse model. Mice lacking Ffar2 specifically on $CD11c^+$ cells showed slightly reduced clinical scores as compared to WT mice, indicating the presence of Ffar2 in DCs is important for their effector functions. These results aim to further dissect the interaction between DCs and SCFAs to be able to use them as novel therapeutic approaches in the future.

P319

PSM peptides from community-associated methicillin-resistant *Staphylococcus aureus* impair the adaptive immune response via modulation of dendritic cell subsets *in vivo*

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DOI: 10.1002/eji.201970300

Introduction: Dendritic cells (DCs) are key players of the immune system and thus a target for immune evasion by pathogens. We recently showed that the virulence factors phenol-soluble-modulins (PSMs) produced by community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) strains induce tolerogenic DCs upon Toll-like receptor activation via the p38-CREB-IL-10 pathway *in vitro*. Objective: Here, we addressed the hypothesis that *S. aureus* PSMs disturb the adaptive immune response via modulation of DC subsets *in vivo*.Materials & Methods: We used a systemic mouse infection model with wildtype and PSM-deletion mutants of *S. aureus* and analyzed DC and T cell subsets by flow cytometry. Results: We found that *S. aureus* reduced the numbers of splenic DC subsets, mainly $CD4^+$ and $CD8^+$ DCs independently of PSM secretion. *S. aureus* infection induced the upregulation of the C-C motif chemokine receptor 7 (CCR7) on the surface of all DC subsets, on $CD4^+$ DCs in a PSM-dependent manner, together with increased expression of MHCII, CD86, CD80, CD40, and the co-inhibitory molecule PD-L2, with only minor effects of PSMs. Moreover, PSMs increased IL-10 production in the spleen and impaired TNF production by $CD4^+$ DCs. Besides, S. aureus PSMs reduced the number of $CD4^+$ T cells in the spleen with impaired priming of Th1 and Th17 cells, whereas $CD4^+CD25^+Foxp3^+$ regulatory T cells (Tregs) were increased. Further, IFN-γ production by $CD8^+$ T cells was reduced by *S. aureus* PSMs. Conclusion: Thus, PSMs from highly virulent *S. aureus* strains modulate the adaptive immune response in the direction of tolerance by affecting DC functions.

P320

Small extracellular vesicle-delivered microRNAs promote IFN-α secretion by plasmacytoid DCs via TLR7

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Question: Aberrant and persistent production of type I interferons (IFNs) represents a characteristic aspect of many autoimmune diseases, including Systemic Lupus Erythematosus (SLE). In these pathologies, self nucleic acids activate pDCs, the main IFN-a producer cell type, and trigger the continuous production of type I IFN via TLR7/9 engagement. However, the nature and origin of pDC-activating self-nucleic acids is not completely characterized.MicroRNAs are short non-coding RNAs that are deregulated in many autoimmune diseases and can be released within extracellular vesicles (EVs), representing possible candidates for pDC activation. Here, we investigate the role of extracellular microRNAs as activators of human pDCs in order to evaluate their potential contribution in autoimmune diseases.

Methods: Freshly purified pDCs were stimulated with synthetic microRNAs or small EVs for 24 hours and cytokine production was evaluated by ELISA. Small EVs from plasma or conditioned media were isolated by serial centrifugations.

Results: We report that small EVs isolated from the plasma of SLE patients can activate the secretion of IFN-α by human blood pDCs, *in vitro*. This activation requires endosomal acidification and is recapitulated by microRNAs purified from small EVs, suggesting that small EV-delivered microRNAs act as self-ligands of innate single strand endosomal RNA-sensors. By using synthetic microRNAs, we identify an "IFN Induction Motif", present in small EVs microRNAs and responsible for the TLR7-dependent activation, maturation and survival of human pDCs.

Conclusions: This study identifies small EV-delivered microRNAs as TLR7 endogenous ligands able to induce pDC activation. Therefore, our results candidate microRNAs as pathogenic players and possible targets in autoimmune reactions.

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The two faces of β_2 integrins: Do the adhesion receptors drive activation or regulation in human dendritic cells?

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DOI: 10.1002/eji.201970300

Introduction: Evidence is mounting that β_2 integrins, adhesion receptors that exist in a continuum between "inactive" and "active", facilitate not only inflammation but are also important in immune regulation. Previous research suggests that β_2 integrin-mediated immunoregulation is prominent in murine dendritic cells (DCs), but a similar role in human cells has yet to be established. Published literature on the role β_2 integrins in dendritic cells (DCs) is limited and conflicting, likely due in part to the fact that DCs express all four β_2 integrin family members, CD11a, CD11b, CD11c or CD11d, which have been shown to differ in functionality in other cell types.

Objectives: Our aim was to investigate the pro-inflammatory and immuno-regulatory roles of β_2 integrin subunits in DCs. To do this, we quantified differences in expression and activation (conformation) of two β_2 integrin α-subunits, CD11a and CD11b, in settings of inflammation as well as tolerance in human DCs.

Patients & Methods: Using flow cytometry, we detected total and activated β_2 integrin subunits (CD11a, CD11b and the pairing subunit CD18) in mature and tolerogenic monocyte-derived DCs (Mo-DCs), as well blood and synovial fluid samples from patients with Rheumatoid Arthritis (RA). Additionally, we explored their respective functional roles for appropriate immune function by assessing T cell priming.

Results: CD11a expression is increased in tolerogenic Mo-DCs suggesting an immunoregulatory role. In RA, an autoimmune disease marked by loss of immune regulation, CD11a expression on DCs is consistently lower compared to healthy controls. Comparing RA synovial fluid to matched peripheral blood shows even lower CD11a expression in the joint. CD11b expression, on the other hand, is lower on tolerogenic Mo-DCs compared to mature Mo-DCs. Blocking CD11b on Mo-DCs reduces their ability to induce T cell proliferation. Furthermore, CD11b expression is higher in synovial fluid DCs, pointing to a potential pro-inflammatory role in the inflamed joint.

Conclusion: Our results suggest that β_2 integrin subunits CD11a and CD11b have opposing roles in human DCs, with CD11a being immunoregulatory, while CD11b might have a pro-inflammatory role. Further functional studies on Mo-DCs and blood-derived DCs are currently under way using agonists and antagonists to fully define the complex interplay between the different β_2 integrins subunits in human DCs.

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Non-invasive imaging of monocytes and macrophages during the onset and progression of abdominal aortic aneurysms by combined 1H/19F MRI

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Question: Abdominal aortic aneurysms (AAA) are a common disease associated with high mortality. Reasons for the formation/progression of AAAs are still elusive, but there is strong evidence that inflammation associated with accumulation of monocytes and macrophages is of crucial importance. Our group has recently utilized perfluorocarbon nanoemulsions (PFC) which are taken up by monocytes and macrophages for detection of inflammation by combined $^{1}H/^{19}F$ MRI [1]. In the present study we aimed to apply this approach to monitor vascular inflammation and AAA-formation in angiotensin II-treated apoE deficient mice.

Methods: ApoE-/- mice were treated with angiotensin II via osmotic minipumps and monitored longitudinally by $^{1}H/^{19}F$ MRI. PFCs were injected immediately and on day 2 and 4 after minipump implantation and $^{1}H/^{19}F$ MRI datasets were acquired on day 2, 4, 7, and on day 10. Flow cytometry and high resolution $^{1}H/^{19}F$ MRI of excised abdominal aortae were also performed.

Results: ^{1}H MRI revealed that angiotensin II treatment led to the formation of AAAs in about 40% of the mice on day 10. AAAs could be detected by analysis of the outer vascular diameter rather than blood flow which was due to aortic dissection and subsequent thrombus formation. Moreover, we observed that AAAs developed at early (day 0-2), intermediate (days 2-7) and late (days 7-10) time points. To monitor vascular inflammation, we intravenously injected PFCs and subjected mice to $^{1}H/^{19}F$ MRI. As expected, we found strong ^{19}F signals in the area of the aneurysm but in some cases ^{19}F signals were also found in areas distant of the AAAs indicating widespread inflammation along the vessel wall. The localization of ^{19}F signals was verified by high resolution *ex vivo* $^{1}H/^{19}F$ MRI of isolated aortae. Histological analysis confirmed the presence of macrophages in this area and flow cytometry revealed higher numbers of immune cells in isolated aortae of AAA mice.

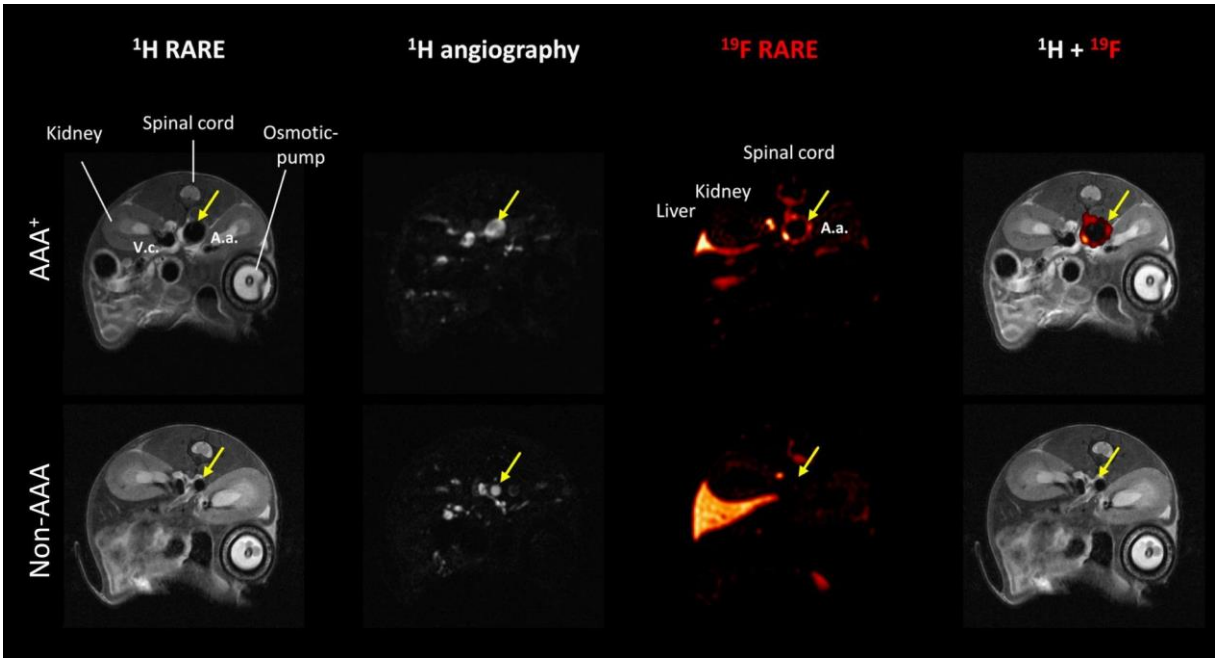
DOI: 10.1002/eji.201970300

Conclusion: PFCs in combination with 1H/19F MRI is suitable to visualize the accumulation of monocytes/macrophages during AAA-formation which may help to further elucidate the pathophysiology of AAAs and to monitor the impact novel therapeutics.

References: [1] Temme, Bönner, Schrader, Flögel. ^{19}F magnetic resonance imaging of endogenous macrophages in inflammation. Wiley Interdiscip Rev Nanomed Nanobiotechnol. 2012

Figure 1: Imaging of vascular inflammation associated with formation of AAAs by ^{1}H/^{19}F MRI

Figure 1



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Ectopic expression of IL-35Ig by dendritic cells induces tolerance via Arginase 1

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Introduction Dendritic cells (DCs) are specialized antigen presenting cells playing a central role in determining the outcome of the immune response, forcing naïve T cells into either activation or differentiation into regulatory T cells (Tregs) depending on DC-extrinsic and DC-intrinsic factors, among which are the cytokine milieu and the immunoregulatory enzymes. Belonging in IL-12 family, IL-35 is a potent suppressive heterodimeric cytokine produced by T and B regulatory cells. Indoleamine 2,3-dioxigenase 1 (IDO1) and Arginase 1 (Arg1) are metabolic enzymes that, expressed by dendritic cells, contribute to immunoregulation.

Objectives The aim of the study is to explore any possible link between IL-35 and the activity of IDO1 and Arg1 enzymes expressed by DCs.

Materials & methods We transfected a single chain IL-35Ig gene construct in murine splenic DCs (DC_{35}) and assessed any IDO1 and Arg1 activities as resulting from ectopic IL-35Ig expression, both *in vitro* and *in vivo*. *In vitro*, modulation of *Ido1* and *Arg1* gene expression was evaluated by real-time PCR; *in vivo*, a negative vaccination strategy exploiting peptide-loaded DC_{35} was set up in order to induce antigen-specific tolerance in mice subsequently challenged with the same peptide in a delayed-type hypersensitivity (DTH) experiment. In all the experiments, DCs transfected with Ig tail (DC_{Ig}) were used as a control of DC_{35}.

Results Unlike *Ido1*, *Arg1* expression was induced *in vitro* in DC_{35}, and it conferred an immunosuppressive phenotype on those cells, as revealed by a DTH assay. In particular, the loss of IDO1 function in DC_{35} ($Ido^{-/-}$ DC_{35}) did not modify

DOI: 10.1002/eji.201970300

the unresponsiveness to skin test following wt DC_{35} administration. On the contrary, Arg1 inhibition in DC_{35} by the specific catalytic inhibitor nor-NOHA reverted the suppressive response to skin test observed with untreated DC_{35}. Moreover, the *in vivo* onset of a tolerogenic phenotype in DC_{35} was associated with the detection of $CD25^+CD39^+$, rather than $Foxp3^+$, regulatory T cells.

Conclusion *Arg1*, but not *Ido1*, expression in DC_{35} appears to be an early event responsible for IL-35Ig–mediated immunosuppression observed in DC_{35}-treated mice.

P325

Ablation of OTUD7B in dendritic cells confers protection against ECM

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Introduction: Cerebral Malaria (CM) is a severe neurological complication of human malaria caused by the parasite *Plasmodium falciparum*. Studies using experimental cerebral malaria (ECM), a murine disease model of human cerebral malaria, have shown that dendritic cells (DCs) play an important role in induction of cerebral malaria and are essential for priming of parasite specific $CD8^+$ T cells, which mediate the disruption of blood brain barrier and cause disease pathology. Cezanne, also known as OTUD7B, is a deubiquitinating enzyme which removes K11, K33, K48, and K63 linked polyubiquitin chains from target proteins, thereby regulating various cellular functions. A recent study has shown that OTUD7B is essential for conferring protection against intracellular bacterial pathogen, *Listeria monocytogenesis*. However the impact of OTUD7B on the course of ECM remains unknown.

Objectives: To study the DC-specific function of OTUD7B in ECM and to identify the molecular mechanism by which OTUD7B regulates DC function and thereby, the outcome of the disease.

Materials and methods: To study the DC-specific function of OTUD7B in ECM, we generated conditional knockout mice in which OTUD7B is specifically deleted in DCs (CD11c-Cre *Otud7b*$^{fl/fl}$). Flow cytometry analysis was done to study the immune cell population in spleen and brain of infected mice. Ex-vivo analysis of cell death was performed using annexin V and 7AAD staining. To identify the signalling pathways regulated by OTUD7B, proteins were isolated from TNF stimulated bone marrow derived dendritic cells (BMDCs) and analysed by western blot.

Results: Upon *P.berghei* ANKA (*Pb*A) infection, CD11c-Cre *Otud7b*$^{fl/fl}$ mice were protected from ECM. Flow cytometry analysis showed a decrease in numbers of splenic DCs and $CD8^+$ T cell population and concomitantly reduced accumulation of pathogenic $CD8^+$ T cells in the brain of CD11c-Cre *Otud7b*$^{fl/fl}$ mice. Furthermore, OTUD7B deficient DCs were more susceptible to infection-induced cell death as assessed ex vivo by Annexin V and 7AAD staining. Western blot analysis from TNF stimulated BMDCs showed increased expression of phospho-MLKL and Cleaved Caspase 3 in OTUD7B deficient BMDCs at 6hrs and 24hrs respectively, which indicate both necroptotic and apoptotic form of cell death.

Discussion: Collectively, our data shows that OTUD7B induces DC cell death and thereby impairs the intracerebral accumulation of disease causing $CD8^+$ T cells, thus protecting the mice from ECM.

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Deciphering the role of dendritic cell subsets in acute kidney injury

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Introduction: Mononuclear phagocytes (MPs) are highly phagocytic immune sentinels that include dendritic cells (DCs), monocytes and macrophages. The origin of kidney MPs has been highly debated because of the large phenotypic overlap of macrophages and DCs in this tissue. The kidney contains at least four subsets of MPs with prominent *Clec9a*-expression history, indicative of dendritic cell origin. These include the two main subsets of conventional DCs, as well as a fraction of cells marked by the monocyte/macrophage marker CD64, which can be

DOI: 10.1002/eji.201970300

further divided in F4/80hiand CD11bhicells. During acute kidney injury (AKI) DCs and other MPs promote kidney damage through the production of pro-inflammatory cytokines, but they also promote tissue repair. However, the role of specific MP subsets in promoting tissue damage and repair has not been investigated.

Objectives: We aim to decipher the identity of MP subsets in the kidney and to clarify their role in acute kidney injury.

Materials & methods: We use fate mappingof MP subtypes, single cell and bulk RNA sequencing to define the origin and transcriptional properties of renal MP subtypes. Inducible and constitutive depletion of DC subsets coupled to functional analysis, microscopy and Histo-Cytometry is used to define the roles of DCs in cisplatin induced AKI.

Results: We demonstrate that in steady state kidney cDC1, cDC2, F4/80hiand CD11bhicells are phenotypically and transcriptionally distinct from each other. In response to stimulation with TLR ligands these populations produce distinct cytokines, indicating that they may respond different to pathogenic stimuli. During cisplatin induced acute kidney injury cDC1 and cDC2 are strongly reduced in kidney from WT mice. Interestingly, F4/80hicells respond to inflammation by downregulating MHCII. Confocal microscopy of kidney sections from cisplatin treated mice revealed that F4/80hicells localize to damaged areas in the kidney, independent of expressing MHCII.

Conclusion: The kidney harbors at least four distinct MP of DC origin that are phenotypically, functionally and transcriptionally distinct from each other. Of these, F4/80hicells adapt to cisplatin induced inflammation by downregulating MHCII in the later stages of AKI, indicating that they may contribute to tissue repair. Future studies will assess the role of DC subsets in AKI by cell depletion, analyzing their localization in the inflamed tissue and by assessing the production of inflammatory cytokines.

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Circulating granulocytes of newborn CFTR$^{-/-}$ piglets have a reduced oxygen-dependent microbicidal activity after bacterial stimulation

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Introduction One of the essential pathways of host defense against bacterial infections in the airways are phagocytosis and oxygen-dependent intracellular killing of pathogens, but in the case of Cystic Fibrosis (CF) these are impaired. It is unclear, however, if this is caused directly by the defective Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) or whether it is only a secondary consequence of chronic inflammation.

Objectives We examined phagocytic potential and oxidative burst of circulating granulocytes from newborn CFTR$^{-/-}$ piglets (first day of life), because at that time point there are not yet any signs of infection nor inflammation in CF.

Material & methods Using flow cytometry, whole blood samples were stimulated with opsonized E. coli, either labelled with fluorescein isothiocyanate to detect their phagocytic uptake or unlabelled to investigate production of reactive oxygen species (ROS) with dihydrorhodamine-123 as a fluorescent substrate.

Results In CFTR$^{-/-}$ piglets there was no difference in frequency of phagocytic and ROS generating granulocytes in comparison to wild-type littermate piglets, however their phagocytic potential and furthermore their oxidative burst activity was significantly reduced as identified by a decreased median fluorescence intensity of fluorescent E. coli and rhodamine-123. We also tested isolated granulocytes for their ROS production capacity following stimulation with phorbol-12-myristate-13-acetate. This experimental set-up allowed us to directly investigate ROS-production, circumventing cellular crosstalk, deficient pathogen recognition or phagocytic uptake as potential reasons for reduced oxidative burst activity observed in whole blood cultures. Indeed, also in these experiments a reduced ROS production was found in granulocytes isolated from CFTR$^{-/-}$ piglets.

Conclusion Thus, our results indicate that the lack of CFTR directly leads to intrinsic alterations in oxygen-dependent microbicidal activity of circulating granulocytes.

DOI: 10.1002/eji.201970300

Complement (P328-P349)

P328

Complement factor C3 derived from myeloid cells participates in defense against *Chlamydia psittaci* in mouse lung infection, but systemic complement from non-myeloid cells plays the paramount role.

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Introduction: Complement (**C**) is an early activated part of the immune system. It interacts with other immunological and inflammatory pathways crosslinking innate and adaptive immunity. Avian strains of the zoonotic intracellular bacterium ***Chlamydia psittaci*** (**Cps**) cause severe pneumonia with systemic spread in man. In mouse lung infection, C3 and its biologically active cleavage product C3a with its receptor are needed for an effective defense against Cps. They seem to stimulate protective adaptive cellular immunity (Bode et. al 2012 and Dutow et. al. 2014). Studies on other pathogens indicate the importance of locally produced C. Thereby, immune cells modulate in an autocrine or paracrine fashion (or even within the C producing myeloid cell) essential immunological functions.

Objectives and Materials & Methods: We wanted to clarify the role of C derived from myeloid or non-myeloid cells in (non-avian DC15) Cps lung infection applying adoptive bone marrow (BM) transfer to irradiated C57BL/6J wild-type (WT) and C3-/- mice. Additionally, we examined by RT-qPCR mRNA-levels of C factors and receptors in the mouse lung epithelial (MLE) 12 cell line.

Results: In severe but non-lethal pneumonia, chimeric WT mice receiving BM cells from WT donors recovered almost completely after 1 to 2 weeks of Cps infection, whereas "$C3^{-/-}$ BM in $C3^{-/-}$ recipients" got increasingly worse. Intriguingly, 2 to 3 weeks p.i., both mixed chimeric mice, i.e. "WT-BM in $C3^{-/-}$-recipients" or "$C3^{-/-}$-BM in WT-recipients", showed an intermediate phenotype e.g. in weight loss. However, challenged with a 3-4 times higher dose of Cps, both WT recipients recovered almost completely and transferred BM from WT mice failed to rescue $C3^{-/-}$-recipients. Moreover, mRNA for various C factors and anaphylatoxin receptors could be detected in MLE-12 cells, and, interestingly, expression of C3-mRNA was upregulated by factor 7 in response to Cps infection.

Conclusion: Apparently, both, circulating and locally, by myeloic cells produced C3 are needed for an optimal defense against Cps. However, systemic C derived from non-myeloid cells such as liver or lung epithelium plays a paramount role in more critical situations with a higher bacterial load, when the pathogen most likely also disseminates from lung to other organs.

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Age-dependent complement expression in a mouse model for Stargardt macular degeneration.

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Background: Stargardt macular degeneration (STGD) is an inherited retinal disease caused by mutations in the ATP binding cassette subfamily A member 4 (ABCA4) photoreceptor gene. Impairment in ABCA4 function is associated with fluorescent lipofuscin accumulation in the retinal pigment epithelium (RPE), progressive photoreceptor degeneration, increased oxidative stress and complement activation. The exact role of complement activation in STGD remains unclear. A previous RPE targeting complement modulating gene therapy in one month old mice reduced complement activation, decreased lipofuscin accumulation and slowed down photoreceptor degeneration. In this study, we investigated age-related transcriptional and protein changes in different complement components in the retina and RPE/choroid in an ABCA4-/- mice, a model for STGD.

DOI: 10.1002/eji.201970300

Methods: Six different retinal cell populations were isolated from wild type and *ABCA4-/-* mice. Quantitative RT-PCR, Western blot and immunostaining were performed to determine complement component expression levels. Systemic complement activation was analysed by ELISA and Western blot. Mice"s were aged between two to eleven months old.

Results: We found aging-associated changes in RPE autofluorescence, glial activity and neuronal loss. Next to that, we demonstrated age-related changes in complement expression in the different retinal cell types, which were comparable in both wild type controls and *ABCA4-/-* mice. Strikingly, our data revealed that *ABCA4-/-* mice expressed increased transcript of *c3* in RPE and decreased transcripts of *cfi* in microglia compared to wild type controls. But, C3 and CFI protein levels were decreased in the murine retinae of *ABCA4-/-* mice during aging compared to wild type controls. We also found C3 changes on protein level in the RPE. Those effects could not be shown after analysing C3 and CFI systemically.

Conclusion: The results of this study give a better insight of complement component expression during aging in retinal cell populations. Besides that, we also showed complement activity changes locally in the eye of *ABCA4-/-* mice compare to control. There is still need to understand the role of complement system regulation in retinal cell populations to identify the most promising complement target. Based on the reported data RPE targeting complement modulating gene therapy might be only effective at early stage of Stargardt disease.

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Complement-induced cardiomyopathy during sepsis in mice is mediated via C3a, C5a and its receptors

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Introduction: Myocardial dysfunction is a severe consequence during sepsis in humans and is associated with an increased mortality. During sepsis, the complement-activation products C3a and C5a are released systemically. C5a was recently shown to be cardio-depressive, which is mainly mediated via its receptors (C5aR1/C5aR2), whereas the effects of C3a and its receptor (C3aR) on the heart are unknown. Furthermore, the mechanisms of complement activation, leading to cardiac dysfunction and cardiomyopathy during sepsis are poorly understood.

Objective: The aim of the study was to investigate cardiac function as well as the role of C3aR during sepsis in mice *in vivo*. Furthermore, the effects of recombinant C3a and of recombinant C5a on cardiomyocytes should be investigated *in vitro*.

Materials & Methods: Male C57BL/6 wildtype, C5aR1-/-and C5aR2-/-mice were objected to sepsis by the cecal ligation-and puncture (CLP). Cardiac function was determined by echocardiography and expression of calcium handling proteins as well as of C3aR in heart tissue was determined. Human cardiomyocytes were treated with recombinant C3a and mitochondrial respiration was determined by Seahorse XFe96 analyzer. Primary isolated rat cardiomyocytes were treated with recombinant C5a and calcium signaling of the cells was determined.

Results: Cardiac function was significantly impaired during sepsis in mice *in vivo*, which was attenuated in absence of either C5aR1 or C5aR2. Furthermore, expression of the key homeostasis-regulating proteins Na+/K+-ATPase, sarcoplasmic-/endoplasmic reticulum ATPase (SERCA) and of sodium-calcium exchanger (NCX) was significantly reduced in heart tissue during sepsis. Also, the expression of C3aR was significantly reduced. In presence of C5a, defective current densities were observed in rat cardiomyocytes. Furthermore, the basal respiration and the spare respiratory capacity of mitochondria from human cardiomyocytes were significantly impaired in presence of C3a.

Conclusion: In the present study, we show that activation of the complement system is involved in cardiac dysfunction during sepsis. The complement activation was mediated via C5aR1 and C5aR2. The anaphylatoxin C3a might also play a critical role, showing cardio-depressive effects *in vitro*, contributing to the development of cardiac dysfunction and cardiomyopathy during sepsis. Therefore, complement therapeutics might be a promising tool to improve cardiac function during sepsis.

DOI: 10.1002/eji.201970300

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C3aR participates to the formation of multinucleated alveolar macrophages *in vitro* and in an experimental allergic asthma model

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Introduction: Under strong pro-inflammatory conditions, macrophages are known to form multinucleated giant cells. So far, such cells have rarely been described in allergic asthma, although granulocyte-macrophage colony–stimulating factor (GM-CSF) released during allergen-driven epithelial response and Th2 cytokines are known triggers of cell multinucleation *in vitro*.

Objectives: We aimed to identify multinucleated cells (MuNCs) in experimental allergic asthma models and to delineate the molecular mechanisms sustaining their formation.

Materials & methods: We used intratracheal (i.t.) models of house-dust-mite (HDM)-induced allergic asthma and interleukin (IL)-33-induced airway inflammation, in wildtype (WT) and various reporter and genetically invalidated mice. Furthermore, we established *in vitro* MuNCs assays, using sorted naïve tissue-associated alveolar macrophages (tAMs) stimulated with GM-CSF and IL-33.

Results: We identified MuNCs in lung cell suspension and in histology sections of WT allergic asthmatic mice. Growth factor and cytokines known to play a key role upon allergen exposure, such as GM-CSF or IL-33, were able to trigger the formation of MuNCs in *in vitro c*ulture of tissue-associated alveolar macrophages (tAMs). Furthermore, microscopic observations and cocultures assays using CSFE- and PKH26-labeled tAMs, revealed that MuNCs arised from both fusion and failed division processes. Although tAMs do not express C3aR at steady state, we observed, using a Tandem-dye (Td)Tomato-C3aR reporter mouse, that they upregulated *de novo* C3aR upon repeated HDM or IL-33 exposure. Interestingly, *in vitro*, GM-CSF and IL-33 increased the abundance of *C3ar1* mRNA suggesting that this receptor could be at play during the formation of MuNCs. Finally, *in vitro* experiments showed that absence of C3aR in tAMs resulted in a significant reduction of MuNCs compared to WT.

Conclusion: We evidenced the formation of MuNCs *in vivo* and *in vitro* upon allergic pulmonary inflammatory conditions. Furthermore, we observed that MuNCs formation depends partly on C3aR, which*, in vivo*, is under the regulatory control of IL-33. Although the functions of MuNCs in allergic asthma are still elusive, our data suggest that C3aR activation in tAMs plays an underestimated role in allergic asthma severity via the control of tAM-derived MuNCs formation.

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Binding of the self-surface marker sialic acid by Factor H (FH) and Factor H-related protein 1 (FHR-1) and its impact on deregulation on host-surfaces

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FHR-1 is the most abundant FHR in human plasma and competes with FH on heavily C3b-opsonized surfaces which is termed "deregulation". The molecules compete each other because the last two C-terminal complement control protein (CCP) domains (*i.e.* FH CCP19-20/ FHR-1 CCP4-5) which bind C3b and polyanionic host cell markers are identical apart from the two amino acids (aa): S1191L and V1197A (*i.e.* FH "SV"; FHR-1 "LA"). We investigated the functional impact of this aa exchange to shed light onto the physiological role of FHR-1.

We recombinantly expressed monomeric wildtype FH18-20 (SV) and wildtype FHR-1 (LA) that dimerises via its two N-terminal domains. Additionally, we prepared truncated FH mutations that resemble the C-terminal FHR-1 domains (*i.e.* FH18-20 (SA), FH18-20 (LV), FH18-20 (LA)) and FHR-1 (SV), a dimer in which CCP4-5 correspond to FH CCP18-20.

Heparin chromatography showed that the aa exchange had no influence on glycosaminoglycan binding. Determining the affinity for C3b by surface plasmon resonance (SPR) elucidated that the dimeric constructs bind better than the monomeric versions and that C3b binding is slightly altered by the aa exchange: FH18-20, FH18-20 (SA) bound equally (KD 9.9/10.2 µM) while constructs that included the S-to-L substitution show slightly higher affinity (FH18-20

DOI: 10.1002/eji.201970300

(LV), FH18-20 (LA) (KD 5.6/ 5.8 μM); dimers FHR-1, FHR-1 (SV) (KD 0.6/1.5 μM)). SPR elucidated that the SV configuration is crucial for binding specifically to α2,3 but not α2,6 linked SIA.

In more physiological conditions on human microvascular endothelial cells (*i.e.* self surface) or sheep erythrocytes (*i.e.* self-like surface) we tested whether SIA-binding correlates with the ability to deregulate FH. Constructs that bind SIA (FH18-20, FH18-20 (SA), FHR-1 (SV)) caused dramatic deregulation on self and self-like surfaces while non-SIA-binding constructs still competed with FH but to a substantial lesser degree.

Taken together, our data demonstrates that the C-termini of FH and FHR-1 are similar but the change of "SV" in FH CCP20 to "LA" in FHR-1 CPP5 abolishes SIA-binding to ensure that the deregulation capacity of FHR-1 does not surpass moderate levels that are dangerous for complement homeostasis on self surfaces.

P333

The Role of the C5a/C5a Receptor 2 Axis in Regulating Uterine Natural Killer Cells during Pregnancy

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Introduction: As a special subset of NK cells prominent in the pregnant uterus, the uterine natural killer (uNK) cells play a critical role in placentation and fetal development during the first trimester of pregnancy. Dysregulated uNK cell functions can lead to serious complications such as preeclampsia and ultimately to miscarriage. The main reason for this is that uNK cells are the principal source of IFNγ and angiogenic factors which must be strictly regulated to ensure a correct remodeling of the uterus and vasculature after implantation. Especially high levels of IFNγ have been shown to cause spiral artery dilation and fetal resorption.

Objectives: We have recently shown that a subset of peripheral NK (pNK) cells expresses the complement receptor C5aR2, without the usual co-expression of C5aR1. Functionally, specific activation of C5aR2 on pNK cells suppresses their IFNγ production *in vitro*. We now hypothesized that uNK cells also only express C5aR2 and that this expression is crucially involved in uNK-mediated IFNγ production, thus controlling placental development and a healthy gestation.

Materials & Methods: Murine uteri were removed on gestation day 7 and used for microscopic evaluation, mRNA extraction or flow cytometry. Different human tissues have been analyzed using the same methods. Functional assays have been conducted to understand the purpose of the different NK cell subsets.

Results: Like in the pNK cells, only a subset of uNK cells expressed C5aR2. In C5aR2-/- mice, the levels of IFNγ in the uterine tissue were significantly higher, showing that in uNK cells as well as in pNK cells the expression of C5aR2 regulates the production of IFNγ. This led to more strongly dilated placental arteries when compared to wildtype mice. Consequently, we found a very low breeding efficiency to be associated with the lack of C5aR2, as evidenced by high numbers of resorptions in C5aR2-/- mice during pregnancy.

Conclusion: Our findings identify a potential novel role for the anaphylatoxin receptor C5aR2 in regulating the uNK cell compartment and indirectly its release of angiogenic factors, ultimately influencing the overall outcome of early pregnancy.

P336

Complement Receptor 3 internalizes apoptotic *Leishmania* promastigotes and triggers LC3 associated phagocytosis in primary human macrophages

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The neglected tropical disease leishmaniasis is initiated by a sand fly inoculum comprised of apoptotic and viable *Leishmania* promastigotes. Apoptotic promastigotes downregulate anti-leishmanial responses involving LC3-associated phagocytosis (LAP) enabling viable parasites to cause disease. In this study, we investigated the LAP mechanism in primary human macrophages (hMDM) induced by apoptotic parasites in more detail. First, we used lentivirally transduced LC3-eGFP-expressing primary human pro- (hMDM1) and anti-inflammatory (hMDM2)

DOI: 10.1002/eji.201970300

macrophages and confocal live cell imaging. Video data analyses revealed LC3 recruitment to occur more frequently and more rapidly towards apoptotic *Leishmania*-containing phagosomes than to compartments harboring viable parasites in hMDM2. In addition, we could demonstrate that apoptotic parasites disappear and are probably degraded after LC3 compartment maturation in HMDM2. To investigate this mechanism in more detail, we magnetically isolated phagosomes from hMDM2 containing apoptotic parasites and analyzed their proteome by label-free mass spectrometry. We quantified more than 2000 host cell-derived proteins and one of the most abundant proteins found was Complement Receptor 3 (CR3). CR3 is expressed on hMDM and could be efficiently blocked with a specific monoclonal antibody. Blocking significantly decreased the uptake of apoptotic parasites in both macrophage phenotypes as analyzed by flow cytometry. Moreover, we found that both LC3 conversion as a marker for LAP maturation and IL-10 secretion induced by apoptotic parasites in hMDM2 is in part CR3-dependent.

Our data demonstrate that LAP is a preferred mechanism for internalization of apoptotic *Leishmania* promastigotes by hMDM2. Moreover, we identified CR3 as a new LAP-inducing receptor.

P337

Incomplete C5 inhibition by Eculizumab accounts for impaired clinical responses in Patients with Paroxysmal Nocturnal Hemoglobinuria (PNH)

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Introduction: Eculizumab inhibits the terminal and lytic complement pathway by inhibiting the complement component C5 and offers substantial clinical benefits for patients suffering from paroxysmal nocturnal hemoglobinuria (PNH) and atypical hemolytic uremic syndrome (aHUS). Previously, we have shown that strong complement activation overrides the C5 inhibition by eculizumab or other stoichiometric C5 inhibitors. We have also demonstrated that residual C5 activity can be observed ex vivo in serum form PNH patients despite of excess amounts of eculizumab.

Methods: We investigate if the ex vivo levels of residual C5 activity (determined in assays of alternative pathway mediated hemolysis of rabbit erythrocytes) in patient sera differ across different PNH patients on eculizumab treatment and whether any differences in residual lytic activity correlate with the clinical responses. We also investigate if clinical signs of strong complement activation in vivo coincide with intravascular hemolysis.

Results: Here we show that the levels of residual C5 activity in ex vivo assays differ markedly (up to 3.4-fold) across sera collected from different PNH patients on eculizumab. This wide variability of residual activities was also found in sera of healthy donors cross-validating the findings in patients. While PNH patients with residual lytic activities of 11-30% exhibited hemolysis levels around the upper limit of normal (i.e. plasma LDH of 250 u/L) as expected for PNH patients on Eculizumab therapy, we found sustained and markedly increased LDH levels of around 400 u/l for the patient with the highest residual C5 activity (37.4%) over the entire observation period of 15 months. This demonstrates that higher residual C5 activity is associated with an incomplete clinical response. Furthermore, the clinical history of nine PNH patients shows intravascular breakthrough hemolysis at the time of documented infections despite of excess amount of eculizumab over C5.

Conclusion: The occurrence of very high levels of residual C5 activity in PNH patients receiving eculizumab is rare, but can impair the suppression of hemolysis. The commonly observed low levels of residual C5 activity seen for most PNH patients can exacerbate during severe infections and thus can cause pharmacodynamic breakthrough hemolysis in eculizumab treated PNH patients. Understanding of this mechanism is important for further optimization of complement inhibitor therapy.

P338

Complement activation as key regulatory mechanism in the pathophysiology of pemphigoid diseases

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DOI: 10.1002/eji.201970300

Pemphigoid diseases (PD) are a heterogeneous group of prototypic organ-specific autoimmune disorders affecting the skin and mucosal tissues. The most frequent PD is bullous pemphigoid (BP) followed by mucous membrane pemphigoid (MMP), while epidermolysis bullosa acquisita (EBA) is the by far rarest entity with an incidence below 1/ million/ year. We have previously developed several mouse models that replicate major immunopathological and clinical characteristics of the human diseases by the injection of rabbit IgG generated against the respective murine target antigens, BP180, laminin 332, and type VII collagen or by immunization of susceptible mice with these proteins. We have recently shown that in all three models, complement is important for formation of skin and mucosae, however, to a different extent. More specifically, in C5aR1-deficient mice, we found clinical disease to be greatly reduced after injection of anti-type VII collagen or anti-laminin 332 IgG, respectively, while in the BP model, C5aR1-deficient mice were significantly less diseased, however, not completely protected compared to wild type animals. Of note, C5aR2-deficient mice were developed significantly more skin lesions after injection of anti-BP180 IgG compared to wildtype animals suggesting an anti-inflammatory role of C5aR2 in murine BP. More recently, we observed no differences in IgG deposition and extent of lesions in both the BP and EBA model between C3-deficient and wild type mice. These data point to a C3-independent activation of C5. Currently, we are pinpointing the cellular sources of C5aR1 and C5aR2 in patients with BP and mouse models of BP and MMP. Furthermore, we will clarify the role of C5a generation using different knock out mice and pharmacological inhibitors.

P339

Shiga Toxin 2a binds to complement components C3b and C5 and upregulates their gene expression in human cell lines

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Question: Complement is a well characterized component of the innate immunity and plays a crucial role in detection and elimination of invading pathogens. Infection with Enterohemorrhagic *Escherichia coli* (EHEC) is a major cause of EHEC-associated Haemolytic Uraemic Syndrome (eHUS) which may progress via uncontrolled activation of complement. Shiga toxin 2a (Stx2a), one of the most potent virulence factors of EHEC, activates complement via the alternative pathway and binds to factor H (fH). Although substantial advancements have been achieved in decoding the disease progression, the role of complement in both gut and blood has only recently begun to be explored. The aim of this study is to investigate the interaction of Stx2a with the pivotal complement proteins C3 and C5 and also whether Stx2a stimulation influenced transcription and synthesis of complement proteins in various cell lines.

Methods: Enzyme-linked immunosorbent assays (ELISAs) were performed to evaluate the binding of Stx2a to the major part of C3, C3b, and C5. An in vitro cytotoxicity assay was performed to evaluate the Stx2a concentrations and time points at which the HK-2, HCT-8 and CiGEnC cells were still resistant to the cytotoxic effects of Stx2a. Further, cells were stimulated for a suitable time point within this period with varied concentrations of Stx2a and reverse transcription-quantitative PCR was employed to analyse the transcription of these complement components in the cell lines.

Results: Stx2a showed dose dependent binding to C3b and C5 when tested by ELISA. Cytotoxicity assay revealed that HCT-8 cells were significantly more resistant towards the cytotoxic activity of Stx2a than CiGEnC and HK-2 cells. RT-qPCR analyses revealed an upregulation of both C3 and C5 transcription with increasing concentrations of Stx2a in HK-2, HCT-8 and CiGEnC cell lines, being most profound in the latter.

Conclusion: STX2a interacts with C3b and C5 and stimulates expression of C3 and C5 in gut as well as kidney cell lines, providing further evidence to the notion that the complement system is involved in the pathophysiology of eHUS. Whether these findings are advantageous or disadvantageous from a host protection perspective remains to be elucidated.

P340

C5a receptor 2 expression controls NK cell cytotoxicity towards tumor cells by regulation of NKp46 surface levels

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DOI: 10.1002/eji.201970300

Objectives: C5a receptor 2 (C5aR2) was initially considered a decoy receptor. This view has been challenged by findings demonstrating that C5aR2 activation induces pro- and anti-inflammatory functions. However, the functional properties of C5aR2 are still enigmatic. Recently, we reported the expression of C5aR2 on murine natural killer (NK) cells, surprisingly without the presence of C5aR1, which is usually co-expressed with C5aR2. We have already shown that specific activation of C5aR2 suppressed IL-12/IL-18-driven production of IFN-γ from NK cells. Another essential NK cell effector function besides IFN-γ production is their cytotoxic activity towards metastatic tumor cells. Especially activating NK cell receptors play an important role in tumor surveillance. An essential member of these receptors is NKp46. We showed in unpublished data that the absence of C5aR2 leads to a strongly increased NKp46 expression in NK cells. This raises the proposition that C5aR2 expression in NK cells negatively regulates NKp46 surface levels and might therefore also be involved in modulating NK cell cytotoxic activity.

Methods: To define the role of C5aR2 in NK cell cytotoxicity, we co-cultured wild type and C5aR2-deficient NK cells with two different tumor cell lines in presence or absence of recombinant C5a. Cytotoxicity was measured via flow cytometric assessment of the degranulation marker CD107a on the NK cell surface and determination of LDH release from lysed target cells.

Results: Mechanistically, we found that C5aR2-deficient NK cells feature a more robust NKp46-dependent cytotoxic activity towards tumor cells than wild type NK cells, that increases in a time-dependent manner.

Conclusion: Our findings demonstrate for the first time C5aR2-dependent regulation of NK cell cytotoxicity as an important effector function via the modulation of the expression of the activating NK cell receptor NKp46. This finding suggests a critical role for C5aR2 in combating metastatic tumor cells by NK cells and thereby constituting a novel therapeutic target in cancer treatment.

P341

Oxidative stress induces complement-dependent pro-angiogenic and -inflammatory response in retinal pigmented epithelium cells

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Oxidative stress in the aging eye is associated with local inflammatory processes. In this study, we investigated the mechanisms of H2O2-induced, complement-dependent inflammatory processes in a human retinal pigment epithelial cell line, ARPE-19.

ARPE-19 viability and integrity were analysed. The expression of components associated with the complement system was determined by qRT-PCR, Western blot, immunofluorescence analyses and secretion of cytokines/ growth factors was measured.

We found that more than 95% of ARPE-19 treated with oxidative stress were but showed disrupted cell-cell junctions. Strikingly, senescent ARPE-19 cells upregulated complement receptors on transcript (*c5ar1*, *cr3*) and protein level (C5aR1) in response to H2O2. This gene modulation was associated with priming of the NLRP3 inflammasome, enhanced secretion of pro-angiogenic factors (VEGF, IL-8) and pro-inflammatory cytokine (IL-6). In the late stage of oxidative stress treatment, we observed significant more transcripts of intracellular proteases (*ctsl*, *ctsb*, *serping1*) and of the complement stabilizer (*cfp*) than in untreated controls. In contrast to previously described protective effects of Olaparib in oxidatively stressed retinae, we observed a more pronounced change towards pro-inflammation in Olaparib-treated than in untreated ARPE-19 cells.

Our results show for the first time a functional link between oxidative stress, complement receptors, pro-angiogenic and -inflammatory responses of ARPE-19 cells. These effects suggested an oxidative stress-associated mechanism of C5aR1-regulation in ARPE-19 cells in connection with upregulated intracellular proteases.

P342

Generation of previously undefined Complement C5 and C3 fragments in jejunum and colon mucus after experimental sepsis as a putative effect of increased proteolytic activity

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DOI: 10.1002/eji.201970300

Introduction- The mucosal immune system is under a tightly regulated environment comprising of the commensal bacterial milieu, the slimy mucosal polysaccharides and constant surveillance by immune cells (Haussner et al., 2019). Like chronic inflammatory responses (Sunderhauf et al., 2017), systemic inflammatory responses (SIRS) have been implicated to disrupt this balance, though the very causes and mechanisms undertaken are not well-defined.

Objectives- In our study, we looked into the content of mucus obtained from a) jejunum and b) colon in an experimental model of sepsis (SIRS with a pathogenic primary cause).

Materials & methods - C57Bl6 male mice aged 8-12 weeks were randomly grouped into two groups viz., sham and cecal ligation puncture (CLP). The study was terminated after 24 hours and the mice were sacrificed. Mucus collected from jejunum and colon of the mice were first processed to obtain its soluble fraction by centrifugation and then tested by ELISA and western blot for complement factors and with fluoro-/chromogenic substrate experiments for protease content.

Results- In jejunum mucus, out of various complement factors tested, activated complement products C3a, C5a and Factor H were found to be decreased in CLP mice compared to the sham mice, though similar trends were not observed in colon mucus in the first instance. However, further testing the mucus soluble fraction from small and large bowel, we found various C5 and C3 fragments with previously undefined molecular weights. This led us to test at first, the jejunum mucus soluble fractions (devoid of any added protease inhibitors) for a global protease activity measurement with a fluorescently labeled casein substrate and then chromogenic substrates for specific pancreatic enzymes like chymotrypsin and elastase. An increased total protease content, trypsin, chymotrypsin and elastase activity was found in CLP mice compared to sham mice.

Conclusion- 24 hours after sepsis, a marked change is seen in the composition of the mucus of the small and large intestine, in terms of indiscriminately cleaved complement products. This could be associated with heightened proteolytic activity in the intestinal lumen after sepsis.

1) Haussner et al. 2019. Front. Immunol.

2) Sunderhauf et al. 2017. Mol Immunol.

P343

Deficiency of the anaphylatoxin receptors C5aR2 and C3aR aggravates hypertensive renal injury.

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Question: The complement is an important part of the immune system, which drives the host defense against microbes and mediates inflammatory responses. Recent experimental data support a role for complement in arterial hypertension. Hypertension and hypertensive end organ damage are not only mediated by hemodynamic injury but also by innate and adaptive immune responses. In line with this view, complement activation may drive the pathology of this through its impact on innate and adaptive immune responses.

During the activation and amplification of the complement cascade, the anaphylatoxins C3a and C5a are released and trigger a pro inflammatory signaling via their corresponding receptors. We recently described that C5a receptor 1 (C5aR1) deficiency ameliorated hypertensive renal injury. However, the role of the second C5a receptor C5aR2 and the C3a receptor (C3aR) in hypertensive end organ damage remain unclear.

Methods: Expression of C5aR2 and C3aR on infiltrating and resident renal cells were determined using tandem tomato knock in mice for either C3aR or C5aR2 by flow cytometry and confocal microscopy. The hypertension model of angiotensin II (Ang II) infusion in combination with unilateral nephrectomy and high salt diet was induced in Balb/c wildtype and C5aR2 as well as C3aR deficient mice. The glomerular filtration rate, albuminuria and morphological damages were determined.

DOI: 10.1002/eji.201970300

Results: Flow cytometric analysis of leukocytes isolated from the kidney of reporter mice showed that C5aR2 is expressed on dendritic cells, macrophages and neutrophils whereas dendritic cells are the major C3aR-expressing population (90%). Using confocal microscopy C5aR2 and C3aR were detected in the kidney only on infiltrating cells but not on resident renal cells. Ang II infusion induced in both knockout mice a significant higher severity of renal injury (albuminuria, glomerular filtration rate, glomerular injury) compared to hypertensive wild type mice. Additionally, the C3aR deficient mice showed a significant higher mortality as well as bleeding compared to hypertensive wild type and the C5aR2 mice.

Conclusion: In summary, our data reveal that C5aR2 and C3aR are expressed on infiltrating cells (mainly dendritic cells) but not on resident cells in the kidney. The deficiency of C5aR2 as well as C3aR increases hypertensive injury in arterial hypertension. This suggests that C5aR2 and C3aR are protective in hypertensive renal injury.

P344

Immunological and complement mediated cardiac damage after asphyxia and hemorrhage in newborn piglets

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Introduction: Asphyxia is a frequent challenge of the peri- and postnatal periods, occurring in approximately 2 of 1000 births and may be associated with severe fetal hemorrhage. In particular, the cardiac consequences of asphyxia are crucial, because cardiac dysfunction and hemodynamic failure may lead to prolonged organ hypoperfusion.

Objectives: The purpose of this study was to evaluate the systemic and cardiac complement activation after asphyxia and hemorrhage (AH) in newborn pigs and the consequences of presence of C5a or C3a on human cardiomyocytes in vitro.

Materials & methods: 15 neonatal piglets underwent AH, followed by resuscitation with volume expansion with either blood or crystalloid. Four hours after return of spontaneous circulation (ROSC), the animals were euthanized and heart tissue and blood samples were collected. We studied the activity of the classical pathway of the complement system by CH-50 method 4 hours after ROSC. Furthermore, we measured local complement factor 5a (C5a) and C5a receptor 1 (C5aR1) in samples of the left ventricle of both treatment groups. Additionally, we cultured human cardiomyocytes (CM) *in vitro* in presence or absence of C5a or C3a and investigated changes in mitochondrial respiration and calcium handling of CM.

Results: Following AH, we observed a significant reduction of the C5aR1 in the left ventricles and a massive local increase of C5a in all treated animals compared to sham animals. The systemic complement activity measured by CH-50 was slightly reduced in the animals with early blood re-transfusion. *In vitro* analysis showed an internalization of C5a in human cardiomyocytes. Furthermore, reduced mitochondrial respiration capacity (basal and spare respiratory capacity) in presence of C5a or C3a was determined. Further, we observed bradycardia of CM in presence of C5a and C3a.

Conclusion: AH leads to systemic activation of the classical complement pathway and cardiac increase of C5a in newborn pigs, which were linked to alterations of the metabolism and functionality of the piglet heart. Therefore, complement therapeutics might be a promising tool to evaluate cardiac dysfunction after AH in newborns.

P345

Properdin-dependent local complement activity of stressed primary human retinal pigment epithelium cells

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Introduction & Objectives: Single nucleotide polymorphisms (SNPs) within genes of the complement system were found to be associated with retinal degeneration (RD). So far, retinal complement activity has been related to systemic

DOI: 10.1002/eji.201970300

complement components and to retinal microglia/macrophages, but human clinical trials targeting the complement system in RD mainly failed. Here, we aim to shed light on the local, retinal pigment epithelium (RPE)-specific complement system and elucidate the immunomodulatory function under stress conditions.

Materials & Methods: RPE cells, isolated and cultivated from different human donor eyes, were apically treated with properdin (FP) under oxidative stress conditions. The expression of complement components and inflammation-associated signaling factors was analyzed in genotyped RPE cells using qPCR, Western Blot and Multiplex−ELISA.

Results: In five of sixteen tested different primary human RPE cell cultures, we identified homogeneous SNPs in genes of the complement system, which were previously associated with an increased risk for age-related macular degeneration. These SNPs were mainly detected in the *CFH*, *C2/CFB* and *CFI* genes.

Treatment of the primary RPE cultures with the positive complement regulator FP under oxidative stress conditions resulted in genotype-dependent mRNA expression changes. We found mainly downregulation in *C1Q*, *C3*, *C3AR1*, *CFP* and *IL-1β* transcripts, upregulation in *CFD* mRNA and mixed expression pattern for *CFB, CFH, C5AR1, CR3* and *FOXP3*.

Strikingly, *CFI* transcripts were up-regulated and *NLRP3* transcripts were down-regulated in all stressed and FP-treated RPE cells, independent of the complement genotype.

In contrast, we detected an increased secretion of C5 and C5a, and a reduced protein secretion of CFI and CFH in supernatants of all FP-supplemented cells compared to untreated cells under oxidative stress. RPE cells positive for three AMD-associated *CFH*-SNPs resulted in increased cell-associated C3, C3a and C5a concentrations and reduced CFI protein expression, after FP treatment, compared to RPE cells with a non-AMD risk genotype.

Conclusion: In conclusion, our data unveiled a local complement activity in cultured primary human RPE cells independent from the systemic complement system. Properdin treatment resulted in an anti-inflammatory mRNA expression pattern in RPE cells, which could subsequently balance the early FP-derived pro-inflammatory protein detection on stressed RPE cells.

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Crucial role of the terminal complement complex in regulated cell death after cartilage trauma

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The innate immune response and particularly terminal complement complex (TCC, C5b-9) deposition is thought to be involved in the pathogenesis of osteoarthritic disease. However, the underlying mechanism of TCC signaling and primarily its possible role in regulating apoptotic (non-inflammatory) or necroptotic (pro-inflammatory) cell death has not been unraveled so far and was first addressed in the following study.

Human cartilage was obtained with consent of donors undergoing total knee joint replacement (n=18). Cartilage explants were prepared from macroscopic intact cartilage, subjected to a blunt impact (0.59 J) by a drop-tower and exposed to complement competent or heat inactivated human serum (HS; 10-30%) with/ without mechanically homogenized cartilage (HG) for 4 d. Additionally, different therapeutics were tested: RIP-Kinase (RIPK) 1-inhibitor Necrostatin-1 (Nec, 40 µM), pan-caspase (CASP) inhibitor zVAD (20 µM), antioxidant N-acetyl cysteine (NAC, 2 mM) as well as TCC-inhibitors aurintricarboxylic acid (ATA, 75 µg/mL) and clusterin (CLU, 30 µg/mL). Unimpacted explants served as controls. Besides cell viability (live dead staining), gene expression of apoptosis- (CASP 3/ 8) and necroptosis-markers (RIPK1/ 3, MLKL) as well as CD59 were determined. For statistical analysis One-way ANOVA was used.

After trauma, cell viability was reduced by 20.8 % ($p \leq 0.0001$) and further decreased by HS in a concentration-dependent manner ([T+30% HS vs T]: -7.6%, $p = 0.048$). Concurrently, gene expression of CD59, RIPK3 and MLKL was significantly enhanced. Addition of HG potentiated the cytotoxic effect of HS as demonstrated in the live dead assay ([vs T] -14%, $p = 0.0002$) and the mRNA levels of necroptosis- and apoptosis-associated markers. Interestingly, these effects required a preceding blunt trauma and were not observed in unimpacted tissue. Both heat inactivation as well as treatments with Nec, zVAD, NAC, ATA or CLU significantly alleviated cytotoxicity of the HS.

DOI: 10.1002/eji.201970300

Overall, it could be proven that the TCC plays a crucial role in regulated cell death after single impact cartilage trauma, which could be circumvent by inhibition of TCC formation or interference with subsequent death signaling, apparently comprising both apoptotic and necroptotic characteristics. Modulation of regulated cell death might be a promising target in preventing posttraumatic cartilage destruction, though further research is needed to specify the TCC-induced pathway.

P348

Molecular mechanisms of better renal regeneration after severe acute kidney injury (AKI) in C5aR2 deficient mice

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Introduction and Aims: Severe acute kidney injury (AKI)has been linked to progressive renal fibrosis. Here,we investigated C5aR1 and C5aR2-/-mice in model of ischemia induced AKI and monitored pathophysiological changes by functional MRI. Molecular mechanisms of C5aR signalling were addressed by high content antibody array.

Methods: Unilateral ischemia reperfusion injury (IRI) of45 min was done in C5aR1, C5aR2 -/- and wild type (WT) mice, end points for tissue retrieval wereat day 1, 7 and 21. C5aR1 and C5aR2 expression was investigated by qPCR. Renal morphology, inflammation, regenerationand renal fibrosis were analyzed by immunohistochemistry and qPCR after IRI. High content antibody array was done at day 1 after IRI. In vitro studies for IL-10 and HO-1 expression were performed with bone marrow derived macrophages (BMDM) after LPS stimulation.

Results: C5aR1 and C5aR2 mRNA was up-regulated at d1, 7 and 21 in WT mice after IRI which showed severe ongoing inflammation and progressive renal fibrosis at d21. C5aR2-/- IRI kidneys developed less fibrosis and showed restauration of the initially impaired renal perfusion from day 7 onwards with enhanced patency of the peritubular capillaries and less myeloid cell infiltration. Tubular proliferation (Ki-67+ cells) was also improved. By high content antibody array revealed enhanced AKT, pAKT and pFGF1 expression in C5aR2-/- IRI kidneys at day1. For validation of the findings western blots were done. Furthermore, enhanced anti-inflammatory IL-10 and HO-1 expression was identified in proximal tubuli and in bone marrow derived macrophages (BMDM) upon stimulation with LPS.

Conclusions: The anaphylatoxin receptors C5aR1 and C5aR2 have distinct roles in mediating AKI. C5aR2 deficiency could be linked to anti-inflammatory macrophage phenotypes via enhanced IL-10 and consecutive HO-1 expression. Clinically, this contributed to enhanced regeneration after IRI and resulted in less renal fibrosis. C5aR2 targeted therapies might be promising in the prevention of IRI induced chronic kidney disease.Further studies on unravelling the distinct roles of the two C5a receptors are needed.

P349

Study of the prognostic significance of the protein C1q in carcinomas

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DOI: 10.1002/eji.201970300

Question. C1q is the first subcomponent of the classical pathway of the complement system, has been shown to perform a diverse range of immune and non-immune functions in a complement dependent as well as complement-independent manner. As a charge pattern recognition molecule of the innate immunity, C1q can recognise a number of self, non-self and altered-self ligands and bring about effector mechanisms designed to clear pathogens and trigger inflammatory response. C1q can be locally synthesised by macrophages and dendritic cells, and thus can get involved in biological processes such as angiogenesis and tissue remodelling, immune modulation, synaptic pruning, and immunologic tolerance. The notion of C1q involvement in the pathogenesis of cancer is still evolving. C1q has been shown to have a dual role in cancer: tumour promoting as well as tumour-protective, depending on the context of the disease studied.

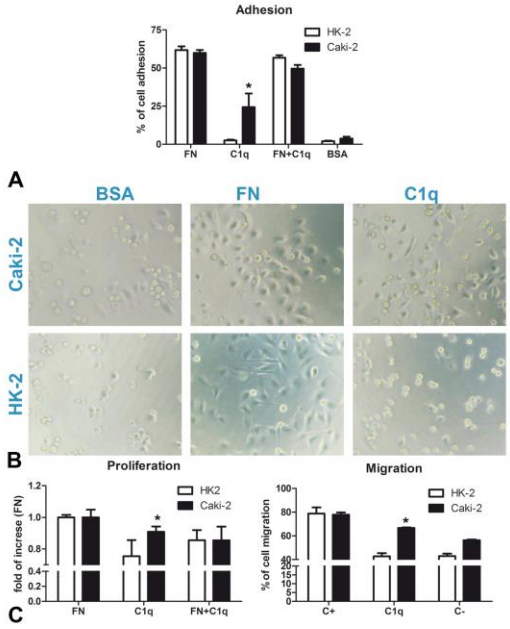
Methods. In the current study, we performed a bioinformatics analysis in order to investigate whether C1q can serve as a potential prognostic marker for human carcinoma. We used the Oncomine database analysis and the survival analysis platforms Kaplan-Meier plotter.

Results. Our results showed that high levels of C1q have a favourable prognostic index in basal-like breast cancer for disease-free survival, and in HER2-positive breast cancer for overall survival, while it showed a pro-tumorigenic role of C1q in lung adenocarcinoma, and in clear cell renal cell carcinoma. Thus, we confirmed via *in vitro* studies the differential capability of cancer cells to interact with C1q, and modulate cell adhesion and migration (figure 1).

Conlusions. This *in silico* study, if validated via a retrospective study at the protein level, can be a step forward in establishing C1q as a new tool as a prognostic biomarker for various carcinoma.

Figure . Effect of C1q on clear cell renal cell carcinoma CCRCC cells adhesion, migration and proliferation. Caki-2 cells **(A)** and HK-2 as negative control cells, were labeled with the fluorescent dye FAST DiI and allowed to adhere to microtiter wells pre-coated with fibronectin, C1q, fibronectin-bound-C1q, or BSA. **(B)** Morphological aspect of one representative Caki-2 and HK-2 cells adhered to fibronectin, C1q, or BSA. **(C)** Effects of C1q on Caki-2 cell migration, and proliferation **(C, D). (C)** Caki-2 cell proliferation. **(D)** FAST DiI-labeled cells were allowed to migrate through a trans-well system using C1q as chemotactic stimulus.

Figure 1



DOI: 10.1002/eji.201970300

Immunotherapy (P350-P374, P399, P400, P404)

P350
Decoding the Human Immune System to Accelerate Next Generation Vaccine and Immunotherapy Development
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Introduction: The old paradigm for R&D is not working against complex infectious diseases and non-communicable diseases of the 21st century. While vaccines have been one of the most effective public health interventions over the last century, during the last 15-years there have been several failures in late stage vaccine and immunotherapeutic development programs against viral, bacterial, parasitic and neoplastic diseases. These failures are due in large part to the lack of understanding of the human immune system and reliance on empiric strategies for vaccine and immunotherapeutic development that are unlikely to be effective in future.

Objective: The Human Vaccines Project (HVP; www.humanvaccinesproject.org) is a non-profit public-private partnership established to accelerate development of next generation vaccines and immunotherapies for major global infectious and neoplastic diseases, by decoding the human immune system. HVP has created a unique model of engaging multinational pharmaceutical partners with key stakeholders from academia, governments and non-governmental organizations, into a human immunology- based, milestone driven, global research consortium. HVP aims to elucidate the "rules of immunity" in humans by conducting strategic, small, and comprehensive exploratory clinical research trials of licensed and experimental vaccines and immunotherapies, to systematically solve the key problems impeding next-generation product development. In this context, HVP aims to discern predictive signatures by systems biology studies of responders vs. non-responders to vaccines and immunotherapies, coupled with frontier super-computing, artificial intelligence and machine learning. In its 3-year startup phase, HVP has already demonstrated the feasibility of its mission, identified preliminary predictive signatures in healthy subjects immunized with Hepatitis B vaccine which can be validated in prospective studies, undertaken the most comprehensive assessment of the B cell and T cell receptor repertoires to date, and is now poised to expand to studies of human immunity in autoimmune diseases and cancer.

Results and Conclusion: In this presentation, recent scientific findings from HVP will be reviewed, and plans for engagement of German science in this global effort highlighted.

P351
A robust human immunophenotyping workflow using CyTOF technology coupled with Maxpar Pathsetter, an automated data analysis software: The Maxpar Direct Immune Profiling System
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Immune profiling is the practice of identifying and quantifying immune populations and their features. Performing immune profiling over time is referred to as immune monitoring. Techniques for immune monitoring in blood are commonly applied in translational and clinical research settings to provide phenotypic understanding of immune states prior to and following treatment. The diversity of immune cell populations demands a high-parameter technique to more fully and efficiently quantify these changes. Mass cytometry, which utilizes CyTOF® technology, is a single-cell analysis platform that uses metal-tagged antibodies and can resolve over 50 markers in a single sample tube without the need for compensation. It is an ideal solution for routine enumeration of immune cell populations. However, development of a robust, highly multiplexed assay requires panel optimization as well as standardization of instrument setup and an easy-to-use yet reliable data analysis solution.

We have developed a sample-to-report solution for human immune profiling using mass cytometry, the Maxpar® Direct™ Immune Profiling System. It includes an optimized 30-marker immune profiling panel provided in a dry, single-tube format, protocols for human whole blood and PBMC staining, a Helios™ data acquisition template, and Maxpar Pathsetter™ software for automated data analysis. The Pathsetter software analyzes FCS 3.0 files generated with the kit and automatically reports cell counts and percentages for 37 immune cell types. It also reports quality

DOI: 10.1002/eji.201970300

metrics such as staining assessment. Pathsetter produces graphical elements such as dot plots and a Cen-se™ map (t-SNE variant).

Here we present assay analytical validation data on repeatability, reproducibility, software precision, and software accuracy*. We also present a performance comparison between dry and liquid formulations of the same antibodies and clones*. This assay provides a robust, complete solution for broad immune profiling using mass cytometry that reduces sources of variability and subjectivity in sample preparation and data analysis.

1 Simoni, Y. et al. Nature 557 (2018): 575–579. 2 Maecker, H.T. et al. 12 (2012): 191–200. 3 NCSS 12 Statistical Software (2018). NCSS, LLC. Kaysville, UT, USA, ncss.com/software/ncss. *Fluidigm reference document 120001-REC-050.

Figure 1

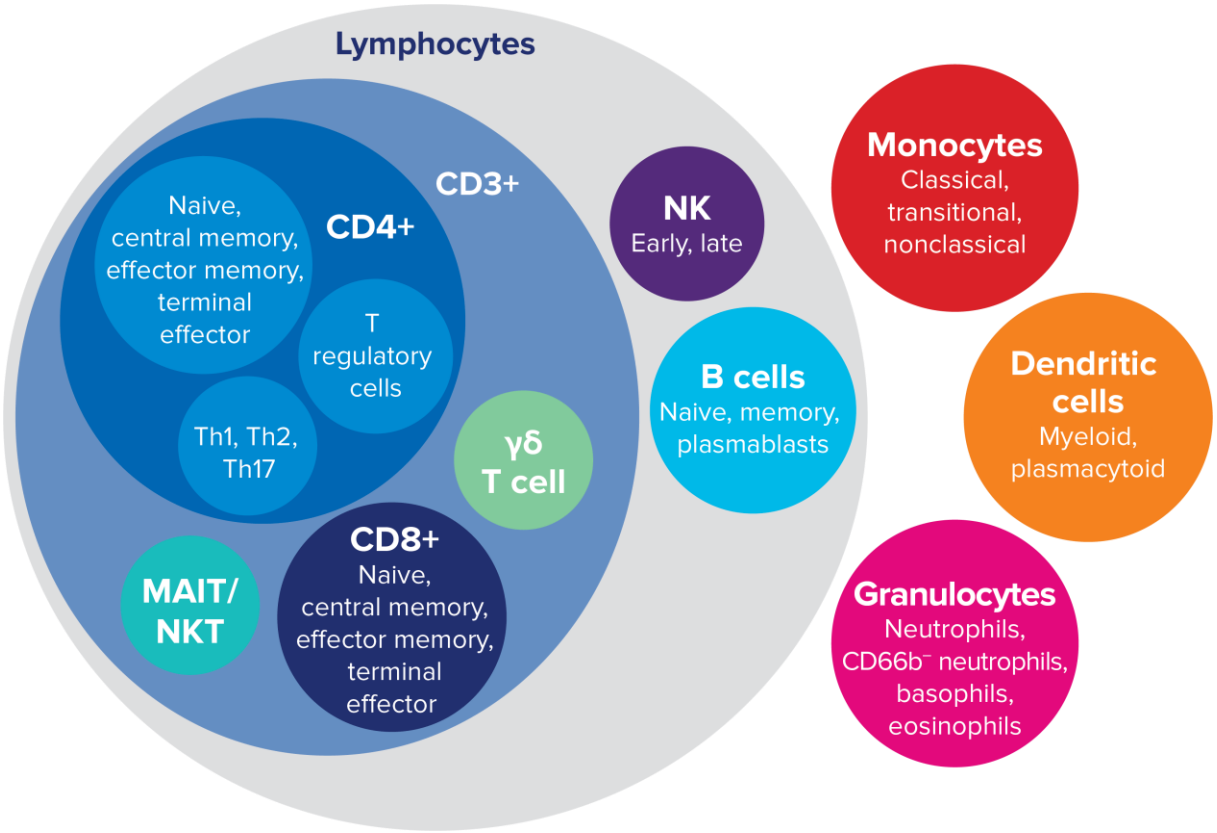
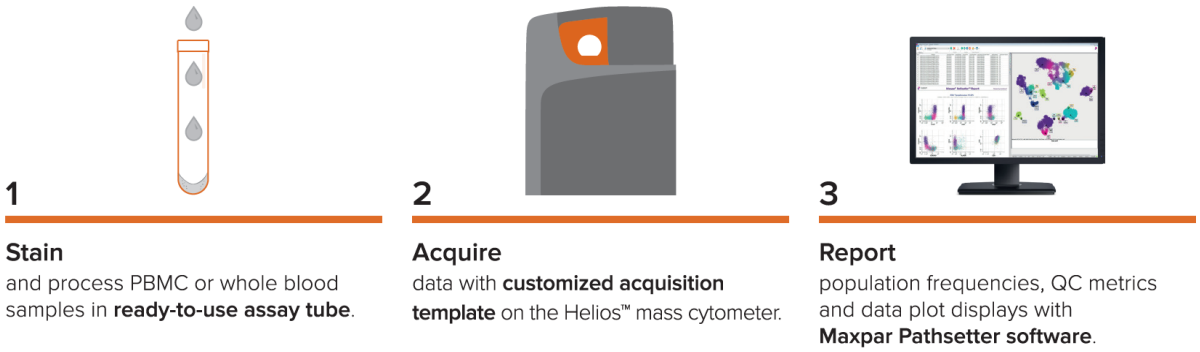


Figure 2

DOI: 10.1002/eji.201970300



P352

Retargeting T cells against leukemia by lipid-specific TCR

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Acute leukemia recurrence following chemotherapy and bone marrow transplantation remains a major unmet clinical need. The transfer of T lymphocytes from bone marrow donors into recipient patients can control leukemia recurrence, although it can lead to detrimental graft versus-host diseases (GvHD). To overcome this problem, it is necessary to transfer into patient donor-derived T lymphocytes that are selectively directed against the malignant cells, but ignore all other tissues. To this aim, we have set up an adoptive immunotherapy strategy that generates donor-derived T lymphocytes selectively redirected against leukemia cells of the recipient by engineering with CD1c restricted TCRs specific for methyl-lysophosphatidic acids (mLPA), a lipid antigen accumulated in malignant cells. Because CD1c molecule is not polymorphic, this strategy is universally applicable to any acute leukemia patient, whose malignant cells express CD1c, which occurs in 45-70% of cases. Co-transduction of a lead chimeric mLPA specific TCR and CD3ζ into primary T cells optimizes expression and generates effectors that kill CD1c-expressing malignant targets *in vitro*, but spare normal B and myeloid cells, and significantly delay leukemia progression in NSG mice. To further improve efficacy and safety of this strategy, we engineer invariant Natural Killer T (iNKT) cells, taking the advantage of their intrinsic anti-tumor activity and ability to protect against GvHD. Further insight into the efficacy and safety of mLPA-specific ACT are being gained in transgenic mice expressing CD1c with a pattern similar to the human one.

P353

The anti-inflammatory effects of whole-body cryotherapy in non-professional athletes

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Introduction: Cryotherapy has gained popularity among athletes across many sports, but is under strict investigation for possible doping-like effects. The main goal of whole-body cryotherapy (CRY) is to counteract inflammatory

DOI: 10.1002/eji.201970300

symptoms following athletic performance, in order to improve physical recovery. Nevertheless, very few data are available on the effects of CRY on immunological and bio-humoral parameters.

Objectives: The study aimed to examine the effect of CRY on several immune parameters, inflammatory markers, body metabolism and on the hormone levels in non-professional athletes.

Materials & methods: We enrolled 10 male non-professional bikers (mean±SD: 44±5 years old) during a training period. Athletes have undergone a once-a-day session of CRY (2 min in a cabin with an atmosphere ranging from -120°C to -170°C) for 3 consecutive days. Before the first and after the last session we collected venous blood. Hematologic parameters, serum chemistry, and plasma hormones were evaluated along with plasma levels of pro- and anti-inflammatory cytokines and chemokines (IL-1beta, IL-2, IL-6, IL-8, IL-10, IL-12p70, IL-18, TNFalpha, IL-1a, VEGF, IL-1ra, CCL2, CXCL13 and CCL19, sFasL). Monocyte phenotype was analyzed by flow cytometry on the basis of the expression of the surface markers CD14, CD16, HLA-DR, CCR5, CCR2 and CXCR4. Lastly, we evaluated the effect of CRY on circulating mitochondrial (mt)DNA, which is released by damaged cell and acts as damage associated molecular pattern.

Results: After CRY treatment, glucose, C protein and S protein decrease while HDL, urea, insuline-like growth factor (IGF)-1 and follicle-stimulating hormone increase. IL-18, IL-8, IL-1RA and CCL2 increased after treatment. The percentage of total, classical, non-classical and intermediate monocytes did not change after CRY, but CD14, CCR5, CCR2 and CXCR4 expression decreased in all monocytes. On intermediate monocytes, only CCR5 decreased. Conversely, CCR5 and CCR2 increased on non-classical monocytes. Finally, plasma levels of mtDNA increased after CRY treatment.

Conclusion: CRY seems to have an anti-inflammatory and a pro-angiogenic effect on monocytes, since CD14 downregulation was observed in monocytes that favors vascular repair. CRY affects also the level of IGF-1, a potent mitogen involved in skeletal muscle growth and regeneration. These observations suggest that CRY could have beneficial effects in inflammatory diseases.

P354

Mesenchymal stromal cells from human umbilical cord prevent the development of lung fibrosis in immunocompetent mice

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Introduction Lung fibrosis is a severe condition resulting from several interstitial lung diseases (ILD) with different etiologies. Current therapy of ILD is rather limited and new anti-fibrotic strategies are needed.

Objectives To evaluate the therapeutic potential of systemically administered hUC-MSC in the well-established model of bleomycin-induced lung fibrosis. Unlike previous studies performed in immunodeficient mice, we aimed at demonstrating the anti-fibrotic activity of hUC-MSC in vivo in bleomycin-treated immunocompetent C57BL/6 mice, in order to make an experimental model representing an immunocompetent human subject affected by SSc-ILD.

Materials And Methods Adult immunocompetent C57BL/6 mice (n=8 per group) were injected intravenously with hUC-MSC (n.= 2.5 × 105) twice, 24 hours and 7 days after endotracheal injection of bleomycin. Upon sacrifice at days 8, 14, 21, collagen content, inflammatory cytokine profile, M2 macrophages infiltration and hUC-MSC presence in explanted lung tissue were analyzed.

Results: Bleomycin-induced pneumonia was largely attenuated by a double infusion of hUC-MSC into the tail vein, with significant reduction of both the inflammatory infiltration and the extent of fibrosis. Following hUC-MSC treatment, the expression of cytokines IL-6, IL-10 and TGF-β was progressively reduced. The attenuation of bleomycin-induced

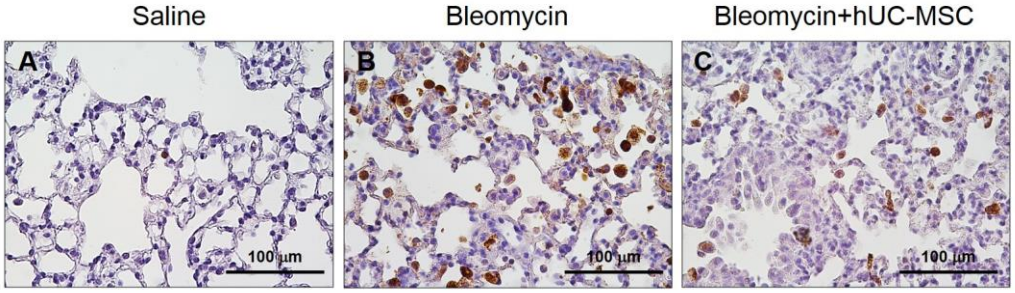
DOI: 10.1002/eji.201970300

fibrosis by hUC-MSC was also associated with down-regulation of M2 macrophages. Only few hUC-MSC were detected from explanted lungs, suggesting a "hit and run" mechanism of action of this cellular therapy.

Conclusion: Systemic administration of a double dose of hUC-MSC significantly reduced bleomycin-induced lung injury (inflammation and fibrosis) in mice through a selective inhibition of the IL6-IL10-TGFβ axis involving lung M2 macrophages. The results suggest a role of excessive proliferation and activation of M2 macrophages in the pathogenesis of lung fibrosis as a consequence of pro-inflammatory stimuli. In conclusion, our data indicate that hUC-MSC possess strong in vivo anti-fibrotic activity in a mouse model resembling an immunocompetent human subject affected by inflammatory ILD, providing proof of concept for ad-hoc clinical trials[1].

REFERENCES 1. Moroncini, G. *et al*. *PLoS One.* 2018 13(6):e0196048

Figure 1



LEGEND Fig.1 Decrease of arginase-I positive M2 macrophages in bleomycin-injured lung tissue upon administration of hUC-MSC

P355

TCR reprogramming for the treatment of refractory adenovirus infection

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[2]DZIF partner site Munich, Munich, Germany
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Introduction: Absent T cell immunity against persistent viruses like Adenovirus (AdV) is a major cause for virus-related mortality in patients after haematopoietic stem cell transplantation. Adoptive T cell transfer has shown to be effective from seropositive individuals, but no routine protocol has been developed for the widespread use of seronegative donors.

Objective: We aimed to redirect T cells by replacing endogenous T cell receptors (TCRs) with an AdV-specific TCR using CRISPR/Cas9 to prevent random integration of conventional retroviral gene transfer. The simultaneous knock-out (KO) of the endogenous TCR will prevent harmful TCR mispairing.

Methods: Primary human T cells were CRISPR/Cas-edited to disrupt TCR α and β chains. KO of the endogenous TCR was confirmed on genetic level by PCR and Sanger sequencing, on protein level by flow cytometry and functionally by intracellular IFNγ staining upon SEB stimulation. AdV-specific TCRs were derived from a patient with a LTDLGQNLLY-specific T cell response. LTDLGQNLLY is known as an AdV major immunodominant HLA-A1-binding T-cell epitope. AdV-specific TCR sequences were integrated into TCR α chain constant region of TCR KO T cells using CRISPR/Cas9 and virus-free DNA templates. LTDLGQNLLY-specific TCR expression was confirmed by pMHC-Streptamer staining. Recombinant T cells were functionally characterized using intracellular IFNγ staining in response to LTDLGQNLLY and cytotoxic capacity after co-cultivation with LTDLGQNLLY presenting target cells.

DOI: 10.1002/eji.201970300

Results: We established stable and highly efficient TCR KO in more than 90% of primary human T cells. TCR KO T cells were unable to produce IFNγ upon stimulation with SEB, which demonstrates the functional disruption of the TCR and we achieved stable knock-in rates of 3.5%. 71% of recombinant AdV-specific T cells produced IFNγ after stimulation with LTDLGQNLLY and, after co-culture with LTDLGQNLLY expressing target cells, TCR engineered T cells showed specific cytotoxic capacity.

Conclusion: In summary, these results demonstrate feasibility of redirecting primary human T cells from seronegative donors by replacing the endogenous TCR with a virus-specific TCR leading to high functionality. The preclinical characterization of these engineered virus-specific T cells reveals that combined CRISPR/Cas mediated TCR KO and knock-in presents a powerful tool for future treatment of refractory viral infections in the immunocompromised host.

P356
The German PID-NET Registry
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Background: The German PID-NET registry was established in 2009 and has been funded by the German Government (BMBF, Support Code: 01GM1517C) until March 2018. Funding until December 2018 was provided by the Care-for-Rare Foundation (www.care-for-rare.org). The PID-NET registry is the only registry in Germany for primary immunodeficiencies (PID). The aim of the registry is to collect clinical and genetic data on patients with PID in order to gain a better understanding of PIDs and to obtain data for clinical trials and research studies.

Methods: PID-NET Germany is an online registry with documentation *via* a web-interface.

Results: At the end of April 2019, there were 3,102 patients registered from 43 centres. From 83 diseased patients, 31 were under 18 years of age, including six SCID patients. Three of the SCID patients underwent a hematopoietic stem cell transplant (HSCT). 64 patients were lost to follow-up, and 12 were discharged.

Out of 2,712 living patients, 1,123 patients (41%) were under 18 years of age. From these living patients, out of 120 SCID patients, 82 patients were under 18 and 24 patients under six years of age. Out of 85 combined ID patients, 43 patients were under 18 and 12 patients under six years of age.

Out of all 3,102 patients, IgG substitution was given to 1,342 patients (26% intravenous, 73% subcutaneous). 465 patients had at least one HSCT and eight patients received gene therapy whereas four of them also had a HSCT.

The most prevalent PID categories were antibody disorders (total: 1,642 patients; <18 y: 405 patients), well defined PID syndromes (total: 359; <18y: 223), and phagocytic disorders (total: 259; <18y: 148).

Among the 1,231 genetically diagnosed patients, most had *Btk* mutations (106 patients), *GP91-phox(CYBB)* (101 patients) *and Del 22q11.2* (87 patients). Categories with over 60% of patients under 18 years of age were autoinflammatory disorders (74%), diseases of immune dysregulation, and other well defined PIDs (64%).

2,136 patients had infections as their presenting symptom, 784 had immune dysregulation, 261 had syndromal manifestations, and 29 had malignancies as presenting symptom. 148 patients had no symptoms at all, 99 of them had been diagnosed based on laboratory abnormalities.

Conclusions: Although we accomplished a significant increase in the number of patients documented, including long-term documentation,we assume that there are still a several thousand patients not yet diagnosed/documented.

P357
Near-infrared photoimmunotherapy targeting CD8 T cells prevents cutaneous graft-versus-host disease
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Introduction*:* Near-infrared photoimmunotherapy (NIR-PIT) represents a novel antibody-based strategy for selective cellular depletion in body locations defined by light-treatment. Originally introduced for EGFR-specific cancer cell treatment, NIR-PIT displayed distinctive depletion prosperity in several mouse models.

DOI: 10.1002/eji.201970300

Objectives: Adapted from these findings, a skin selective depletion of an antibody-targeted cell type has a great potential of becoming a therapeutic option.

Materials & methods: Here, we report on a method for the skin selective depletion of CD8 T cells and its therapeutic effect in a humanized mouse model of acute cutaneous graft-versus-host disease (aGvHD). In immunodeficient NSG (NOD.Cg-*Prkdcscid Il2rgtm1Wjl*/SzJ) mice transplanted with human skin, aGvHD was induced by intravenous injection of mismatched peripheral blood mononuclear cells.

Results: Humanized acute GvHD mice displayed a strong T cell infiltration, inflammation and epithelial skin graft rejection. Skin specific ablation of target CD8 T cells was performed by the combined function of an anti-CD8-mAb-photosensetizer conjugate activated by near-infrared light. Our data demonstrate selectively killing skin localized CD8 T cells while sparing systemic immunosuppression. The effect proved to be highly efficient by preventing the manifestation of cutaneous aGvHD as studied by histopathology, granzyme B expression and elafin expression.

Conclusion: We demonstrates the importance of CD8 T cells during acute allogeneic skin graft rejection and emphasize that near-infrared photoimmunotherapy can serve as a novel skin selective therapeutic approach for research and clinical use.

P358

Introducing a novel switchable CAR platform with reduced CAR size for immunotherapy of tumors

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Introduction: Recently the use of chimeric antigen receptor (CAR) modified T cells in the immunotherapy of tumors has become a promising approach. CAR T cells are able to recognize tumor-associated antigens (TAAs) in a major histocompatibility-complex (MHC)-independent manner. Although highly efficient, the inability to regulate the activity of CAR T cells can cause severe side effects and thus needs to be considered in future developments. Here, we introduce the RevCAR system – a novel switchable modular universal CAR system having a minimal size to overcome the obstacles of conventional CAR therapy.

Objectives: In order to improve the controllability of CAR T cells a modular CAR system, which allows switching the activity of CAR T cells repeatedly "ON" and "OFF", was generated. Furthermore, to avoid unspecific side effects and minimize tonic signaling of conventional CAR T cells, the extracellular single chain variable fragment (scFv) was removed. Thus, resulting RevCARs have a smaller size allowing "gated" targeting strategies, e.g. by facilitating simultaneous transduction of two independent CARs with different specificities and split motifs, which could further improve the safety of CAR T cells.

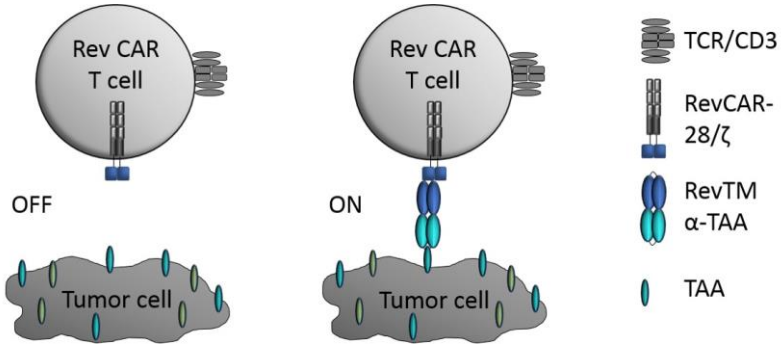
Materials & methods: In order to reduce the size of the artificial receptor the original idea was to replace the extracellular scFv domain of a conventional CAR with a small peptide epitope and to engage the resulting RevCAR T cell via a bispecific target module which we termed RevTM. For proof of concept two small peptide epitopes were selected and the respective RevCARs constructed. In addition, a series of different RevTMs was generated. On the one hand the RevTM recognizes one of the two peptide epitopes on the other hand the RevTM can be directed to any potential TAA.

Results: Until now a series of RevTMs was constructed and functionally analyzed. RevCAR T cells armed via the respective RevTM were able to efficiently lyse their respective target cells in a peptide epitope and target specific, as well as target module dependent manner. These data are supported by the analysis of cytokine secretion from RevCAR T cells which was only observed in the presence of both target cells and the respective RevTM.

Conclusion: Taken together these results demonstrate the high anti-tumor efficiency of the novel RevCAR platform which is characterized by a small size, an improved safety, easy controllability as well as high flexibility.

DOI: 10.1002/eji.201970300

Figure 1



P359

Impact of cytokine secretion and response to mesenchymal stem cells (MSCs) varies with sources

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Introduction: Cytokines can stimulate MSCs as well as secrete cytokines for various regenerative functions. Though MSCs are known for their immunomodulation, their interaction with cytokines of paracrine or indigenous origin and subsequent impact on differentiation is not clear with sources of MSCs.

Objectives: To investigate whether there is any difference in the functional modulation property of MSCs isolated from various source and on their cytokines profile.

Materials and Methods: We isolated MSCs from umbilical cord blood (UCB), dental pulp (DP) and liposuction material (LP) and compared their ability to respond to activated neutrophils and directly to LPS. Cytokine profiling (IL-1α, IL-2, IL-4, IL-6, IL-8, TNF-α, IFN-γ, TGF-β), cellular proliferation and differentiation to osteoblast were assessed.

Results: The results of the cytokine secretion in response to the activated neutrophil co-culture were largely comparable, while the LSMSCs cytokine response with TNF-α and IFN-γ was high owing to its mature cellular phenotype compared with UCBMSCs and DPMSCs. There was no change observed with the viability and proliferation between LP/DP/UCB MSCs when co-cultured with activated neutrophils. Direct activation of MSCs with LPS further served as control however did not induce a rapid proliferation, while cell death was evidenced in all the three types of MSCs. We observed an elevated TGF-β in all the three MSCs while significantly higher concentration is DPMSCs. IL-2 expression was not observed with any of the MSCs pre or post activation with neutrophils or LPS. Further when MSCs post neutrophil exposure were induced for osteogenic differentiation, though all the MSCs devoid of the sources differentiated, we observed a rapid and significant turnover of DPMSCs positive of osteogenic markers than LP and UCB MSCs with corresponding increase in the TGF-β even during stress indication progenitors have higher threshold than adult differentiated cells during repair.

Conclusion: Taken together these results suggest that cytokines whether it is indigenous or paracrine has an impact on functional aspects of MSCs irrespective of their sources.

DOI: 10.1002/eji.201970300

P360

Delay of acute Graft-versus-Host Disease after allogeneic bone marrow transplantation by molecular targeting of anti-apoptotic proteins in donor T cells

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Introduction: Graft-versus-Host-Disease (GvHD) remains one the most frequent complication following Bone Marrow Transplantation (BMT). The mitochondrial apoptotic pathway is crucial for regulation of cell proliferation or apoptosis. That process is controlled by the BCL-2 family of proteins. The competitive binding of α-helical BH3-domains to the BH3-binding grooves of either pro- or anti-apoptotic proteins controls the induction of apoptosis. By targeting the BH3-binding domains of the pro-survival molecules in T cells using BH3-mimetics ABT-737 and ABT-199, we aim to induce apoptosis in alloreactive T cells.

Methods: Apoptosis and Mitochondrial Outer Membrane Permeabilization (MOMP) rates were assessed by flow cytometry. Mice were treated either with ABT-199 intraperitoneal or control-vehicle for 10 consecutive days, starting from 24h after BMT. Mice were scored daily for signs of clinical GvHD.

Results: Following the incubation of resting and activated CD8+ T cells with BH-3 mimetics, we report a greater induction of apoptosis in activated as compared to resting CD8+ cells. However, this differential effect is abrogated in the presence of high IL-2 levels. In addition, we provide evidence of upregulation of the pro-survival proteins BCL-2, BCL-xL and MCL-1, and in parallel, a downregulation of the pro-apoptotic protein PUMA in proportion to the concentration of added IL-2. This data hints towards an antagonistic effect of IL-2 on the induction of apoptosis via BH-3 mimetics. Furthermore we could show a greater resistance of regulatory T cells (Tregs) to BH-3 mimetics compared to conventional CD4+ cells both in vitro and in vivo, suggesting different regulatory elements regulating the mitochondrial apoptosis pathway in Tregs. Using two murine acute GvHD models, we demonstrate that treatment with ABT-199 led to a significantly delayed onset of GvHD and in one model a prolonged survival after BMT.

Conclusion: We report a differential effect of the BH3-mimetics on CD8+ T cells, and a different expression profile of BCL-2 proteins depending on their activation state. Furthermore, we show a delayed GVHD onset following BMT in ABT-199- treated mice. The mechanisms of this delay is likely conferred by Tregs which are enriched following BMT. However, we observed a functional antagonism of IL-2 signaling towards the induction of mitochondrial apoptosis in T cells and this might be the reason for incomplete GvHD-prevention.

P361

Regulatory T cells engineered with chimeric antigen receptors as an immunotherapy candidate for type 1 diabetes

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Epidemiological data show a steady increase in the prevalence of autoimmune diseases in Western society over the last decades, for example Type 1 diabetes (T1D). Nowadays, 300,000 people in Germany have T1D with more than 30,000 children and adolescents under the age of 19. T1D develops in patients with genetic predisposition under the influence of various environmental factors. Current medical option is a long-life administration of insulin, a cure is not possible yet.

Adoptive immunotherapy with *ex vivo* expanded, polyspecific regulatory T cells (Tregs) is a promising treatment for graft-versus-host disease. Animal transplantation models have demonstrated that the adoptive transfer of allospecific Tregs offers greater protection from graft rejection than that of polyclonal Tregs. This finding is in contrast to those of autoimmune models, where adoptive transfer of polyspecific Tregs had very limited effects, while antigen-specific Tregs were promising. However, antigen-specific Tregs in autoimmunity cannot be isolated in sufficient numbers.

DOI: 10.1002/eji.201970300

Chimeric antigen receptors (CARs) can modify T cells and redirect their specificity toward needed antigens and are currently clinically used in leukemia patients. A major benefit of CAR technology is its "off-the-shelf" usability in a translational setting in contrast to major histocompatibility complex (MHC)-restricted T cell receptors. We used CAR technology to redirect T cell specificity toward insulin and redirect T effector cells (Teffs) to Tregs by Foxp3 transduction.

Our data demonstrate that our converted, insulin-specific CAR Tregs (cTregs) were functional stable, suppressive and long-lived *in vivo*. This is a proof of concept for both redirection of T cell specificity and conversion of Teffs to cTregs (Tenspolde et al. 2019).

Tenspolde, M., K. Zimmermann, L. C. Weber, M. Hapke, M. Lieber, J. Dywicki, A. Frenzel, M. Hust, M. Galla, L. E. Buitrago-Molina, M. P. Manns, E. Jaeckel and M. Hardtke-Wolenski (2019). "Regulatory T cells engineered with a novel insulin-specific chimeric antigen receptor as a candidate immunotherapy for type 1 diabetes." J Autoimmun.

P362

T cell receptor avidity-dependent T cell fate during infection and tumor disease

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Introduction: During infection and tumor disease, the composition of the T cell receptor (TCR) repertoire of antigen-specific CD8+T cells changes over time. Upon recall infection, at late time points of chronic infections or during tumor disease, TCR repertoire evolution often leads to dominant T cell clones, but it is incompletely understood whether and how these T cell populations are selected based on avidity.

Objectives: We aimed to create an experimental system which enables investigation of TCR avidity-dependent repertoire evolution during infection and tumor disease. Specifically, we developed an experimental approach that allows to follow the trajectories of T cells with different antigen-specific TCRs and avidities within a polyclonal population. With this experimental tool, avidity based consequences during *in vivo*antigen challenge are explored upon adoptive transfer of TCR-transgenic T cells.

Materials & Methods: We built up a library of TCRs with different avidities against the same epitope SIINFEKL, and developed a novel TCR repertoire tracking system, which allows us to study TCR avidity-dependent T cell fate in OVA mouse models of infection and tumor disease.

Results: Upon recall infection (*Listeria monocytogenes*OVA), during chronic infection (IE2 OVA murine Cytomegalovirus) and during tumor disease (subcutaneous MC38 OVA tumors), we observed that TCR avidity determined T cell fate in a highly context-dependent manner, and that TCR repertoires do not necessarily evolve linearly towards high avidity.

Conclusion: Whether high avidity TCRs are present or absent at a given disease state is relevant for optimal choices of immunotherapeutic strategies. Deciphering TCR avidity-dependent T cell fate therefore not only adds to our understanding of basic immunology, but also has profound translational implications.

P363

The Bacteria Product Violacein has an Immunostimulatory Effect and Acts Via TLR8

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DOI: 10.1002/eji.201970300

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Introduction: Violacein is a natural purple pigment produced by several Gram-negative bacteria, such as *Chromobacterium violaceum* and *Janthinobacterium lividum*. A variety of biological activities have been reported for this compound, such as: antibacterial, anti-viral, antitumor, antileukemic and others. This wide range of biological activities has attracted interest to understand its mechanism of action with the purpose of finding a potential application as a therapeutic agent.

Objectives: Study the effect and the molecular mechanism of activation of the bacterial product violacein on immune cells.

Materials & methods: We studied the effect of violacein on different immune cell lines, namely THP-1, MonoMac 6, ANA-1, Raw 264.7 cells, as well as on human peripheral blood mononuclear cells (PBMCs). We obtained evidence of the molecular mechanism of activation by determining the mRNA expression pattern upon treatment with violacein in Raw 264.7 cells. Furthermore, the effect of violacein was studied using TLR7/8-transfected HEK-293 cells with a NF-κB-luciferase reporter plasmid and we studied the suppression of the immunostimulatory effect of violacein in PBMCs with a specific hTLR8 antagonist, CU-CPT9a. Finally, we investigated the interaction of hTLR8 with violacein *in silico.*

Results: A stimulation of TNF-α production was observed in murine macrophages (ANA-1 and Raw 264.7), and in PBMCs, IL-6 and IL-1β secretion was detected. The mRNA expression pattern indicated that violacein caused activation of pathways related with an immune and inflammatory response. Additionally, our data utilizing TLR-transfected HEK-293 cells indicate that violacein activates the human TLR8 (hTLR8) receptor signaling pathway and not human TLR7 (hTLR7). Furthermore, we found that the immunostimulatory effect of violacein in PBMCs can be blockade by CU-CPT9a. Finally, according to *in silico* analysis, violacein could bind to hTLR8 in a similar fashion to imidazoquinoline compounds.

Conclusion: We found that violacein present an immunostimulatory effect in PBMCs and some murine cell lines. In addition, our results shown that violacein is activating immune response pathways and in more detail, we obtained evidence that violacein is acting via TLR8 in PBMCs and could bind to hTLR8 in similar fashion to imidazoquinoline compounds according to *in silico* analysis. For this reasons, we propose that violacein could have potential contribution in future immunotherapy strategies.

P364

A mouse model-based screening platform for the identification of immune activating natural products for novel cancer immunotherapies

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Introduction: The most abundant human diseases worldwide include cancer and bacterial infections. Immunotherapy now serves as an alternative for traditional approaches especially in anti-cancer treatment. Nonetheless, the emergence of resistances causes a strong need to find new drugs. Drug development can be inspired by natural products which comprise many highly bioactive molecules.

Objectives: In this work, a library of 240 natural products derived from endophytic fungi and marine sponges was analysed for suitability for immunotherapy according to a newly established screening pipeline.

Material & methods: Natural products underwent various screenings by using primary immune cell cultures from bone marrow of cytokine reporter mouse models. Compounds were analysed for toxic vs. stimulatory capacities on these immune cells and for direct beneficial bioactivities against tumour cell lines or pathogens.

Results: Natural products were defined to be promising for immunotherapeutic drug development if they fulfil three criteria: First, they have to be non-toxic for target immune cells. Secondly, compounds should exert additional bioactivities such as direct toxicity against cancer cells. Lastly and importantly, they have to exhibit immune activating properties. Thus, we are looking for novel compounds that modulate immune effector functions and simultaneously target tumours and pathogens, consequently reducing the risk of the appearance of resistances.

DOI: 10.1002/eji.201970300

Here, 240 natural products were screened for compliance of the abovementioned guidelines. Three compounds were found so far to fulfil these conditions and show immunostimulatory capacities assessed by IL-12p40 and T cell activation assays. Following this, target finding approaches will be applied to identify the underlying molecular mechanisms before potential biochemical optimization and further testing in *in vivo* tumour and infection mouse models.

Conclusion: The presented pipeline enables an efficient and reliable screening of natural products for immune activating capacities and thus represents a useful tool for the selection of novel, promising compounds for further immunotherapeutic drug development.

P365

Survival and immune reconstitution of syngeneic, haploidentical and allogeneic hematopoietic stem cell transplantation in *Atm*-deficient mice

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Hematopoietic stem cell transplantation (HSCT) seems to be an encouraging opportunity for a curative therapy to restore immunity and prevent the development of hematologic malignancies in the genetic instability syndrome Ataxia-telangiectasia (A-T). However, experience in the conditioning regimen is limited and no transplantation strategy for A-T patients exists. Conditioning regimen and donor selection are critical factors in the clinical setting of HSCT and incur substantial risks, especially in A-T. The aim of this study was (1) to evaluate whether different approaches of HSCT are feasible in regard to graft versus host response (GvHD) and sufficient concerning immune reconstitution (2) and to de-escalate the toxic effects of the conditioning regimen by reducing the dose of cyclophosphamide (CP).

T cells from syngeneic, allogeneic and haploidentical donor mice were used to determine GvHD induced T cell proliferation in a mixed lymphocyte reaction (MLR). *Atm*-deficient mice were treated with CP or reduced CP in combination with Fludarabine (FLU) and transplanted with $5x10^6$ CD90.2 depleted bone marrow donor cells from 129/SvEv GFP-transfected wildtype mice (syngeneic) or from mice of the F1 generation of 129/SvEv wildtype mice and C57BL/6 mice (haploidentical), or from C57BL/6 mice (allogeneic). Tracking of GFP-positive donor derived cells was performed using flow cytometry and *Atm* PCR. Oxidative stress and damage were detected by a RT Profiler PCR Array and 8-Hydroxy-2′-deoxyguanosine.

MLR resulted in an increased proliferation of allogeneic donor T cells compared to syngeneic and haploidentical donor cells. Response was lower on dendritic cells isolated from *Atm*-deficient mice compared to wildtype controls. *In vivo* results showed the restoration of T cells in *Atm*-deficient mice accompanied by a prolonged life span and through reduction of thymic tumors. However, allogeneic stem cell transplantation was accompanied with a higher mortality rate, compared to the haploidentical and syngeneic setting. Decreased antioxidative capacity and a higher DNA-damage were seen in CP treated *Atm*-deficient mice.

Haploidentical HSCT seems to be a feasible strategy for A-T. Our data provided further evidence for the high sensitivity against ROS-inducing agents in A-T and this fact needs to be taken into consideration in the choice of the host-conditioning strategy.

P366

CD16 aggregation in high affinity conditions by tumor-targeting mAb obinutuzumab promotes a PI3K/mTOR-dependent priming of Natural Killer cells for IFN-gamma production, associated to miR-155 upregulation

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DOI: 10.1002/eji.201970300

Introduction: Anti-CD20 monoclonal antibodies (mAbs) are used in the treatment of B-cell malignancies and autoimmune disorders. Their clinical efficacy largely relies on Natural Killer (NK) cell-mediated Antibody Dependent Cellular Cytotoxicity (ADCC) based on the recognition of IgG-opsonized cells by the low affinity receptor for IgG FcgammaRIIIA/CD16. Beside ADCC, CD16 ligation also results in production of cytokines such as IFN-gamma, which promotes the development of adaptive immune responses. Obinutuzumab is a glycoengineered anti-CD20 mAb with a modified Fc domain and enhanced affinity for CD16. Relevant for this study is our previous observation that the sustained CD16 ligation in optimised affinity conditions by obinutuzumab-opsonized targets enhances the capability of human NK cells to produce IFN-gamma in response to a subsequent stimulation with cytokine or target.

Objectives: To identify the molecular mechanisms underlying the enhanced IFN-gamma production of obinutuzumab-experienced NK cells.

Materials & Methods: Primary human NK cells were co-cultured with biotin-loaded anti-CD20-opsonized cells, recovered by negative selection on streptavidin beads and analysed.

Results: Our data show in obinutuzumab-experienced NK cells an increased IFN-gamma production in response to the gamma chain cytokines IL-2 and IL-15, which associates to increased miR-155 levels, but not to the upregulation of IFN-gamma mRNA levels. A similar trend was observed in NK cells from Chronic Lymphocytic Leukemia (CLL) patients upon interaction with obinutuzumab-opsonized autologous CLL. In obinutuzumab-experienced NK cells, the increased miR-155 expression associates to reduced levels of the miR-155 direct target SHIP-1, which acts in constraining PI3K-dependent signals. Moreover, downstream to PI3K, the phosphorylation status of mTOR effector, S6K, resulted amplified in response to IL-2 or IL-15 stimulation. Further, the treatment with mTOR inhibitor, rapamycin, or p110PI3Kdelta inhibitor, idelalisib, prevents the enhanced IFN-gamma response, thus highlighting the relevance of PI3K/mTOR pathway in the increased cytokine responsiveness.

Conclusion: Our data unveil an unknown capability of CD16 to mediate a PI3K/mTOR-dependent NK cell priming for IFN-gamma production associated to the modulation of miR-155. The enhanced IFN-gamma competence may be envisaged in a potentiated immunoregulatory role of NK cells in a therapeutic setting.

P367

A novel mouse model for superagonistic anti-CD28 monoclonal antibody-induced cytokine release syndrome in humans

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Superagonistic anti-CD28 monoclonal antibodies (CD28-SA) do not require parallel stimulation of the T cell receptor complex to fully activate T cells. While CD28-SA treatment of mice with autoimmune/inflammatory diseases led to CD4+ Foxp3+ regulatory T cell (Treg)-mediated protection, healthy human volunteers injected with saturating amounts of CD28-SA (100 µg/ kg BW) developed a severe cytokine release syndrome (CRS) due to activation of effector/ memory CD4+ T cells. Apart from the relatively increased suppressive potency of Treg from mice compared to humans, it has so far been unclear whether the lack of a true memory CD4+ T cell compartment in cleanly housed mice or a general unresponsiveness of mouse effector/ memory CD4+ T cells explains the failure of mouse models to predict CD28-SA-induced CRS in humans. We, therefore, transferred *in vitro* differentiated TCR-transgenic OT-II Th1 cells into diphtheria toxin-treated wild-type or Treg-depleted DEREG-C57BL/6 recipient mice. Injection of CD28-SA into Treg-depleted recipient mice activated the transferred OT-II Th1 cells leading to the release of high amounts of IFNg into the circulation. Titration of CD28-SA in this model *in vivo* revealed that, similar to humans, about tenfold more CD28-SA (6 mg versus 600 µg/ kg BW) was required to escalate from minimally detectable increases in systemic IFNg to very high concentrations in the serum. Apart from monitoring mouse Th1 cell responses to CD28-SA stimulation *in vivo* we also defined *in vitro* conditions mirroring *in vivo* responses. Transfer of Th1 cells into cleanly housed recipient mice *in vivo* and our novel *in vitro* assay will, thus, facilitate pre-clinical testing of immunomodulatory reagents. It will, further, help to better understand the molecular requirements for CD28-SA-mediated effector/memory CD4+ T cell activation *in vivo*.

DOI: 10.1002/eji.201970300

P368

Generation of a cell-based safety mechanism for CAR T cell therapy

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Introduction: Adoptive immunotherapy based on genetically engineered T cells armed with chimeric antigen receptors (CARs) has shown tremendous clinical success in patients with B cell malignancies. However, the high sensitivity of these engineered cells towards their targets also bears the risk of severe off-target toxicities. Therefore, it is necessary to develop strategies that improve the safety of CAR T cell therapy. In this context, especially the removal of transferred cells in case of adverse events can be seen as a promising approach to revert long-term CAR T cell related toxicities in patients who are in stable remission.

Objectives:Hence, our goal is to develop a novel efficient cellular safeguard mechanism in order to specifically eradicate therapeutically transferred CAR T cells in case of developing adverse effects. This goal could be achieved by using a second CAR T cell (anti-CAR CAR T cell) which recognizes an additional tag introduced into the primary CAR construct.

Materials & methods: For this purpose, we generated CARs targeting a short peptide-sequence (StrepTag®) included in the extracellular domain of anti-CD19 CARs. So-called anti-CAR CAR T cells were tested for cytokine release upon exposure to their target cells, as well as for their *in vitro* killing capacity. *In vivo* functionality was evaluated by co-injection of CD19 and anti-CAR CAR T cells first in immune-compromised RAG2/IL2rg knock-out mice and later in immune-competent C57BL/6 mice, where successful B-cell reconstitution was used as additional readout. Finally, B-cell recover was evaluated in a clinically relevant setting where anti-CAR CAR T cells were administered after CD19 CAR T cells in C57BL/6 mice with stable B cell aplasia.

Results: StrepTag was first introduced into the hinge region of CD19 CARs, with no alterations of CAR functionality. We then engineered anti-CAR CAR-T cells which specifically recognized StrepTagged anti-CD19 CAR T cell. Besides, we observed that the generated anti-CAR CAR T cells released effector cytokines after stimulation with their target cells and showed high *in vitro* killing efficacies. More importantly, we found that the effector CAR T cells are also capable of killing their target cells in *in vivo* models with following successful B cell reconstitution.

Conclusion: In conclusion, we could show the feasibility of the proposed cellular safeguard mechanism and its clinical relevance in reverting long-term, CAR-T cell related toxicities.

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Induction of anti-tumor responses via antigen targeting of dendritic cells *in vivo*

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Multiple groups have shown that antigen targeting by using recombinant antigen-conjugated antibodies against different endocytic receptors specifically expressed on DCs is an excellent method to trigger and modulate immune responses *in vivo*. By taking advantage of the subset-specifically expressed endocytic C-type lectin receptors DEC205 present on splenic CD8+ DCs or DCIR2 on CD11c+CD8- DCs, it is possible to predominantly trigger antigen-specific CD8+ or CD4+ T cell responses, respectively. By this targeting approach the survival of tumor challenged mice is prolonged accompanied by a reduced tumor growth. Recently, we demonstrated FcγRIV, present on both classical DC subsets in murine spleen, to be effective in the induction of concomitant CD8+ and CD4+ T cell responses. In this project, we focus on two central aspects:

Potential of FcγRIV targeting: Even though this receptor is expressed on an array of immune cells, the induction of immune responses by targeting of FcγRIV is solely dependent on DCs. Since this receptor is found on both, CD8+ and CD8- DCs, and induced potent T cell responses, we set out to determine, if the targeting of this receptor is superior compared to targeting approaches via DEC205 and DCIR2 in murine tumor models *in vivo*.

Mechanism of tumor protection by DCIR2 targeting: Antigen delivery via DEC205 induces CD8+ T cells capable of killing tumor cells directly. In contrast, DCIR2 targeting triggers only minor T cell responses in naïve mice. In the

DOI: 10.1002/eji.201970300

current project, we want to obtain mechanistic insights in the anti-tumor responses induced by DCIR2 targeting. We hypothesize that the minor induction of CD8+ T cells by DCIR2 targeting is sufficient to kill a small number of tumor cells. As a consequence, other tumor specific antigens are released and trigger de novo immune responses (CD8+ T cells and/or antibodies), which can in turn explain the observed protection. To investigate this, we are using several tumor cell lines to study cross-reactivity and the tumor-associated-epitope spreading after the initial immune response.

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P370

Establishment of a vaginal HPV16 E6$^+$/E7$^+$ tumor model in MHC-humanized A2.DR1 mice

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Introduction: We recently demonstrated the therapeutic efficacy of amphiphilic peptide vaccinations in a subcutaneous HPV16 (human papillomavirus 16) tumor model in MHC-humanized A2.DR1 mice. The mucosa, where HPV infections naturally occur in humans, is more difficult to access for T cells than the subcutis. To better mimic the situation in patients, we now aim to establish the tumor model in the anogenital mucosa. This orthotopic location will allow for a more detailed understanding of the requirements for successful therapeutic HPV16 vaccination.

Objectives: The desired tumor model has to express the vaccination target proteins HPV16 E6 and E7, which also cause cell immortalization. Moreover, an activated oncoprotein is required for tumorigenicity and luciferase for tracking of tumor growth. For this project, we follow two paths: first, we adapted our HPV16-positive tumor model PAP-A2 for the orthotopic setting and second, we developed a new tumor model based on lung cells from A2.DR1 mice.

Materials & methods: Both cell types were transduced with HPV16 E6 and E7, transfected with luciferase and - in the case of the lung cells - with activated H-Ras to render them tumorigenic. The cells were tested for tumorigenicity subcutaneously as well as intravaginally.

Results: Western blot analysis confirmed the expression of E6, E7 and H-Ras, and luminescence measurements proved luciferase expression. After validation of tumorigenicity of subcutaneously injected cells, cells were instilled intravaginally into A2.DR1 mice. Subsequent monitoring of the genital region revealed the growth of tumors in the vaginal cavity.

Conclusion and Outlook: Once the new orthotopic tumor model is completely established in A2.DR1 mice, it will be used to test novel therapeutic vaccination approaches against HPV16-positive tumors *in vivo* and to examine strategies to induce the migration of tumor-reactive T cells to mucosally located tumors. This can be either achieved by directly inducing tissue-resident T cells on-site or to influence the migration of vaccination-induced T cells to the mucosa.

Taken together, the new HPV16 E6$^+$/E7$^+$ orthotopic tumor model in MHC-humanized A2.DR1 mice will help to develop effective therapeutic HPV16 vaccinations by studying and influencing the trafficking of T cells to the mucosal tumor site.

DOI: 10.1002/eji.201970300

P371

Expansion of human CD4+ T cells *in vitro* by bead-bound conventional anti-CD28 monoclonal antibodies

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The most widely applied protocols for *in vitro* expansion of human T cells, including those used for immunotherapeutic purposes, rely on monoclonal antibodies (mAb) with specificity for CD3 and the co-stimulatory protein CD28. Anti-CD28 mAb, with the exception of so-called superagonistic anti-CD28 mAb like TAB08 (TGN1412), are believed not to be able to induce full T cell activation and expansion. Here we describe a novel method using non-superagonistic, i.e. conventional, anti-CD28 mAb coated on Dynabeads (bead-bound conventional anti-CD28; BBC-28) capable of activating and expanding human CD4+ T cells *in vitro*. BBC-28 did not induce preferential expansion of CD4+ Foxp3+ regulatory T cells over Foxp3- conventional CD4+ T cells as has been observed upon superagonistic anti-CD28 mAb stimulation of human CD4+ T cells *in vitro*. Accordingly, BBC-28-expanded human CD4+ T cells fully retained the capacity to proliferate and produce effector cytokines in response to clinically relevant recall antigens. Currently, we are testing the impact of BBC-28 stimulation on the cytotoxic activity of human CD4+ and CD8+ T cells against cancer cells focusing on malignant melanoma. Molecularly, we noted that the activity of the acid sphingomyelinase (ASM) catalyzing cleavage of sphingomyelin into ceramide and phosphocholine provides a cue regarding the form of signaling required for conventional anti-CD28 mAb to induce T cell proliferation: Conventional anti-CD28 mAb in solution induced a strong increase in enzymatic activity of the ASM, while immobilizing these antibodies on beads (BBC-28) prevented induction of ASM activity. This study was supported by grants from the DFG (FOR2123/P02) and the BMBF (031L0156C).

P372

TCR:pMHC koff-rate as a functional parameter for selecting T cell receptors with therapeutic value for adoptive cell therapy

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Question: Clinical success of adoptive cell therapy (ACT) with TCR-engineered T cells is still limited by the restricted number of available TCRs. Besides some difficulties to access relevant tumor sites, the spatiotemporal distribution of tumor-specific T cells in patients is still unknown. Peripheral blood of healthy donors, instead, represents a more reliable source as it is accessible and the antigen unexperienced TCR repertoire should contain T cells for any antigen specificity. However, naïve antigen-specific T cell populations are of low frequency and contain a substantial fraction of T cells with low avidity TCRs. Our aims are therefore i) to develop a workflow for reliable isolation and extraction of TCRs from the naïve repertoire and ii) to evaluate TCR structural avidity as a predictor of TCR functionality.

Methods: Antigen-specific CD8+ T cells are single cell sorted according to pMHC multimer staining. After short-term feeder-free expansion, antigen specificity and TCR structural avidity of single-cell derived clones are analyzed by pMHC multimer re-staining and TCR:pMHC koff-rate measurement, respectively. Paired TCR-α and TCR-β chains of candidate clones are sequenced and the full TCR finally re-expressed in T cells for functional validation (i.e. cytokine release upon antigen stimulation and koff-rate).

Results: For proof-of-concept, we screened naïve-like T cells from an HLA-A*02 yellow fever (YFV) vaccinated healthy donor for TCRs against the A2/YFV-NS4b212-22 epitope; as expected, most of the re-expressed TCRs showed high avidity and functionality. Assessing the naïve repertoire of HLA-A*02 healthy donors, high avidity T cells could be obtained with specificity for the tumor associated antigen (TAA) A2/MART1 (A27L)26-35, in accordance with limited thymic presentation of the antigen. On the other side, among the TCRs isolated from populations specific for the thymically presented TAA A2/Her2/neu369-377 only one functional TCR was successfully identified. Furthermore, we were also able to identify neo-antigen specific TCRs from extremely rare populations, such as T cells specific for the tumor suppressor antigen A2/RNF43. Remarkably, TCR koff-rate predicted T cell functionality.

Conclusions: In conclusion, isolation of TCRs valuable for ACT from rare antigen-specific CD8+ naïve T cells is feasible and the TCR:pMHC koff-rate is a reliable parameter for screening of functional TCRs.

DOI: 10.1002/eji.201970300

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Enhancement of early human B cell development by JAK inhibition

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Background: JAK inhibition treatment with tofacitinib modulates the signaling of several cytokines involved in lymphocytes development and activation and has been proved to be effective in Rheumatoid arthritis (RA). Severe combined immunodeficiency patients with JAK3 or common γ chain mutations show defective B cell activation and memory formation. Hence, tofacitinib treatment may directly impact B cell function.

B cells develop from HSC. Mouse B cell development depends on IL-7 signaling, in human on pre-B cell receptor signaling. Though several cytokines play a role in the regulation of early B cell development. Hence the impact of JAK inhibition on early B cell development needs to be studied.

Objective: To assess the impact of JAK inhibition on early B cell development *in vitro*.

Methods: We used an *in vitro* model in which $CD34^+$ cells isolated from cord blood are cultivated subsequently in SCF, FLT3L and IL6, then SCF, Flt3L and IL7 and finally in cytokine-free medium. The culture reproduces all stages of development from common lymphoid progenitors to immature B cells. We performed the culture in the presence of 100 nM tofacitinib.

Results: Tofacitinib treatment enhances commitment into B cell lineage from $CD34^+$ cells and leads to increased numbers of pre-B and immature B cells in comparison to untreated cells. In presence of tofacitinib the expression of lineage specification and commitment transcription factors occurs earlier and results in enhanced commitment in the early phase of differentiation. Patients treated with tofacitinib show increased peripheral B cell numbers at 8-12 weeks after start of therapy.

Conclusions: Our data indicate that JAK inhibition may promote early B cell development by enhancing the commitment of lymphoid precursors to the B cell compartment, contributing to a temporary increase in relative and absolute numbers of B cells in peripheral blood of treated patients. These data contribute to our understanding of human B cell development, prompt us to further analyze the quality of B cell output from the bone marrow in JAK inhibited patients, and may provide cues to understand the outcome of JAK inhibition treatment in rheumatic diseases.

P374

Short-term expansion of BK-virus-specific T-cells from immunosuppressed patients after kidney transplantation indicates potential for adoptive T-cell therapy

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Introduction: Patients after kidney transplantation are continuously immunosuppressed to avoid graft rejection. As a consequence, patients are highly susceptible to polyomavirus BK (BKV) infections, which cause BKV-associated nephropathy with graft failure in up to 30% of cases. Therapeutic options are limited and mainly based on reduction of immunosuppression, which, however, leads to acute graft failure in about 10% of patients. Recently, an association between reduced BK viral load and reconstitution of BKV-specific T-cells (BKV-T-cells) were seen in patients after kidney transplantation, emphasizing the importance of BKV-specific immunity.

Objectives: We aimed to verify whether BKV-T-cells derived from immunosuppressed transplanted patients with or without acute BKV viremia can be expanded ex vivo as a prerequisite for the adoptive transfer of BKV-T-cells.

DOI: 10.1002/eji.201970300

Patients and Methods: For the ex vivo short-term expansion (STE), isolated mononuclear cells of 11 healthy donors (HD), 11 patients without (BKV-) and 11 patients with BK viral load (BKV+) were stimulated at day 0 and 6 with BKV-specific peptide pools and with interleukin 15 at day 9. After 12 days, cell products were characterized via IFN-g-ELISpot and intracellular staining of activation markers (such as IFN-g and TNF-α) including detailed analyses of T-cell phenotypes via flow cytometry. Furthermore, cytolytic activity and potential alloreactivity of expanded BKV-T-cells against autologous and allogeneic target cells were assessed via flow cytometry.

Results: STE revealed highly specific BKV-T-cell spot forming colonies (SFC)/105 cells) from healthy donors (558±138/105 cells), patients without (504±141/105 cells) and with BK viral load (501±101/105 cells) without significant differences between these groups. Generally in all groups, predominantly CD4+ T-cells were expanded (HD: 59%±9 vs BKV+: 60%±15 vs BKV-: 65%±11) compared to CD8+ T-cells (HD: 33%±6 vs BKV+: 33%±12 vs BKV-: 27%±10) among CD3. In all 3 groups, CD8+ and CD4+ T-cells showed an effector-memory (CD62L-/CD45RA-) and central-memory (CD62L+/CD45RA-) phenotype, known to induce fast and long-term immunity, respectively. Expanded CD8+ as well as CD4+ BKV-T-cells were "polyfunctional" as determined by secretion of different cytokines such as IFN-g and TNF-a.

Conclusion: Successful ex vivo expansion and characterisation of patient-derived BKV-specific T-cells is a prerequisite for a potential follow-up study treating patients with BKV viremia.

P399

Consistent, long-term PD1 blockade using a syngeneic, engineered anti-mPD1 antibody: Superior tools for consistent and more meaningful immunotherapy research

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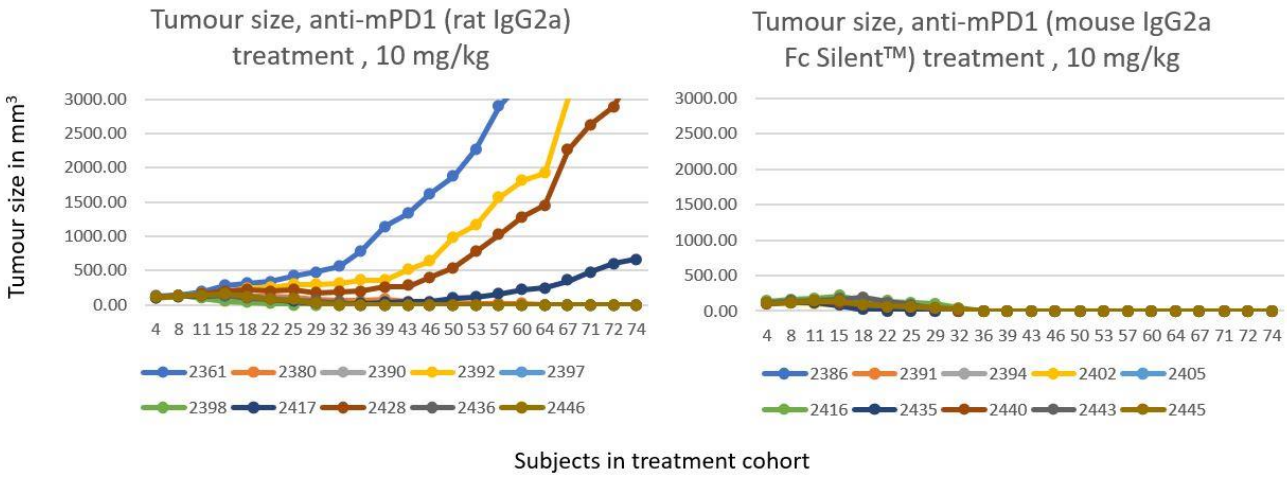
Immune-checkpoint blockade has established itself as an effective cancer therapy, with anti-PD1/PDL1 and anti-CTLA4 antibodies being used to treat diverse cancers. However, basic research into the biology underlying treatment response, particularly in long-term treatment, is often hampered by the quality of reagents available, as most antibodies used *in vivo* in mice are of rat or hamster origin, and are therefore immunogenic, leading to underlying immune activation. Anti-drug antibodies are rapidly generated against these antibodies to inconsistent responses within cohorts as well as gradual lack of response to the treatment. In this study, we compare the efficacy of the anti-mouse PD1 clone RMP1-14 in its original rat IgG2a format against an engineered mouse IgG2a Fc Silent™ version at different doses over a period of 74 days in a non-immunogenic HEP1-6 murine hepatocellular carcinoma model. Syngeneic mouse IgG2a Fc Silent™ antibodies showed better dose-efficacy and more homogenous treatment responses. These results show how recombinant antibody engineering can be applied to improve even gold-standard experimental procedures, allowing researchers to gain a clearer view of *in vivo* biology without the artefacts induced by immunogenic reagents. Moreover, improvements to animal welfare, through the use of smaller injection boli and reduction of adverse immunological reactions, must also be considered. We conclude that, where available, researchers should opt to use syngeneic antibodies in *in vivo* applications.

Figure 1

DOI: 10.1002/eji.201970300



P400

Selective inhibition of the transcription factor nuclear factor of activated T-cells (NFAT) in Graft-versus-Host disease

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Introduction: Patients suffering from hematologic malignancies are often treated with allogenic hematopoietic stem cells transplantation (HSCT) from a healthy donor. T cells within the graft eradicate tumor cells and thereby prevent tumor relapses known as graft-versus-leukemia (GvL) effect. However, donor T cells might also attack nonmalignant tissue of the host with life threating consequences called graft versus-host-disease (GvHD). To prevent and treat GvHD, patients receive cyclosporine A or tacrolimus, which not only often cause severe adverse effects, but also interfere with the anticipated GvL. Both drugs inhibit the phosphatase calcineurin, thus suppressing activation of 'nuclear factor of activated T-cells (NFAT). Our group found that absence of one or two members of the NFAT family in T cells prevents harmful GvHD, but preserves the valuable GvL (Vaeth et. al. 2015)

Objective: Instead of broad immune suppression, we aim to target individual NFAT members specifically by CRISPR/Cas9 in primary murine and human T cells before allogenic stem cell plus T cell transplantation and evaluating the edited T cells for their efficacy to mitigate GvHD while still conferring the GvL effect.

Methods: Primary CD3 T cells from C57BL/6 mouse expressing Cas9, luciferase and CD90.1 were stimulated and electroporated with guide RNAs specific for NFATc1 or NFATc2. Knock out efficiency (protein loss) was measured in flow cytometry. Together with CD90.2 bone marrow, edited T cells will be transferred into irradiated CD90.2 BALB/c mice for an acute GvHD model. Weight loss, disease scoring and *in vivo*/*ex vivo* bioluminescence imaging analysis will be performed.

Results: We have successfully established CRISPR/Cas9-mediated knockout in a mouse lymphoma cell line and primary mouse T cells with an efficiency of 80%. We have observed significant reduction in NFAT target gene expression for e.g. IL-2, IFN-γ and CD40L resembling T cells isolated from NFATc1 or NFATc2 deficient mice. We have also observed that stimulated and electroporated T cells are able to expand *in vivo* in the BALB/c mouse model and causes acute GvHD.

Conclusion: We have successfully optimized a method to knockout individual NFAT members with 80% efficiency in mouse primary T cells. With this, the project will allow us to explore the possibility of specific NFAT deletion in donor T cells ahead of HSCT for the benefit of patients with hematologic malignancies, preventing GvHD, while maintaining GvL.

DOI: 10.1002/eji.201970300

P404

SpotMix™ peptide pools – a new approach for rapid antigen target identification

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Introduction: Enhancing specific immune responsiveness by vaccination is a therapeutic approach to treat infectious diseases and cancer. Its success, however, depends on the identification of suitable target antigens and epitopes. Stimulation with proteins or whole protein-spanning, overlapping peptide pools (PepMixTM) has been successfully used for that purpose. However, this approach comes with a significant effort in terms of time, labor and cost, in particular if many potential candidate proteins have to be analyzed.

Objectives: Based on a method for the highly parallel synthesis of multiple peptides in low quantities, we developed a novel and fast protocol which permits the production of so called SpotMixTM peptide pools that can be used in commonly used T-cell assays like ELISpot and flow cytometry.

Materials & methods: We isolated PBMC from different CMV positive donors and performed ELISpot experiments to detect Interferon gamma (IFNg) responses upon stimulation with single peptides or peptide pools belonging to CMV antigen pp65 generated by different synthesis strategies.

Results: The stimulation of PBMCs with PepMixTM and SpotMixTM peptide pools revealed comparable results. Furthermore, using the Matrix pooling strategy for pp65 SpotMixTM peptides we show an efficient way to identify distinct T-cell epitopes within pp65.

Conclusion: The SpotMixTM peptide pools are optimal tools for various applications like antigen target identification, pathogen-spanning T-cell epitope discovery, immunogenicity testing or Neo-epitope prioritization, allowing assay implementation within a considerably shorter time frame.

Regulatory and helper T cells (P375-P397)

P375

The alarmin IL-33 drives a ST2+ Treg-mediated anti-inflammatory immune response during immune-mediated hepatitis

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Introduction: In patients with liver disease, serum levels of the alarmin IL-33 are elevated, indicating an immunomodulatory function of IL-33 in hepatic inflammation. In Concanavalin (Con)A-induced immune-mediated hepatitis, IL-33 is released by necrotic hepatocytes and induces immune responses by signaling through the IL-33 receptor ST2. We have shown previously that IL-33 pre-treatment protected from immune-mediated hepatitis, suggesting an immunosuppressive role of IL-33 in liver disease. Objectives: Since regulatory T cells (Tregs) expressing ST2 respond to IL-33, we aimed at investigating the IL-33-driven ST2+ Treg response in the inflamed liver.

Materials & methods: To induce immune-mediated hepatitis, mice received ConA and were analyzed 24 hours later. To address the immunosuppressive effect of IL-33 on disease pathology, mice were treated with IL-33 on three days before ConA challenge. The phenotype of hepatic Tregs was determined by flow cytometry. Tregs from IL-33-treated FIR-tiger mice were adoptively transferred into C57BL/6 mice, which received ConA one day later.

Results: In homeostasis, the frequency of ST2+ Foxp3+ Tregs was elevated in the liver compared to the spleen. Hepatic ST2+ Tregs expressed higher levels of Foxp3 than ST2- Tregs and the frequencies of ST2+ Tregs expressing ICOS, KLRG1, CD103, TIGIT, GITR, and CTLA-4 were increased, indicating an activated phenotype of this Treg subset in the steady-state. Moreover, hepatic ST2+ Tregs were characterized by expression of GATA3, the epidermal growth factor amphiregulin, and the anti-inflammatory cytokine IL-10. In immune-mediated hepatitis, the frequency of ST2+ Tregs was enhanced that up-regulated expression of CD25, ICOS, CD103, TIGIT, GITR, CTLA-4, and PD-L1, and maintained high IL-10 expression. Moreover, ST2-/- mice developed more severe immune-mediated hepatitis,

DOI: 10.1002/eji.201970300

despite an elevated frequency of Foxp3+ Tregs, underlining the importance of the IL-33/ST2 pathway for hepatic immune regulation. IL-33 pre-treatment before induction of hepatitis increased the frequency of activated hepatic ST2+ Tregs compared to ConA-treated mice, which was associated with reduced liver inflammation and necrosis. Moreover, transfer of IL-33-elicited Tregs before ConA challenge potently suppressed immune-mediated hepatitis.

Conclusion: The immune regulatory function of IL-33 in immune-mediated hepatitis might be driven by expansion and recruitment of a highly activated Treg population expressing ST2.

P377

Influence of early-life perturbations on phenotype and maintenance of neonatally-generated Foxp3+ regulatory T cells

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Introduction: The first wave of Foxp3$^+$ regulatory T cells (Tregs) generated in neonates has been reported to be stably maintained in various organs for long-term, display a distinct phenotype compared to Tregs produced during adulthood and be indispensable for life-long prevention of autoimmunity.

Objectives: As neonates are particularly susceptible to infections, we asked if infections acquired at neonatal age might affect the generation of the first wave of Foxp3$^+$ Tregs, resulting in long-lasting consequences for immunological tolerance.

Materials & Methods: Neonatal (day 0 old) Foxp3^{EGFPCreERT2}Rosa26^{YFP} mice were treated with the TLR agonists LPS or Poly(I:C) to mimic transient infections with bacteria or viruses, respectively. Subsequently, mice received repetitive injections of Tamoxifen on day 1, 4 and 7 to label neonatally-generated Foxp3$^+$ Tregs with permanent YFP expression. PBS-treated littermates receiving repetitive Tamoxifen injections served as controls. At selected time points later in life, we analyzed the tissue distribution, stability and phenotype of neonatally-generated Foxp3$^+$ Tregs by flow cytometry.

Results: In both TLR agonist- and PBS-treated mice neonatally-labeled YFP$^+$ Tregs preferentially accumulated in non-lymphoid when compare to lymphoid organs. Yet, one or three weeks after birth barely any differences between the tested groups were observed regarding thymic Treg development as well as maintenance in peripheral lymphoid and non-lymphoid organs. Surprisingly, at later time points (six and twelve weeks after birth) the frequency of YFP$^+$ Tregs in both lymphoid and non-lymphoid organs decreased significantly in TLR agonist-treated mice when compared to PBS-treated controls. This decrease was not due to a loss of Foxp3 stability as similar frequencies of Foxp3$^+$ cells were observed among YFP$^+$ cells between the tested groups. However, substantial differences regarding the expression of tissue-residency markers like KLRG1, CD69 and ST2 as well as markers for effector Treg subsets like T-bet, Gata3, CD44 and CD25 were found.

Conclusions: Together, our study demonstrates that early-life perturbations alter the phenotype of neonatally-generated Tregs in the adult phase, suggesting that infections acquired at neonatal age might have long-lasting consequences for immunological tolerance, prevention of autoimmunity and other non-canonical functions of tissue-resident Tregs.

P378

T cell intrinsic and DC-mediated effects of PARP-1 in Foxp3 Treg versus Th17 cell differentiation

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Introduction. PARP-1 (poly(ADPribose)polymerase-1) is an enzyme involved in DNA damage detection/repair, inflammation and cell death. Recent findings, including ours, unveiled the role of PARP-1 in lymphocyte activation and differentiation. PARP-1KO mice are resistant to the induction of several inflammatory/immune-mediated diseases while PARP inhibitors reduce the disease burden in colitis, EAE, rheumatoid arthritis and allergy.

Objectives. To understand the role of PARP-1 in the balance between effector and regulatory T cell differentiation and response.

DOI: 10.1002/eji.201970300

Materials & methods. Naïve CD4, Treg and dendritic cells (DC) were purified by immunomagnetic sorting from WT or PARP-1KO C57Bl/6 mice and stimulated with anti-CD3/CD28 in the presence of TGFb1 and/or IL2 and/ or IL6. mRNA were analyzed by RT and real time PCR; cell phenotype by flow cytometry with surface/intracellular/intranuclear staining. RAG2KO recipient mice and WT vs PARP-1KO congenic mice were used in bone marrow chimera competitive assays.

Results. We had found that PARP-1KO mice display increased number of regulatory CD4+Foxp3+ T cells (Tregs) in central as well as peripheral lymphatic organs compared with WT controls. PARP-1KO Tregs were functional as assessed both *in vitro* and *in vivo*. While in a chimera competitive assay PARP-1KO thymocytes generated higher numbers of Tregs compared with WT cells, we wondered whether conversion of naïve CD4 cells to inducible Tregs (iTregs) was also affected. Purified naïve CD4 cells from PARP-1KO mice, stimulated *in vitro* with CD3/CD28 and TGFb1, expressed Foxp3 mRNA at higher levels and generated a greater number of Foxp3+ iTregs than the WT counterpart. Interestingly, *in vitro* differentiation of purified naive CD4 cells to Th17 cells, as induced by CD3/CD28, TGFb1 and IL-6, was not affected by PARP-1 deficiency. In peripheral lymphatic organs, conversion to Foxp3+ iTregs occurs upon stimulation by DCs in a tolerogenic context. Noteworthy, we found that purified WT naïve CD4 cells, stimulated with CD3 and TGFb1 in the presence of either WT or PARP-1KO DCs, generated comparable numbers of Foxp3+ iTregs. At variance, WT DCs induced a higher frequency of IL17+CD4+ cells compared with PARP-1KO DCs.

Conclusion. Altogether, these results demonstrate that PARP-1 plays an important role in the balance between regulatory and Th17 cell differentiation with the involvement of both CD4 T cell intrinsic and DC-mediated effects.

P379

Regulating microvesicle transport at the immunological synapse

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Introduction: The immunological synapse (IS) of T cells is the organizing compartment establishing (i) the formation of the TCR signalling network and (ii) polarized immune responses including the release of IL-2 and transexocytosis of microvesicles (MVs) towards antigen-presenting cells (APCs). We and others have previously identified a unique TCR network assembly at the IS discriminating Tconv- and Treg cells. Furthermore, by using phosphoproteomics we revealed a set of nine cytoskeleton regulators that belong to the TCR signalling network and likely orchestrate signal component recruitment and MV transport at the T cell IS.

Objective: This project aims to clarify the role of the newly identified cytoskeleton regulators in controlling MV transport at the IS of T cells. Functional studies shall focus on candidate regulators that selectively allow the modulation of TCR signalling and related polarized immune responses.

Methods: The CRISPR/Cas9 technology was used to generate mutant cell lines that are now studied in T cell/B cell conjugates using superantigens. Microscopy and Multi-epitope cartography (MELC) analysis supports the profiling and identification of IS formation phenotypes and variant MV transport. Targeted proteome approaches and multiplex/qPCR-based cytokine profiling are used for in-depth characterization of MV contents and T cell responses, respectively.

Results: We have generated four mutant T cell lines, of which we are now focussing at LIC1 and DBNL. The dynein motor subunit LIC1 was found to affect the spatial organization of the cSMAC and to selectively modulate the secretion of 2 out of in total 27 tested cytokines. DBNL alters both the cSMAC and the pSMAC without greatly interfering with upstream TCR signalling events. At the moment, we collect proteome data to fully understand the TCR-induced and LIC1- and DBNL-dependent immune responses. Furthermore, we have established and will present two analytical strategies to identify the MV inventory that is transexocytosed upon the conjugation of T cells with APCs, and that we again found to discriminate Tconv- and Treg cells.

Conclusion: Cytoskeleton regulators constitute a promising target to manipulate MV transport, MV cargo and T cell immunity. Here, we will present and discuss first novel immune regulatory pathways for conventional T cells.

DOI: 10.1002/eji.201970300

P380

Imaging of regulatory T cell dynamics and interactions with conventional T cells in skin-draining lymph nodes

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Regulatory T (Treg) cells expressing the transcription factor Foxp3 are pivotal for the maintenance of immune homeostasis and tolerance.This hypothesis is underlined by the fact that several autoimmune and inflammatory pathologies in humans are associated with functional defects in Treg cell populations. Animal models also revealed that depletion of Treg cells leads to allergic disease or autoimmune responses that are accompanied by uncontrolled lymphoproliferation, for example of Foxp3-negative conventional T cells (Tconv). There exists already brilliant two-photon imaging data, visualizing a contact between Treg and Tconv cells in in skin-draining lymph nodes (SDLN) of ovalbumin (OVA) immunized mice.

Based on experimental limitations, it is not fully understood whether resting or proliferating Treg cells interact with Tconv cells within distinct micro-compartments of SDLNs. In order to clarify this aspect, we used the experimental model of leishmaniasis. In this model, parasite-derived antigens are presented by lymph node-homing cutaneous dendritic cell (DC) subsets. In contrast to OVA-immunization models, experimental leishmaniasis is suitable to characterize adaptive immunity to skin-derived antigens during an inflammatory driven immune response. Thus, basic immunological questions concerning the development and maintenance of T cell subsets within SDLNs can be answered.

SDLNs of infected C57BL/6 mice were analyzed at day 10 after infection. Software-based multicolor histo-cytometry studies were performed to highlight cellular rendezvous *in situ*. As expected, we were able to detect proliferating Treg cells within the paracortex of the SDLNs. However, detailed cell-cell-neighborhood analysis revealed that proliferating Treg cells exclusively form cluster with expanding Tconv cells. Such clusters of proliferating Treg and Tconv cells are formed in delineated regions around antigen-presenting DCs. Continuing imaging flow cytometer analysis supported the concept that proliferating Treg cells can form specialized immunological synapses with Tconv cells of distinct subtypes, indicating a high level of immune regulation.

Our data therefore support the concept that proliferating Treg cells interact preferentially with primed Tconv cells within dedicated cell clusters.

P381

FOXO4 and AHR control a stress-induced immune-mediated host protection via secretion of IL-22

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Introduction: Epithelial barrier integrity is of outermost importance to protect the human body from external harm. To ensure this protection, defense mechanisms against pathogens are needed, but at the same time the initiation of repair mechanisms following inflammation induced tissue damage is crucial. One of the key cytokines involved in tissue regeneration is interleukin (IL)-22. During inflammation, cells capable of IL-22 production are recruited into barrier organs to restore inflammation mediated barrier damage. The aryl hydrocarbon receptor (AHR) has been described to regulate IL-22 expression in T cells. Previous results from our group indicated that another transcription factor belonging to the Forkhead box O (FOXO) family, FOXO4, is expressed in human T cells.

Objectives: FOXO4 has been mainly described as tumor suppressor and regulator of senescence and longevity. In line with this, Foxo4 mRNA is expressed in various tissues at low level and downregulated in most tumors. FOXO4 mRNA and protein have been detected in mouse CD4+ T cells and our group could assign FOXO4 expression to human T cells of the Th22 subset. Despite this, a role of FOXO4 in shaping T cell phenotype and function has not been performed so far.

DOI: 10.1002/eji.201970300

Methods:We used a lentiviral transduction system to modify FOXO4 expression in CD4+ effector T cells and in naïve T cells that were subsequently cultured under Th22 polarizing conditions. FOXO4 expression was evaluated by qRT-PCR and western blot analysis. Cytokine secretion of restimulated T cells was assessed by ELISA. Protein-protein interactions were analyzed by Co-Immunoprecipitation. Further, a scratch assay was performed to assess the role of FOXO4 mediated IL-22 secretion in wound healing.

Results: FOXO4 expression is induced during *in vitro* differentiation of Th22 cells. Moreover, IL-22 secretion of vitro differentiated Th22 cells overexpressing FOXO4 was increased while FOXO4 knockdown in these cells led to decreased IL-22 secretion. Further, we showed that IL-22 secretion was co-dependent on AHR. A physiological role of FOXO4 dependent effects in IL-22 secretion was confirmed by scratch assays showing decreased artificial wound healing with supernatants from FOXO4 deficient T cells.

Conclusion:Here, we now show that oxidative stress and pro-inflammatory cytokines stabilize FOXO4 expression in human T cells allowing cooperation with AHR to regulate IL-22 in effector cells and differentiating naïve T cells.

P382

CD4+ Foxp3+ regulatory T cell-mediated immunomodulation by pharmacological inhibition of the acid sphingomyelinase in humans

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Lack of acid sphingomyelinase (ASM) activity, catalyzing the cleavage of sphingomyelin to ceramide and phosphocholine, is associated with increased activity and frequency of Foxp3+ regulatory T cells (Treg) among CD4+ T cells in mice *in vivo* and *in vitro* (Hollmann et al., JI 197:3130, 2016). Therefore, blockade of ASM activity might constitute a new therapeutic mechanism to correct numeric and/or functional Treg deficiencies in diseases like multiple sclerosis or major depression. In the present study, we first performed *in vitro* experiments with human T cells from healthy human blood donors using two widely prescribed antidepressants with high (sertraline) or low (citalopram) capacity to inhibit ASM activity. In human PBMCs (peripheral blood mononuclear cells), similar to our findings in mice, we observed an increase in the frequency of Treg among human CD4+ T cells upon inhibition of ASM activity. Furthermore, looking into the mechanism behind this effect, we found that inhibition of ASM activity was associated with changes in the expression and turn-over of CTLA-4, a key inhibitory molecule expressed by Treg. We then assessed the effect of pharmacological ASM inhibition on human Treg *in vivo* by prospectively analyzing the composition of CD4+ T cells in patients treated for major depression. Our data show that pharmacological inhibition of ASM activity was superior to anti-depressants with little or no ASM-inhibitory activity in normalizing CD45RA-CD25high effector Treg frequencies among CD4+ T cells in patients treated for depression. Moreover, correlating our data with the clinical response (HAMD week four < 50% HAMD upon admission), we found that an early increase in effector Treg frequencies among CD4+ cells during the first week of treatment identified responders. In summary, the frequency of (effector) Treg among CD4+ T cells in mice and humans is increased after inhibition of ASM activity suggesting that ASM blockade might beneficially modulate autoimmune diseases and depression-promoting inflammation. This study was supported by a grant from the DFG (FOR2123 project P02).

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Role of Histone Methylation in Foxp3+ Treg Cell Development and Autoimmunity

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CD4+Foxp3+ regulatory T (Treg) cells develop both, in thymus (tTreg) and peripheral lymphoid tissues (pTreg) and prevent catastrophic autoimmunity throughout life. A common hallmark of Treg cell development is the induction of Foxp3 expression upon appropriate antigen and cytokine stimulation, with Foxp3 acting as a stabilizer/amplifier of

DOI: 10.1002/eji.201970300

Treg cell-specific gene expression, including the *Foxp3* gene itself. Besides several conserved non-coding regions (CNS1-3) that fine-tune *Foxp3* gene expression, efficient DNA demethylation of CNS2 and promoters of Foxp3 targets are essential for the stability of a Foxp3+ Treg cell phenotype. Global mapping studies further indicated the establishment of a Treg cell-specific epigenetic landscape during development, but the functional relevance of such epigenetic regulation, in particular histone methylation, has remained poorly understood. Here, we have analyzed the role of two members of the H3K4 methyltransferase family, Mll1 and Mll2, in the biology of Foxp3+ Treg cells. Constitutive deletion of Mll1/Mll2 in adolescent BAC.Foxp3GFP-Cre x Mllfl/fl mice resulted in moderate aberrations in thymic and peripheral Foxp3GFP-Cre+ tTreg cell compartments. In Mll1/Mll2 double-deficient mice, peripheral Foxp3GFP-Cre+ tTreg cells were severely abrogated during age progression, with individual lymph nodes being enlarged due to increased effector T cell numbers, but immune tolerance was largely maintained by Foxp3+ Treg cells that escaped Mll deletion due to the lack of transgenic BAC.Foxp3GFP-Cre expression. Strikingly, heterozygous Foxp3-driven deletion of Mll1 & Mll2 promotes aggressive autoimmune diabetes in adult NOD BAC.Foxp3GFP-Cre x Foxp3IRES-RFP x Mll1/2fl/wt mice, whereby diabetes incidence strongly correlates with homozygous MHC class II I-Ag7 expression. Ongoing studies focus on the molecular mechanisms underlying this novel function of Mll1 & Mll2 in Foxp3+ Treg cell development and autoimmunity.

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Role and Regulation of innate IL-1α production by Human T helper cells in health and disease

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Over the last few years Th17 cells have been recognized as major drivers of inflammatory diseases. Th17 cells also display heterogeneity and plasticity, which translates into distinct functions in settings of health and disease and which can be exploited for therapeutic purposes. Previously, we"ve identified the existence of pro-inflammatory and anti-inflammatory human Th17 cell subsets, which differ in their ability to produce IL-10, their microbial antigen specificities and their priming requirements (Zielinski CE et al. Nature 2012). Novel insights indicate that the pro-inflammatory Th17 cell subset can also produce IL-1α, an innate danger signal and alarmin that might confer pathogenicity to this T cell population. The expression of IL-1α by an adaptive immune cell subset therefore prompted us to investigate its characteristics and regulation in detail. We found that the molecular machinery driving the Th17 cell identity is coupled to IL-1a expression . Surprisingly, we could demonstrate a role for the NLRP3 inflammasome for the secretion of IL-1a but not IL-1b. Its regulation followed different rules than those in innate immune cells. Taken together, we demonstrate that IL-1a represents a novel, so far overlooked effector cytokine of human Th17 cells that is regulated by an alternative mode of inflammasome activation. This demonstrates that innate signaling mechanisms can be adopted by adaptive T cells to exert pro-inflammatory functions. Future work will reveal the impact of this T cell program for human health and disease.

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Foxp3 specific activation of Nrf2 results in loss of immune tolerance

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Introduction: Metabolic processes, toxic insults, infections and inflammation lead to situations where pro-oxidants outbalance the anti-oxidative system in cells, a state of ""oxidative stress"" is reached. Oxidative stress can activate a variety of transcription factors, most importantly Nrf2. However, the specific function of Nrf2 in regulatory T cells (Tregs), the central regulators of immune homeostasis, is unclear.

Objectives: We aimed to study how oxidative stress influences Tregs by analyzing the key transcription factor of the oxidative stress response - Nrf2.

Material and Methods: Consequences of constitutive Nrf2 activation in $Foxp3^{+}$ cells was analyzed with $Foxp3^{cre}Keap^{fl/fl}$ mice, T cells from $Nrf2^{-/-}$ mice were used to analyze consequences of a Nrf2 deletion. Mixed bone marrow chimeras were created to confirm Treg-intrinsic effects of Nrf2.

DOI: 10.1002/eji.201970300

Results: A Treg specific activation of Nrf2 resulted in an autoinflammatory phenotype with enhanced effector T cell activation and immune cell infiltrates in the lung. Bone marrow chimeras revealed that Nrf2 activation intrinsically in Tregs leads to a loss of Treg mediated tolerance *in vivo*. Moreover, Nrf2 activation in Tregs downregulated Foxp3 expression, but enhanced their glucose uptake and mTOR activity, thus mimicking a metabolic phenotype that is known to be associated with impaired lineage stability and cell functioning.

Conclusion: We hereby demonstrate for the first time the deleterious consequences of Nrf2 activation in Tregs and our study therefore makes an important contribution towards a better understanding of Nrf2 mediated immune functions.

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Bach2 controls T follicular helper cells by direct repression of Bcl-6

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The generation of highly specific memory B cells and long-lived plasma cells takes place during the germinal center (GC) reaction and depends on help of a specialized T cell subset named T follicular helper (Tfh) cells. Consequently, Tfh cells are essential for inducing long-lasting immunity against pathogens, e.g. after vaccination, whereas many autoimmune diseases are associated with dysregulated Tfh cell numbers. Therefore, molecules regulating the maintenance of Tfh cells are of special clinical interest. Much about the molecular regulation of Tfh cells can be learned from B cells, since the transcriptional networks defining the GC B cell and Tfh cell phenotype share many similarities. The prime example is Bcl-6, which has a key role for both the development of GC B cells and Tfh cells.

Another important transcription factor to maintain the phenotype of GC B cells is Bach2. Bach2 is highly expressed in GC B cells and cooperates with Bcl-6 to suppress expression of Blimp-1, thereby preventing terminal differentiation of GC B cells into plasma cells. Interestingly, next generation sequencing revealed that Bach2 is strongly downregulated in Tfh compared to non-Tfh cells. Ectopic overexpression of Bach2 in Tfh cells resulted in a rapid loss of their phenotype and subsequent breakdown of the germinal center response.

To understand the underlying molecular mechanisms we performed transcriptome analysis shortly after Bach2 overexpression. This revealed 16 potential downstream targets of Bach2 in Tfh cells, among them the signature cytokine IL-21, the co-inhibitory receptor TIGIT and the transcriptional repressor Bcl-6. Further analysis finally identified Bcl-6 as the decisive downstream target of Bcl-6.

In stark contrast to the regulatory network in germinal center B cells, Bach2 in Tfh cells is not co-expressed with Bcl-6 at high levels to inhibit the antagonizing factor Blimp-1. Instead, ChIP sequencing data and functional promoter studies revealed that Bach2 suppresses Bcl-6 by direct binding to the promoter. Using ChIP for Irf-4 we could show that Bach2 replaces an activating complex of Batf and Irf-4 at the Bcl-6 promoter. Thus, the transcriptional network regulated by Bach2 in Tfh cell is completely different from GC B cells.

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Expansion of regulatory T cells in a model of experimental colitis is driven by cognate antigen recognition

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Introduction: Inflammatory bowel disease (IBD) is a major health burden with no effective cure and frequent need for lifelong treatment. The increased incidence of IBD in industrialised nations has been linked to changes in lifestyle, hygiene, diet and microbiota.

Objectives: Multiple immune pathways have been implicated in IBD, in particular dysregulation of regulatory T cells (Tregs) in the colon. However, it is currently not understood if the suppression of inflammation by colonic Tregs depends on cognate antigen recognition.

Materials and Methods: The enormous TCR repertoire complexity limits the usefulness of TCR repertoire sequencing in comparing patterns of clonal CD4 T cell selection between individuals. To overcome this limitation, we

DOI: 10.1002/eji.201970300

modified the adoptive T cell transfer colitis model in mice by transferring highly similar collections of clonally expanded Tregs to multiple individuals. Dominant clonotypes in the colons of individual mice were identified using full repertoire TCRα sequencing.

Results: Our data demonstrate that expansion of Tregs in the colon is highly dependent on TCR specificity. The Treg-repertoire is reproducibly restricted to a few clonotypes, most of which are highly expanded in the colon. These dominant clonotypes are shared between different individual recipients, suggesting specificity to a common antigen. We are currently characterizing the specificity of these Treg clones, with particular emphasis on microbiota derived antigens.

Conclusions: The combination of transferring harmonized repertoires to individuals and full repertoire TCRα sequencing allowed us to better understand the selection process of Tregs in the colon. Fully functional Tregs are selected and expanded through recognition of a common antigen.

Our data offer new insights into the mechanisms of suppression of gut inflammation and may lead to development of novel therapeutic approaches for treatment of IBD.

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Crosstalk of intestinal stem cells and T cells

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Introduction: The human intestinal epithelial layer fulfills an important barrier function to protect the human organism against microbes including viral infections. Mucosal immune cells contribute to the maintenance of the epithelial barrier and prevention of invasive infections. The organoid technique represents a novel three-dimensional (3D) model system that recapitulates many physiological relevant features of the *in vivo* tissue and enables studies of primary human intestinal epithelial cells *in vitro* (Sato et al., Nature, 2009). However, the current organoid cultures do not allow for co-culture with immune cells due to different medium requirements of intestinal stem cells and T cells.

Objectives: Establishment of an intestinal organoid - T cell co-culture system to investigate the mechanisms underlying the crosstalk between intestinal stem cells and immune cells.

Materials and Methods: Maintenance of stem cells in intestinal organoids depends on specific niche factors. To set up the co-culture system, we first investigated whether the organoid medium and its individual components interfered with T cell survival and proliferation.

Results: We observed that intestinal T cells were able to proliferate in T cell and organoid medium and exhibited similar survival rates. In contrast, peripheral blood T cells cultured in intestinal organoid medium showed comparable survival rates as in normal T cell medium, but their proliferation was drastically reduced. When investigating the effect of individual components of the organoid medium on peripheral blood T cell performance we identified the p38 inhibitor SB202190 as a component that negatively affected T cell proliferation.

Conclusions: Our data show that intestinal T cells are adapted to intestinal niche factors and suggest that intestinal T cells can be used for co-culture experiments using intestinal organoid medium. Co-culture settings for organoids and peripheral blood T cells require further medium optimization to increase peripheral blood T cell proliferation. In general, the co-culture system of intestinal organoids and T cells will be a powerful tool to investigate their crosstalk in the future, providing insights in T cell responses upon bacterial or viral infection of intestinal organoids.

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Rolling circle translation of a circular RNA in T helper lymphocytes

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DOI: 10.1002/eji.201970300

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Question: Circular RNAs (circRNAs) have gained considerable interest in the course of their re-characterization as endogenous RNA species which is present in various cell types including T cells. However, the function and biological impact of circRNAs still remain poorly understood. In this project we aim at identifying and characterizing the expression of circRNAs in T helper (Th) lymphocytes with a focus on molecular mechanisms of function.

Material and Methods: Analyzing total RNA sequencing data from naive and activated murine Th lymphocytes under Th1, Th2 and Th17 polarizing condition, we discovered a highly abundant circRNA (circ11607907). Using circ-specific qRT-PCR, retroviral overexpression, immunoblotting, dCas9-VP64-mediated selective promoter activation and polysome profiling, we investigated the expression and molecular function of this circRNA candidate.

Results: The circ11607907 showed high expression in naïve Th cells, which strongly decreased with T cell activation and is further reduced upon repeated activation of Th1 cells. The corresponding mRNA expression follows a similar pattern, yet with a stable intermediate expression in Th1 cells. Interestingly, one transcript variant harboring an alternative exon 1 resembled the drastically reduced expression upon T cell activation. Selectively activating promoter regions of the host gene *Ikzf1* revealed specific up-regulation of the circRNA and the alternative exon 1 but not the protein coding mRNA upon targeting promoter 1a but not 1b.

Dissecting the circ11607907 sequence, we found an infinite open reading frame with a potential for rolling circle translation. Indeed, ectopic overexpression of the circRNA in NIH 3T3 cells resulted in the expression of cryptic Ikaros proteins which could be abolished by mutating the start codon or introducing a stop codon. Using polysome profiling revealed that the endogenous circ11607907 can be detected in the polysomal fractions indicating that it also translated. Currently, we aim at deciphering the role of these cryptic proteins in Th cells activation.

Conclusions: Our data suggest that in naïve Th cells and during early T cell activation circ11607907 expression is a controlled process based on specific transcriptional regulation and the usage of an alternative transcript. Once generated the circRNA can be translated into cryptic Ikaros proteins in a rolling circle fashion. Taken together, this points at a specific function of the circ11607907 in Th lymphocytes.

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The role of regulatory T cells in experimental autoimmune glomerulonephritis

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Anti-glomerular basement membrane (anti-GBM) disease is characterized by antibodies and T cells directed against the Goodpasture antigen, the noncolla- genous domain of the □3-chain of type IV collagen [□3(IV)NC1] of the GBM. Consequences are the deposition of autoantibodies along the GBM and the development of crescentic glomerulonephritis (GN) with rapid loss of renal function. Forkhead box protein P3 (Foxp3)□regulatory T (Treg) cells are crucial for the maintenance of peripheral tolerance to self-antigens and the prevention of immunopathology.

Here, we use the mouse model of experimental autoimmune GN to characterize the role of Treg cells in anti-GBM disease. Immunization of DBA/1 mice with □3(IV)NC1 induced the formation of □3(IV)NC1- specific T cells and antibodies and, after 8–10 weeks, the development of crescentic GN.

Immunization resulted in increased frequencies of peripheral Treg cells and renal accumulation of these cells in the stage of acute GN. Depletion of Treg cells during immunization led to enhanced generation of □3(IV)NC1-specific antibodies and T cells and to aggravated GN. In contrast, depletion or expansion of the Treg cell population in mice with established autoimmunity had only minor consequences for renal inflammation and did not alter the severity of GN.

In conclusion, our results indicate that in anti-GBM disease, Treg cells restrict the induction of autoimmunity against □3(IV)NC1. However, Treg cells are inefficient in preventing crescentic GN after autoimmunity has been established.

DOI: 10.1002/eji.201970300

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IFN-γ-IRF1-axis as a regulator of glucose uptake and effector cell function in Th9 cells

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Introduction: T helper 9 (Th9) cells, characterised by their Interleukin 9 (IL-9) production, are involved in antitumor response and in immunity against helminths. They also contribute to autoimmunity, atopic dermatitis, colitis and asthma. We have previously shown that the transcription factor IRF 1, which is upregulated by interferon-gamma (IFN-γ), suppresses IL-9 production, by counteracting IRF4-driven *Il9*-promoter activity and histone modifications in Th9 cells. Besides an impact on IL-9 production, IRF1 also suppressed Th9 differentiation at the genome-wide level. As Th9 differentiation is regulated by glucose metabolism, we speculated on the impact of IRF1 on glucose metabolism.

Objectives: We hypothesize that the suppression of Th9 differentiation through IFN-γ-IRF1 axis could be a result of altered glucose uptake. Therefore, we are analysing the effect of IFN-γ-IRF1 axis on the glycolysis, on the expression of the important glucose transporters GLUT1, GLUT3 as well as on the glucose uptake.

Materials and Methods: Lymphocytes from C57BL6 (WT) and IRF1 knockout (KO) mice were differentiated in the presence or absence of IFN-γ to Th9 cells and the expression of GLUT1 and GLUT3 transporters were analysed by flow cytometry. For glucose uptake we used the glucose fluorescent analogon 2-NBDG.

Results: In comparison to IRF1KO Th9 cells, WT Th9 cells treated with IFN-γ, display a decreased glucose uptake as well as GLUT1 and 3 expression. Consistently, IRF1KO Th9 cells display increased glycolytic capacity as compared to IFN-g treated WT Th9 cells. IRF1 directly binds to *Slc2a3* regulatory elements, and probably by competing with IRF4 suppresses glycolysis in Th9 cells.

Conclusion: IRF1 regulates Th9 differentiation and IL-9 production by influence of glycolysis, besides a direct effect on the *Il9* promoter.

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Visualizing MHC-II restricted response to rodent malaria.

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Introduction: Malaria is caused by different *Plasmodium* species that can infect a variety of animals including men and rodents. Upon infection of the host, the parasites traverse through a complex life cycle, including a blood stage and a liver stage of infection. Even though the host"s immune response towards each of these stages is incompletely understood, CD4 T cell responses are known to play an important role in mediating immunity towards Plasmodium infection throughout the parasite"s life cycle.

Objectives: This project aims to examine the evolution of an MHC II-restricted response to a novel MHC II-restricted epitope in *Plasmodium* infection of B6 mice and to further characterise the protective capacity of this specificity.

Material & methods: To track CD4 T cell mediated immune responses, we made use of a recently discovered MHC-II restricted epitope that is expressed by both rodent and human Plasmodium species and is presented by the MHC II IAb haplotype of B6 mice. This epitope, abbreviated as YYI, is recognized by the PbT-II transgenic T cells. As a non-infective priming of PbT-II cells an anti-Clec9A antibody is used attached to the YYI epitope. In an initial step, GFP-expressing PbT-II transgenic T cells were injected into naïve B6 mice that were primed with Clec9A-YYI and then infected with PbA liver-stage parasites.

Results: 2-Photon intravital imaging of the liver revealed formation of PbT-II cell clusters 24 hours after parasite challenge. These clusters mostly contained cells with a patrolling phenotype and were no longer detectable 48h after liver-stage parasite infection. While transfer of naïve PbT-II cells did not lead to improvement of the outcome of liver or blood stage infection, in vitro differentiated and activated Th1 or Th2 PbT-II cells could reduce the blood parasite burden and prevent pathology after *Plasmodium* infection.

DOI: 10.1002/eji.201970300

Conclusion: These results highlight the potential of PbT-II cells to mediate protection against Plasmodium infection. Future studies will reveal, whether and how MHC-II restricted immunity operates and potentially reveal new strategies for effective vaccine design.

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Spatio-temporal regulation of c-myc translation is a mechanism of diversification during CD8 T cell differentiation

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Introduction: Asymmetric cell division (ACD) plays crucial roles in differentiation and development. In CD8 T cells, ACD can result in daughter cells with distinct functional cell fates. We found that during the first division of an activated T cell, c-Myc can sort asymmetrically in the two daughter cells. Further, asymmetric amino acid transporter distribution, amino acid content, and TORC1 function correlate with and influence c-Myc expression. The functional consequences of different c-Myc levels in activated T cells include altered proliferative potential, different metabolism, and distinct responses to antigen in vivo. However, it remains unclear how asymmetric assortment of this short-lived nuclear factor is achieved.

Objective: During T cell activation, engagement of T cell receptors creates a synapse that rapidly recruits the microtubule organizing center (MTOC) of the cell directly over the contact site. We hypothesized that sustained polarization of cytosolic components during T cell activation direct c-Myc asymmetry.

Methods: To visualize the translation of c-Myc we employed super-resolution (STORM) microscopy of nacent c-Myc peptides in association with polysomes and developed a statistical method to assess the spatial distribution of c-Myc translation. Using pharmacological and genetic approaches we probed signaling pathways regulating the spatial distribution of c-Myc translation.

Results: Using super-resolution microscopy, we identified microtubule-associated LAMP1+ vesicles associated with active TORC1 that accumulate near the MTOC, resulting in polarized phosphorylation of TORC1 substrates. These result in the activation of translation initiation factors required for c-Myc translation, including eiF4A and eiF4B. Inhibition of eIF4A or TORC1 activity effectively equilibrated c-Myc levels during ACD, indicating a critical role for both in polarized c-Myc translation. Strikingly, we found that the association of c-Myc protein with polysomes was strongly correlated with proximity to the MTOC. That is, the translation of c-Myc is polarized during T cell activation, and we suggest that upon cell division, this polarization is responsible for delivering c-Myc protein predominantly to the proximal daughter nucleus. This novel process appears to influence the divergent fate of the daughter cells of the first T cell division.

Conclusion: Our results raise the intriguing possibility that CD8 T cells utilize spatial regulation of protein translation as a mechanism of diversification following activation, and may represent a general mechanism in other forms of ACD.

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c-Rel and IκBNS are crucial for Foxp3+ CD25− regulatory T cell precursor generation

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Introduction: Regulatory T (Treg) cells play a key role in peripheral tolerance preventing autoimmune disorders. In the classical thymic Treg cell development model, TCR-mediated CD25 induction takes place in Treg cell precursors. Subsequently, γc-mediated stimuli lead to the differentiation of those cells in CD25+ Foxp3+ mature Treg cells. Next to this path, an alternative route has been proposed, in which CD25− Foxp3+ cells develop into mature CD25+ Foxp3+ Treg cells. Molecularly, the two components of the NF-κB pathway c-Rel and IκBNS control Foxp3 induction during classical Treg cell development. However, their contribution to the alternative Treg cell development route is unclear.

DOI: 10.1002/eji.201970300

Objectives: To elucidate the role of NF-κB, particularly c-Rel and IκBNS, in the alternative route of thymic Treg cell generation.

Materials & methods: c-Rel-deficient, IκBNS-deficient and IκBNS-c-Rel-double deficient (DKO) mice as well as Foxp3DTR-eGFP reporter mice were used. *Ex-vivo* cell analysis by flow cytometry as well as *in vitro* Treg cell maturation assays were performed in this study

Results: We observed comparable reduction of alternative Treg cell precursors in IκBNS- deficient, c-Rel-deficient and DKO mice, indicating that c-Rel and IκBNS contribute to the development of those cells. Similar to conventional Treg precursors, we detected expression of CD122 in alternative Treg precursors. In Treg maturation assays, we noticed impaired CD25 induction in c-Rel-deficient cells in contrast to IκBNS and wild type cells.

Conclusion: Both IκBNS and c-Rel are essential for Foxp3 induction during Treg development. IκBNS is dispensable for CD25 expression in both Treg cell development routes. Despite CD25 absence in alternative Treg cell precursors, IL-2 receptor subunit beta-chain (CD122) is expressed in these cells, being responsive to γc cytokine signalling. Taken together, our data demonstrate that c-Rel and IκBNS are required for the generation of alternative Treg precursors.

DOI: 10.1002/eji.201970300

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DOI: 10.1002/eji.201970300

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DOI: 10.1002/eji.201970300

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DOI: 10.1002/eji.201970300

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DOI: 10.1002/eji.201970300

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DOI: 10.1002/eji.201970300

