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O101

The psoriasis risk allele HLA-Cw0602 shows increased susceptibility to chronic/recurrent tonsillitis and points to shared T cell epitopes in these diseases

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Pharyngeal tonsillitis is one of the most common upper respiratory tract disorders in humans where Group A streptococcus is considered to be clinically the most important bacterial pathogen. While many patients experience only occasional throat infections with a relative short duration a subset of patients suffer from chronic/recurrent tonsillitis. The reason for this is not yet fully understood but there seems to be a genetic predisposition to the condition. To search for new genetic biomarkers in pharyngeal tonsillitis we performed a MHC/HLA-focused genome wide association study using Immunochip platform. Over 190,000 SNPs were analyzed in a cohort of 96 Finnish patients subjected to tonsillectomy due to chronic/recurrent tonsillitis and 504 healthy donors. Genetic association between the cases and controls was evaluated by PLINK program and the imputation of the classical HLA-alleles from SNP genotypes was done by using HIBAG. We found that the psoriasis risk allele HLA-Cw*0602 significantly increased susceptibility to tonsillitis (OR = 2.37, $P = 0.03$). This finding provides a novel genetic explanation for the known link between streptococcal throat infections and onset and pathogenesis of psoriasis. The result supports the theory of molecular mimicry between streptococcal M protein and skin keratins, and points to presentation of shared antigenic T cell epitopes by HLA-Cw6 in both diseases.

O102

Type I interferon and receptor activator of nuclear factor Kappa B signaling within the central nervous system

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There is a growing interest in understanding the role of Type I interferons (IFN), which include IFN-beta, in the central nervous system (CNS), as they play an important role in regulation of inflammation. IFN-beta has been shown to protect against multiple sclerosis (MS) and experimental autoimmune encephalomyelitis (EAE), an animal model of MS. Type I IFN are induced by engagement of immune receptors including Toll-like receptors and members of the Tumor Necrosis Factor Receptor (TNFR) family, involving the activation of nuclear factor kappa B (NF-kB) and interferon regulatory factors. TNFRs are expressed in the CNS and member of this family have been proposed as possible therapeutic targets. Receptor activator of NF-kB (RANK) belongs to the TNFR family, and RANK signaling has been shown to induce IFN-beta. We have investigated RANK as a novel receptor for induction of IFN-beta in the CNS and its functional relevance. We showed that RANK message is expressed in the CNS, and that microglia, known to be a major source of Type I IFN, expressed RANK. Administration of human recombinant RANK ligand directly into the cerebrospinal fluid of reporter mice that express luciferase under the control of the IFN-beta promoter, revealed induction of IFN-beta within hours. IFN-beta expression was detected in CD45/CD11b positive cells. RANK expression was increased in the CNS of mice at peak of EAE suggesting its involvement in EAE pathogenesis. Our finding suggest that RANK- IFN-beta signaling within the CNS plays a role in regulation of CNS inflammation.

O103

Serotonin and tryptophan metabolites in APECED syndrome

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Introduction: Aromatic L-amino acid decarboxylase (AADC) and tryptophan hydroxylase (TPH) autoantibodies (Ab) are commonly found in Autoimmune Polyendocrinopathy-Candidiasis-Ectodermal Dystrophy (APECED). They are associated with the lack of gastrointestinal enteroendocrine cells.

Methods: We assessed the serum levels of serotonin (5-HT) and tryptophan (Trp) metabolites and compared them to the presence or absence of anti-TPH and AADC Ab (ELISA) in APECED patients. As 5-HT is a neurotransmitter involved in depression, we assessed the current mental health of the patients with a self-reported inventory for measuring possible depression (RBDI).

Results: Twenty-four patients filled the RBDI questionnaire (18 women, mean age 43). Four patients had moderate depression. 54% had AADC Ab, 46% TPH-1 Ab and 33% had both. RBDI score was not related to circulating Ab. 5-HT levels were significantly lower in patients with anti-TPH-1 Abs and with both TPH-1 and AADC Ab^+. The other metabolite levels (XA, HIAA, AA, IAA) were also disturbed. We found a positive correlation between fatigue, 3-hydroxykynurenine and Dopa levels and negative correlation between sleep disorders and Trp levels, but no correlation between the total RBDI score and 5-HT levels.

Discussion: Our study confirms a relationship between TPH-1 Abs and AADC Ab on 5-HT and various Trp metabolites levels, as a consequence of the lack of neuroendocrine cells in the gut. The clinical impact of such decrease has to be determined, but an impact on the gut microbiota is suspected.

Conclusion: APECED patients with TPH-1 and AADC Ab have lower levels of 5-HT and Trp metabolites.

O104

Infant microbiota promotes altered immune maturation depending on presence of lactobacilli and later life allergic sensitization

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Early life intestinal microbiota is important for immune maturation in the gut. We have previously shown in a cohort of Swedish children that the presence of certain species of *lactobacilli* in the intestine early in life is associated with reduced risk of allergic sensitization later in life, despite allergic heredity. We further wanted to investigate how the specific intestinal flora in the first weeks of life affected the development of the immune system.

Therefore stool samples collected from 2 week old children within the cohort were used to inoculate germ-free mice. The stool samples were pooled according to presence of certain *lactobacilli* as well as allergic heredity and known allergic sensitization of the individual children later in life. The immunological profile of the offspring of the inoculated mice was subsequently characterized. In order to assess mucosal immune responses, leukocytes were isolated from the intestinal tract and analyzed using FACS.

We found that proportions of the $CD4^+$ T-cell compartment differed in mice depending on the flora they had been exposed to. Frequencies of $FOXP3^+$ and $RORgt^+$ cells, indicative of regulatory T-cells and T helper 17 cells respectively, varied. Overall, early life intestinal flora had major effects on immune development.

O105

Activation of plasmacytoid dendritic cells in colon-draining lymph nodes during C. rodentium infection involves pathogen-sensing and inflammatory pathways distinct from $CD103^+$ DC

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Dendritic cells (DC) bear the main responsibility for initiation of adaptive immune responses necessary for

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antimicrobial immunity. In the gut, CD103$^+$ migratory DC traffic from small intestinal lamina propria to small intestinal mesenteric lymph nodes (siMLN) and induce regulatory and antimicrobial immunity there. Immune surveillance the large intestine and the role of CD103$^+$ DC therewith are less well characterized.
We identified three small mesenteric lymph nodes, distinct from small intestinal lymph nodes, which drain lymph specifically from the colon, and studied DC responses to the attaching and effacing pathogen *C. rodentium* in these. Transcriptional profiling of CD103$^+$ DC and the lymph-node resident pDC populations during steady-state conditions revealed activity of distinct sets of genes in these two DC subsets, both in small intestinal and colon-draining lymph nodes. *C. rodentium* activated DC especially in colon-draining lymph nodes, and gene expression changed in pDC more profoundly than in CD103$^+$ DC. Among the genes most upregulated in pDC were C-type receptor CLEC4E, IL-1-receptors (IL1R1-2), proinflammatory cytokines (IL1a, IL-6) and TLR6. The coMLN and siMLN also showed differential lymphocyte response to bacterial signals as seen with increased IFNγ production by CD4$^+$ cells in the coMLN, but not in the siMLN.
Our results indicate that colon immune surveillance is distinct from that of the small intestine in terms of draining lymph nodes, and identify pDC as active sentinels of colonic inflammation and/or microbial dysbiosis.

P101

The role of Furin1 in Drosophila immunity

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Many important biological processes involve proteolysis of larger protein precursors by proprotein convertase enzymes. We have investigated the role of the proprotein convertase family member Furin1 of *Drosophila melanogaster* in innate immunity. Furin1 expression was knocked down in the fat body by crossing transgenic UAS-Furin1-RNAi flies with C564-GAL4 or Fb-GAL4 flies. Adult flies from these crosses and control crosses were infected with Gram negative *Enterobacter cloacae* or Gram positive *Micrococcus luteus* plus *Enterococcus faecalis* and their survival was monitored. Our preliminary results indicate that flies with decreased expression of Furin1 in the fat body are more sensitive to bacterial infection than control flies. This is possibly due to the lack of induction of antimicrobial peptide expression via the Toll and Imd signalling pathways, as analysed by quantitative RT-PCR. The fat body structure of Furin1-RNAi × Fb-GAL4; UAS-GFP larvae appeared normal when compared to the w1118 × Fb-GAL4; UAS-GFP control cross larvae. Both RNAi-mediated knockdown of Cactus and overexpression of IMD rescued the antimicrobial peptide expression from flies with decreased levels of Furin1 in the fat body. This suggests that Furin1 may function upstream of the Toll pathway member Cactus and the IMD pathway member IMD. Further studies will be carried out to elucidate the mechanism for Furin1 mediated regulation of the immune signalling pathways.

P102

The effect of bacterial citrullination on host gene expression pattern

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Porphyromonas gingivalis is one of the major periodontal pathogens involved in the development of chronic periodontitis. *P. gingivalis* is also the only know prokaryote to express the enzyme peptidyl arginine deiminase, necessary for protein citrullination. In this study we investigated the impact of PPAD on fibroblast gene expression.
Fibroblasts were infected with wild-type (ATCC33277) and PPAD knock-out (ATCC C351A) and RNA was purified to generate a full expression profile using microarray analysis. Further, ELISA was used to analyse the presence of IL36γ in keratinocyte cultures infected with wild type and knock-out bacteria.
Microarray analysis revealed that *IL36γ* was upregulated following wild type infection. In contrast, it was found that *IL36γ* was the most down-regulated gene following infection with the PPAD knock-out bacteria. This was confirmed by the decrease of IL36γ production in keratinocyte cultures infected with PPAD knock-out.
The lack of PPAD enzyme during infection of fibroblast further resulted in a depletion two IL-2 related genes: positive regulation of IL-2 production and positive regulation of IL-2 biosynthesis process.
Taken together, this data indicates a novel regulatory function of the PPAD enzyme where the presence of

bacterial PAD prevents a down-regulation of IL36γ, and possibly IL-2, production by host cells.

P103

Staphylococcus aureus and lactobacilli oppositely influence the maturation and activation of FOXP3+ CD4 T-cells

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Introduction: The gut microbiota influences immune maturation during early life and associates with the development of immune-mediated diseases. $CD4^+$ $FOXP3^+$ T-regulatory cells, which are important for immune homeostasis and tolerance, are influenced by the gut microbiota. Lactobacilli and *Staphylococcus (S.) aureus* are common bacteria in the neonatal gut, and related to allergy-protection or allergy-risk. *In vitro*, lactobacilli dampen immune activation induced by *S. aureus*.
Aims: To investigate if early life colonization with lactobacilli and *S. aureus* influences the maturation and functional responses of $FOXP3^+$ T-cells and to examine how soluble products from these bacteria affect $FOXP3^+$ T-cells *in vitro*.
Methods: RT PCR was used to detect and quantify bacterial DNA in faeces from infants and associated with immune data at age two. PBMC from children at age two, age seven and adults were analysed *ex vivo* or after stimulation with cell free supernatants (CFS) from *Lactobacillus* (*L.*) *reuteri* DSM 17938 or/and *S. aureus* 161:2. The PBMC were analysed by flow cytometry and ELISA.
Results: *S. aureus*-colonization associated with a higher percentage of $FOXP3^+$ cells that expressed IL-10 and CD161, a marker connected to cytokine-producing capacity. *In vitro* stimulation with *S. aureus*-CFS induced expression of CD161, IL-10 and IFN-γ by $FOXP3^+$ cells. In opposite, lactobacilli-colonization associated with a lower percentage of IL-10-expressing $FOXP3^+$ cells after stimulation. *L. reuteri*-CFS also dampened *S. aureus*-induced activation of $FOXP3^+$ cells *in vitro*.
Conclusions: Species in the early gut microbiota are differentially linked to the development and function of $FOXP3^+$ cells later in life.

P104

Effect of fractions and compounds from marine organisms on immune responses

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Marine sponges produce a wide range of secondary metabolites and recently there has been an increased focus on exploring them as a source for natural products. The objective of this study was to determine the anti-inflammatory properties of fractions/compounds from *Halicondria sitiens*.
Halicondria sitiens was collected from the ocean around Iceland. Fractionation of extracts from the sponge was carried out using solvent partitioning and the active fractions obtained were fractionated further by size and polarity. Human monocyte-derived dendritic cells (DCs) were matured with TNF-α and IL-1β and stimulated with LPS for 48 h in the presence or absence of fractions/compounds. The effects of the fractions/compounds on maturation and activation of the DCs were evaluated by measuring their secretion of cytokines by ELISA.
Three fractions/compounds obtained from preparative HPLC of the $CHCl_3$ fraction of *H. sitiens* show promising immunomodulatory effects on DCs. One decreased DC secretion of IL-6, IL-12, IL-27 and IL-10 without affecting their secretion of IL-1ra, another one had no effect on IL-6, IL-12, IL-27 and IL-10 but increased secretion of IL-1ra, and the third one decreased secretion of IL-12 and IL-10 without an effect on IL-6 and IL-1ra. One of these fractions also affected the morphology of DCs causing them to take on an elongated or pointed shape.
These results demonstrate that *H. sitiens* contains several bioactive compounds that may impact activation of Th1 and/or Th17 cells and have possible applications in the treatment of autoimmune disease.

P106

Does the secreted molecules of the gut bacterium Faecalibacterium prausnitzii inhibit the fat accumulation of hepatocytes in vitro?

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Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease worldwide. NAFLD is a

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disease where the gut microbiota seems to play an important role (reviewed in 1). We have recently discovered that the abundance of fecal *Faecalibacterium prausnitzii* is lower in the NAFLD patients (2). Moreover when *F. prausnitzii* was intragastrically introduced to the NAFLD mouse model, the prevention of fatty liver through the mechanisms that involved reduced adipose tissue inflammation as well as improved insulin signaling was observed. Therefore it was agreeable to evaluate whether the growth media of *F. prausnitzii* possess the same effect on hepatocytes *in vitro*.

Human hepatocyte-derived cell line HepG2 was used in the experiments and the fat accumulation was induced with oleic-palmitic acid with the ratio of 2:1 since this is considered to be a good model for human steatotic liver (3). The fat accumulation in HepG2 cells was measured with Oil red O staining. Fat-overload was also successfully induced by sucrose and glycerol although the effect was less prominent.

The growth media supernatant of *F. prausnitzii* did not inhibit the HepG2 fat accumulation *in vitro* in this experimental model. Even though the positive health effect mediated by the *F. prausnitzi*i did not apply *in vitro*, it is important to unravel the possible mechanisms further to fully understand its role in NAFLD and also in other diseases. Based on the earlier and current results, it is likely that the effects of *F. prausnitzii* on liver are mediated by the adipose tissue.

1. Kirpich IA, et al. *Clin Biochem*. 2015;**48**:923–30.
2. Munukka E, et al. *J Hepatol* 2014;**61**:132–8.
3. Gómez-Lechón MJ, et al. *Chem Biol Interact* 2007; **165**:106–16.

P107

The role of innate immunity in Type 1 Diabetes (T1D)

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Objectives: DIPP-PAMP (Pathogen Associated Molecular Patterns) project is a part of Finnish DIPP (Type 1 Diabetes Prediction and Prevention) study where we screen newborns for T1D risk genotypes and follow them for the emergence of islet autoantibodies and T1D. The aim of the DIPP-PAMP project is to study how innate immunity is involved in human T1D pathogenesis.

Methods: Monocyte derived macrophages were stimulated with CpG-DNA alone or complexed to HMGB1. The secretion of Type 1 interferons and expression of the genes for TLR9 pathway were studied. Six adolescent T1D cases and age- and sex matched controls and 27 children positive for T1D-associated autoantibodies and 27 autoantibody negative control children were included. Nine of the 27 autoantibody positive children progressed to T1D during the study. The expression levels of selected genes were studied using quantitative RT-PCR. The statistical significance was tested with the method of Kenward-Roger.

Results: IFN-α secretion was elevated in CpG-DNA stimulated macrophages of T1D patients. IFN-α level was modulated when the macrophages of T1D patients were treated with CpG-DNA complexed to HMGB1. In gene expression studies of DIPP-PAMP children, *IRF5* and *IRF7* genes were differentially regulated in the non-progressors (autoantibody positive and did not progress to T1D) when compared with the progressors (autoantibody positive and progressed to T1D).

Conclusions: Previous studies show that Type I interferon signature can be temporally detected in the whole blood of T1D progressors before appearance of autoantibodies. This preliminary study further confirms the role of Type I interferon signaling in T1D pathogenesis.

O201

Mannose receptor (MR) have a protective role in mannan induced psoriasis (MIP)

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Psoriasis and psoriasis arthritis are both poorly understood, but common diseases in human. They are induced by unknown environmental factors and affects both skin and joints. Mannan induced psoriasis (MIP) is a novel mouse model for psoriasis arthritis (induced by a single injection of mannan) causing arthritic symptoms such as swelling and redness of the paws and the characteristic lesions of psoriasis. The model is dependent upon macrophage produced reactive oxygen species (ROS) and $\gamma\delta$T cells. However, the receptor, important for the recognition and initiation of the disease, is unknown. This study focused on investigating one of the receptors binding mannan; the mannose receptor (MR). MR is a C-type lectin present on myeloid cells and lymphatics. To investigate its importance for MIP we utilized MR knockout mice, showing that the lack of MR leads to more severe arthritis compared to the wild type. We further concluded that a mutation leading to ROS deficiency will, as previously shown,

exacerbate the disease. The importance of MR as a protecting pathway is diminishing if the mouse lacks ROS; either the protective pathway activated by MR is dependent upon ROS or the severity of MIP in a ROS deficient organism is too severe and the protective effect is therefore lost. In conclusion, these results indicate that MR scavenges mannan and preventing it from binding to other receptors in the mouse, for example mannan binding lectin, and results in protection. Further studies are needed to conclude which receptor is important for the initiation of MIP.

O203

Leukocyte protein Trojan, as a candidate for apoptotic regulatory role

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Trojan is a novel, leukocyte-specific protein, cloned from chicken (Gallus gallus) embryonic thymocytes. The molecule is a type I transmembrane protein with an extracellular CCP domain, followed by two FN3 domains. Its cytoplasmic tail has a predicted MAPK docking region with two PKA phosphorylation sites.

In thymus, Trojan is expressed on DN and SP cells, but diminishes from the surface of selection-undergoing DP thymocytes. This expression pattern, similar to that of the anti-apoptotic IL-7Rα chain and BCL-2, made us hypothesise an anti-apoptotic function for Trojan. Our studies with a chicken T cell line showed that upon apoptosis induction, Trojan expression rises on the surface of surviving cells. When sorted, the cells with no surface expression of Trojan appeared much more susceptible to apoptosis, compared to the cells expressing the molecule.

Cells, transfected to overexpress Trojan showed elevated levels of intracellular calcium, suggesting the protein is able to transmit cytoplasmic signals. The mechanistic nature of Trojan-induced signalling is a target for future investigation.

Trojan belongs to a novel protein family that includes Mystran, a receptor-type tyrosine phosphatase and Thracian, a transmembrane protein with an ITAM. We discovered the family in other avian species, reptiles and coalecanth fish. We observed positive evolutionary selection within the extracellular regions of the proteins. This adaptation was likely a response to ligand changes, association with other surface molecules or as a means of evading pathogen challenges. In contrast, the cytoplasmic tails of the proteins had stayed mostly unchanged, suggesting conserved signalling mechanisms.

O204

Integrative molecular profiling during human induced regulatory T cell (iTreg) generation reveals novel regulators of FOXP3

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Regulatory T cells (Tregs) suppress other immune cells and are critical mediators of peripheral self-tolerance, preventing autoimmune disease but hampering tumor rejection. Therapeutic manipulation of Tregs is subject to numerous clinical investigations with promising outcomes in first in-man trials of adoptive Treg transfer. The number of naturally occurring Tregs (nTregs) is minute, encouraging the complementary approach of inducing Tregs (iTregs) from naïve T cells. Mouse studies exemplify the importance of peripherally induced Tregs along with applicability of iTreg transfer in disease models.

Yet, mechanisms and experimental procedures governing human iTreg generation are incompletely understood. We therefore established and compared different protocols of human iTreg generation using TGF-β in combination with other compounds. Tregs induced by these protocols expressed several Treg signature molecules, and we identified a novel combination of TGF-β, retinoic acid and rapamycin to induce human iTregs with superior suppressive activity *in vitro*.

Using five different induction protocols in parallel, we performed deep molecular profiling using RNA sequencing and high resolution proteomics during iTreg induction over time. The time-dependent transcriptomic and proteomic data were subject to bioinformatics analysis. By integrative analysis of these data, we short-listed 40 novel candidate molecules potentially important for iTreg induction. Those new candidate molecules were validated by a targeted shRNA screen: Knockdown of several of these molecules led to reduced induction of FOXP3, the master transcription factor of Tregs.

Our analysis of the molecular mechanisms ruling iTreg generation may have important implications for our understanding and ability to treat cancer, autoimmune and inflammatory diseases.

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O205

Vitamin D regulates human neutrophil anti-microbial defense and inflammatory response to Streptococcus pneumoniae

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Background and Aim: *Streptococcus pneumoniae* is a major cause of pneumonia, septicaemia, and meningitis worldwide. Despite being such a dreadful pathogen, pneumococci are also common colonizers of the upper respiratory tract of healthy children. Vitamin D deficiency strongly correlates to disease severity in pneumonia patients. We investigated the effect of vitamin D on the immune response of human neutrophils to pneumococci.

Results: We found that the active hormonal form of vitamin D_3 induced upregulation of pattern recognition receptors, TLR2 and NOD2 as well as release of the antimicrobial peptides, HNP1-3 and LL-37 in infected neutrophils, which correlated with increased killing of serotype 4 pneumococci in a vitamin D receptor dependent manner. Strikingly, vitamin D supplementation of sera from patients with respiratory tract infections enhanced the bacterial clearance by neutrophils. Isogenic pneumococcal mutant strains deficient in capsule and pneumolysin were more susceptible to neutrophil killing. The pro-hormone, 25-hydroxyvitamin D_3 exerted limited effects alone, but IFN-γ priming restored its activity in neutrophils by upregulating the converting enzyme, CYP27B1. Further, vitamin D downregulated pro-inflammatory cytokine (IL-6, IL-8 and IL-12) production by infected neutrophils and induced the anti-inflammatory cytokine IL-4, which might contribute to reduced apoptosis of infected neutrophils.

Impact: Our results provide molecular clues to explain the link between vitamin D deficiency and susceptibility to pneumococcal infections and open new avenues for therapeutic development of vitamin D or suitable analogues as an adjunct alongside antibiotics to treat patients with pneumococcal infections.

P201

Efficacy of cell wall-deficient spheroplasts against experimental murine listeriosis

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Various strategies adapted to develop an efficient vaccine against foodborne pathogen, *Listeria monocytogenes*, have met with little success. Spheroplasts (bacterial cell devoid of cell wall) are likely to undergo membrane–membrane fusion, leading to the delivery of their content to the cytosol of antigen-presenting cells, thus facilitating MHC class I antigen processing and presentation. In this study, we evaluated the prophylactic potential of Listeria spheroplast-based vaccine against experimental murine listeriosis in comparison with heat-killed Listeria (HKL) and archaeosome-entrapped Listeria whole-cell protein (LWCP). Compared with HKL, the spheroplast-based vaccine was found to evoke better Th1 response as exhibited by the presence of type 1 cytokines in the host (interferon-c and IL-12) and a high IgG2a/IgG1 ratio. Robust lympho-proliferative efficacy was apparent in both spheroplast-immunized and archae-osome-entrapped LWCP-immunized groups. The upregulation of costimulatory and effector memory markers upon immunization with spheroplasts was found to be at par with that evoked by archaeosome-entrapped LWCP-immunized group. Central memory response in gated $CD8^+$ T cell was much higher in spheroplast-immunized animals when compared with archaeosome-entrapped LWCP group. The data presented here clearly demonstrate that spheroplasts evoked a robust immune response and offer better prophylactic potential against *L. monocytogenes*.

P202

Characterization and mechanistic dissection of the differentiation of $GM\text{-}CSF^+$ $CD4^+$ T cells

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$CD4^+$ T helper (Th) cells are crucial players in the immune system mainly by producing cytokines. Attempts have been made to classify them to subsets based on the signature cytokines. Subsets such as Th1 and Th17 are involved in the establishment of autoimmune diseases such as Multiple

Sclerosis (MS) and its mouse model Experimental Autoimmune Encephalomyelitis (EAE), which are driven by the T cell-released cytokines IL-17, IFN-γ, IL-22 and GM-CSF. Of these, so far the only cytokine shown to be necessary for the induction of EAE is GM-CSF produced by autoreactive T cells. Furthermore, in humans the fraction of GM-CSF$^+$ cells within CD4$^+$ T cells has been shown to be elevated in MS patients compared to controls in cerebrospinal fluid.

Despite their important role in disease, the differentiation mechanism of GM-CSF$^+$ CD4$^+$ T cells and its triggering factors are not entirely known. We aim to identify the cytokine signals and stimulation conditions required for the differentiation of human GM-CSF$^+$ CD4$^+$ T cells and the characteristics of this population, such as co-expression of other T cell markers.

Recently we identified novel inducing conditions for human GM-CSF$^+$ CD4$^+$ T cells and characterized this cell population. We are currently investigating the chromatin and transcriptome of this cell population by ATAC-seq and RNA-seq during *in vitro* induction to find transcription factors and pathways regulating their differentiation. We analyze and integrate these data through computational methods. These studies will help to gain a better understanding of the T cell related pathogenesis of autoimmune disease.

P203

IFNβ therapy induces a monocyte specific block of miR-150 maturation with a parallel reduction of miR-150 in plasma

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MicroRNA (miRNA) has emerged as an attractive class of biomarkers due to their stability and sensitivity of detection. Several miRNAs have been proposed as diagnostic and prognostic biomarkers in inflammatory diseases, and some miRNAs have also been reported as treatment markers for autoimmune disease. However, the common use of complex mixture of cells, e.g., whole blood, have led to high variability and little overlap between published data. To avoid this, we have sorted immune cells into CD3$^+$ (T cells) CD14$^+$ (monocytes) CD15$^+$ (neutrophils) CD19$^+$ (B cells) followed by quantification of miRNA expression. To have the complete picture of the mechanisms of miRNA expression and transportation we also measured levels of primary miRNA in cells and levels of mature miRNA in plasma.

Many systemic autoimmune diseases are characterized by an interferon (IFN) signature where type I IFN levels are increased in patients compared to healthy controls. The strength of the IFN signature correlates with disease activity. IFNs control the gene expression of so called interferon-regulated genes (IRGs). It has been suggested that one of the controlling factors of IRG expression is microRNAs (miRNA). We measured levels of several miRNAs in multiple sclerosis (MS) patients before and after treatment with Avonex® (IFN-β1a) and found that levels of miR-150 are strongly decreased in blood plasma and in monocytes after IFN stimulation but not in neutrophils, T cells or B cells. We also observed that levels of primary miR-150 transcripts were not changed upon the stimulation with IFN-β. The findings strongly point to miR-150 selective maturation regulation by IFN-β.

We are currently examining other possible miRNAs reported to be involved in IFN responses and those specific to autoimmune diseases. We hope to reveal miRNAs that are regulated by IFN type I and examine the cell type specific mechanisms of IFN regulation.

P204

Role of chromatin organizer SATB1 during development of regulatory T cells

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Development of regulatory T cells (Tregs) is one of the crucial mechanisms of immune tolerance and homeostasis. Lineage commitment is dependent on the cell type specific transcription factors and chromatin modifying machinery. Foxp3 is the master regulator of Treg development and is known to regulate key genes involved in regulatory T cell function such as CTLA4, GITR. SATB1 is a T lineage-enriched chromatin organizer that orchestrates expression of multilple genes important for T-cell development and differentiation. SATB1 is upregulated upon activation in Th2 cells and it is involved in the compaction of chromatin loops that induce the expression of cytokine genes on the *Il-4* locus. Interestingly, SATB1 is downregulated in FOXP3-dependent manner in Tregs. Ectopic expression of SATB1 in Tregs results in effector phenotype. Therefore it is important to study the regulation of SATB1 during early stages of Treg development. We show that SATB1 is abundantly expressed during early stages of thymic Treg development. We then performed FACS sorting of Treg precursors from FOXP3-GFP transgenic mice and differentiated them *in vitro* into Treg cells. Our study revealed upregulation of SATB1 during early stages of thymic Treg development when these cells do not express FOXP3. Treg precursors that receive high strength of TCR signal reveal high Nur77 expression. Interestingly, SATB1 is upregulated in Nur77-expressing CD25$^+$ GITR$^+$ CD4SP cells. Thus, TCR signal positively regulates SATB1 expression in

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these cells. Downregulation of SATB1 in progenitors of Tregs seems to be critical for the generation of FOXP3 positive Tregs.

P205

β-glucan induces reactive oxygen species (ROS) dependent splenomegaly in mice

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β-glucan is a polysaccharide that can be found on cell walls of bacteria and fungi as well as in oat, barley and seaweed. It is known as a strong immune stimulant and has been used as a treatment against tumors. Depending on its structure, the β-glucan will bind to different receptors. However, little is known about the pro-inflammatory effects induced by different molecular variants of this polysaccharide. Previous studies by our lab have shown that reactive oxygen species (ROS) are important signaling components in the immune response. In this study, mice received 1,3-1,6- β-glucan (*Saccharomyces Cerevisiae*) intraperitoneal, and 7 days after a development of splenomegaly was noticed. ROS deficient mice were proven to have significantly more severe splenomegaly compared to wild type. When ROS deficient mice were exposed to β-glucans with either 1,3-, 1,4- or 1,6-glycosidic bond, only the 1,3 and 1,6 β-glucans caused splenomegaly, and 1,4 glycosidic bond did not lead to spleen enlargement.. In conclusion, the glycosidic bond of the β-glucan determines its ability to induce splenomegaly in mice in a ROS dependent manner. Nevertheless, further studies will validate the structural importance of β-glucan for the induction of splenomegaly.

P206

Localization and distribution of fibrinogen C domain containing 1 (FIBCD1) in human tissues

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Introduction: We have previously identified and characterized fibrinogen C domain-containing 1 (FIBCD1) as a homotetrameric type II transmembrane protein. FIBCD1 is a member of the fibrinogen-related protein (FReP) family, and is thought to play a crucial role in the innate immune system (Schlosser et al, 2009; Thomsen et al, 2011). FIBCD1 functions as an endocytic receptor, binding chitin and other acetylated compounds with high affinity. We hypothesize that FIBCD1 serves as a pattern recognition molecule for acetylated compounds, found in various pathogens such as helminths and fungi. We have previously shown that FIBCD1 is present at mucosal surfaces in the lung and large intestine.

Aim: The present study investigates the distribution and localization of FIBCD1 in various healthy human tissues.

Results: We used a monoclonal antibody directed towards the FIBCD1 ectodomain in an immunohistochemistry-based analysis and demonstrate that FIBCD1 protein is highly expressed at the apical surfaces of the epithelium throughout the gastrointestinal tract, in the uterus, testis, bladder, gallbladder and the salivary glands. To a lesser extent, FIBCD1 is expressed in the pancreas, the spleen and the tonsils. Moreover, using quantitative real-time PCR we demonstrate that FIBCD1 mRNA is highly expressed in the gastrointestinal tract, the lung, the adrenal gland and the testis, which is in coherence with our immunohistochemical findings.

Conclusion: FIBCD1 is present at the apical epithelial surfaces of various mucosal tissues. This strengthens our hypothesis that FIBCD1 is a novel player involved in the innate immune responses of the mucosa.

O301

The multiple sclerosis susceptibility genes TAGAP and IL2RA are regulated by vitamin D in CD4⁺ T cells

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Multiple sclerosis (MS) is an inflammatory, demyelinating disorder of the central nervous system that develops in genetically susceptible individuals. The majority of the MS-associated gene variants are located in genetic regions with importance for T cell differentiation. Vitamin D is a potent immunomodulator, and vitamin D deficiency has

been suggested to be associated with increased MS disease susceptibility and activity. Vitamin D acts through its vitamin D receptor (VDR), which binds vitamin D response elements (VDRE) within regulatory regions of its target genes, thereby affecting gene transcription. In $CD4^+$ T cells, we have analyzed *in vitro* vitamin D responsiveness of selected VDRE-containing MS-associated genes. We identify *IL2RA* and *TAGAP* as novel vitamin D target genes. The vitamin D response was observed in samples from both MS patients and controls, and was not dependent on the genotype of MS associated SNPs in the respective genes. From published VDR chromatin immunoprecipitation-sequencing data, we have now selected additional MS-associated genes to be analyzed for vitamin D responsiveness in $CD4^+$ T cells.

O302

Salivary IgA from the sublingual compartment as a novel non-invasive proxy for intestinal immune induction

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Whole saliva IgA appears like an attractive non-invasive readout for intestinal immune induction after infection or vaccination, but has failed to show consistent correlation with invasive markers and IgA in feces or intestinal lavage. For reference, we measured antibodies to live bacteria in 30 healthy volunteers who were orally infected with wild-type enterotoxigenic *Escherichia coli* (*BMC Infect Dis* 2014;14:482–9). The response against these bacteria in serum, lavage and lymphocyte supernatants (ALS) was compared with that in parotid and sublingual/submandibular secretions sampled by absorbing sponge (Salimetrics®). Strong correlation occurred between IgA levels against the challenge bacteria in sublingual fluid and lavage ($r = 0.69$, $P < 0.0001$) and ALS ($r = 0.70$, $P < 0.0001$). In sublingual fluid, 93% responded with more than a 2-fold increase in IgA antibodies against the challenge strain, whereas the corresponding response in parotid secretions was only 67% ($P = 0.039$). With >2-fold ALS as a reference, the sensitivity of a >2-fold response for IgA in sublingual fluid was 96% compared with 92% in intestinal lavage and only 67% in parotid fluid. To exclude that flow rate variations influenced the results, we used albumin as a surrogate marker. Our data suggested that IgA in sublingual fluid, rather than whole saliva with its variable content of parotid fluid, is a preferential non-invasive proxy for intestinal immune induction. Although we have not directly shown this possibility, our findings suggest that IgA plasma cell precursors generated in GALT home more consistently to the sublingual/submandibular than to the parotid glands, in keeping with the compartmentalization of the mucosal immune system.

O303

Effects of exopolysaccharides from Cyanobacterium aponinum from the Blue Lagoon in Iceland on anti-CD3/anti-CD28 stimulated T cells

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Regular bathing in the Blue Lagoon in Iceland has been shown to have beneficial effects on psoriasis. *Cyanobacterium aponinum* is dominating in the Blue Lagoon's microbial ecosystem. We have shown that exopolysaccharides from *C. aponinum* from the Blue Lagoon (EPS-Ca) increased IL-10 secretion by human monocyte-derived dendritic cells *in vitro* and co-culturing allogeneic T cells with dendritic cells matured in the presence of EPS-Ca led to reduced ratio of $IL\text{-}17^+$ $ROR\gamma t^+$/$IL\text{-}10^+$ $FoxP3^+$ in the $CD4^+$ T cells. However, the effects of the EPS-Ca on stimulated T cells has not been determined.
Human naive $CD4^+$ and $CD8^+$ T cells were isolated from PBMCs and stimulated with anti-CD3ε and anti-CD28, either separately or at a CD4:CD8 ratio of 2:1, for 72 h in the presence or absence of EPS-Ca. The concentration of cytokines in the supernatants was measured by ELISA.
$CD4^+$ T cells stimulated in the presence of EPS-Ca secreted less IL-10 than $CD4^+$ T cells stimulated in the absence of EPS-Ca, however, EPS-Ca did not affect their secretion of IL-13, IL-17 or IFN-γ. EPS-Ca had no effect on cytokine secretion by $CD8^+$ T cells. When both $CD4^+$ and $CD8^+$ T cells were stimulated together their cytokine secretion pattern was similar to that observed for stimulation of only $CD4^+$ T cells.
These results demonstrate that although EPS-Ca enhances the potential of dendritic cells to direct T cell activation into Treg cells, the EPS-Ca does not directly induce secretion of the Treg-associated cytokine IL-10 by the T cells.

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O304

The role of BCR ligation in Id-driven T-B collaboration

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B cells process their endogenous BCR and present V-region derived idiotypic (Id) peptides on their MHC class II molecules to Id-specific CD4^{+} T cells. This activity causes Id-driven T-B collaboration, a mechanism which could be important for immune regulation, but has also been shown to underlie immunopathology. The importance of specific BCR ligation in Id-driven T-B collaboration has not been explored yet.

To study the contribution of BCR ligation to Id-driven T-B collaboration, we have developed a new animal model through crossing a novel knock-in mouse carrying the rearranged heavy chain VDJ of the MOPC315 BCR (VDJ_H^{315} KI) with a novel mouse that expresses the λ2 light chain carrying a MOPC315-derived idiotypic sequence stimulatory to I-E^{d} restricted CD4^{+} T cells ($V\lambda2^{315\ m}$ mouse). The $V\lambda2^{315\ m}$ mouse was generated through replacement of the germline Vλ2 exon 2 with a synthetic Vλ2 fragment which carries mutations for amino acid positions 94, 95 and 96 that encode the idiotypic determinant.

In $VDJ_H^{315} \times V\lambda2^{315\ m}$ mice, the prevalence of B cells that harbor the reconstituted Id^{+} BCR is ~1.5%, which is the frequency for λ2^{+} B cells in wild type-mice. This indicates that the gene replacement procedure did not affect physiological recombination frequency and the expression levels of Vλ2. Using a series of *in vitro* and *in vivo* experiments relying on $VDJ_H^{315} \times V\lambda2^{315\ m}$ mice, we have established that Id-driven T-B collaboration is dependent on specific ligation of the Id^{+} BCR. Our results indicate that ligation induces a concurrent proliferation of Id^{+} B cells and Id-specific T cells, germinal center formation, plasma cell differentiation and the production of high levels of Id^{+} IgG antibodies.

Apart from an importance in immune regulation, these processes may be involved in the development of Id-driven autoimmune diseases and B cell cancers in animal models.

O305

Determination of proinsulin T cell epitopes restricted by type 1 diabetes-associated HLA molecules

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Type 1 diabetes (T1D) is caused by a T-cell mediated destruction of pancreatic beta cells. Several antigens have been implicated as targets of the autoimmune CD4^{+} T-cell response in T1D but recent studies suggest that (pro)insulin may be the most important autoantigen. The HLA class II region has the strongest impact on the genetic risk of T1D and the DR3-DQ2 and DR4-DQ8 are the main risk haplotypes for the disease. Hence, the identification of proinsulin epitopes restricted by HLA class II molecules encoded by the DR3-DQ2 and DR4-DQ8 haplotypes is important for the understanding of the disease pathogenesis.

In this study, we have used a well-established T-cell cloning method based on CFSE-dilution to generate proinsulin-specific T-cell clones from healthy individuals who carry only the high-risk DR3-DQ2 and/or DR4-DQ8 haplotypes. Epitope specificity of the T-cell clones was determined by stimulating them with individual peptides spanning the proinsulin sequence, and HLA-restriction was determined by using anti-DR, -DQ and -DP antibodies and HLA class II-homozygous antigen presenting cells.

So far, we have generated 26 unique T-cell clones that recognize at least eight distinct epitopes within proinsulin. With our approach we have confirmed the existence of previously reported DR4-restricted epitopes and also identified novel DR3- and DQ2-restricted epitopes. The identification of the T-cell epitopes targeted by CD4^{+} T cells in T1D may be employed in the development of both antigen-specific therapies as well as better T-cell assays for immune monitoring in clinical trials.

O306

Optimal T cell activation and B cell antibody responses in vivo require the interaction between LFA-1 and kindlin-3

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Introduction: Kindlin-3 is an important integrin regulator that binds to the beta2 integrin cytoplasmic part. It is

mutated in the rare genetic disorder, LADIII, that is characterized by defective neutrophil trafficking and platelet function, leading to recurrent bacterial infections and bleeding. We have previously reported that the mutation TTT/AAA of the beta2-integrin cytoplasmic tail, which abolishes the binding of kindlin-3 to the integrin, results in impaired integrin activation to its high affinity state, and therefore reduced integrin function. Whether or not the integrin/kindlin interaction regulates T or B cell activation *in vivo* is unclear at present.

Materials and Methods: TTT/AAA-beta2-integrin knock-in (KI) mice and T cell receptor transgenic (OT-II) KI mice, where the integrin/kindlin connection has been disrupted were used.

Results: Basal T cell activation status in these animals *in vivo* is normal, but they display reduced T cell activation by wild type antigen-loaded dendritic cells *in vitro*. In addition, T cell activation *in vivo* is reduced. Basal antibody levels are also normal in TTT/AAA-beta2-integrin knock-in mice, but B cell numbers in lymph nodes, and IgG and IgM production after immunization are reduced.

Conclusions: We show that the integrin/kindlin interaction is required not only for trafficking of immune cells, but also for T cell activation and B cell antibody responses *in vivo*. These results imply that the immunodeficiency found in leukocyte adhesion deficiency type III patients, in addition to being caused by defects in neutrophil function, may also, in part, be due to defects in lymphocyte trafficking and activation.

P301

Toll-like receptors in primary Sjögren's syndrome

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Primary Sjögren's syndrome (pSS) is a systemic autoimmune disease of unknown etiology. It is characterized by chronic inflammation in salivary and lacrimal glands resulting in dry mouth and eyes, and the presence of autoantibodies. Many patients suffer from various additional symptoms due to extraglandular manifestations. The female to male ratio is 9:1, and the age of onset is usually between 40 and 60 years of age.

We analysed Toll-like receptor (TLR) expression and function in peripheral blood mononuclear cells (PBMC), B cells and B cell subsets. TLR7 and -9 were expressed at similar levels in B cells of pSS patients and healthy controls. Naïve B cells expressed less TLR7 and -9 compared to memory and pre-switched memory B cells. We next analyzed the effect of B cell stimulation via TLR7 and -9. In general we found upregulated levels of surface markers and cytokines after stimulation with both TLR7 and -9 ligands in pSS patients and controls. B cells from pSS patients secreted higher amounts of IL-8, IL-15, IFN-α, IL-1RA, MCP-1 and IL-2R in both unstimulated and stimulated cells. We also found less intracellular IL-10 in pre-switched memory B cells in pSS patients compared to healthy controls. Evaluating expression levels of all 10 TLRs in PBMC, we found that pSS patients expressed less TLR9 and more TLR8 at mRNA level, and less TLR5 and more TLR7 at protein level compared to controls. These results indicate that TLRs might indeed be involved in the pathogenesis of pSS.

P302

The role of CD73 on afferent lymphatics

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The ectoenzyme CD73 (ecto-5′nucleotidase) is widely expressed throughout the body. It is important for the regulation of inflammatory responses and for cell migration. CD73 further catalyses the hydrolysis of AMP and thereby is one of the most important producers of extracellular adenosine. While it is expressed on the lymphatic endothelium, the role it plays there has not yet been deciphered.

As lymphatic CD73 does not seem to affect cell trafficking, we aim to study whether CD73 expressed on afferent lymphatics exerts immunosuppressive effects on dendritic cells migrating towards the draining lymph nodes.

To answer this, human monocyte derived dendritic cells (DCs) are activated *in vitro* and the effect of different substances such as anti-inflammatory adenosine on their maturation is analysed. In addition, dendritic cells are co-cultured together with a CD73 expressing lymphatic endothelial cell line before comparing their maturation markers. In *in vivo* models, dendritic cells from CD73 deficient animals and their respective wild types are used to check for a possible effect of lymphatic CD73 on the maturation of these cells. This is done in the steady state, following the injection of an inflammatory substance to the footpad as well as after FITC ear painting.

First results indicate that only MHCII is downregulated following the exposure to the adenosine agonist NECA. In

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the *in vivo* experiments, we found similar differences in the MHCII expression as KO animals showed increased levels.

P305

Characterization of secreted molecules from lactobacilli with systemic immune-modulatory activity

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Lactobacilli are commensal lactic acid-producing bacteria with the capacity to influence and modulate mucosal and systemic immunity *in vitro* and *in vivo*. Early-life colonization with lactobacilli is associated with decreased risk of allergy development and supplementation of certain lactobacilli spp are known to ameliorate gut inflammatory diseases and improve epithelial barrier function. However, the direct mechanism of these bacteria on systemic immune responses remains elusive.

Human PBMC were stimulated with *S. aureus* cell free supernatant (CFS) in the presence or absence of lactobacilli-CFS. Pro- and anti-inflammatory cytokine responses were evaluated using ELISA and flow cytometry. Classical biomolecular/chemical techniques were employed to characterize and identify the bioactive molecules present in the lactobacilli-CFS.

We show that CFS of multiple lactobacilli spp dampen *S. aureus*-induced IFN-γ and IL-17A secretion. Size fractionation suggests that molecules of different size differentially dampen these cytokines. Heat-inactivation of the CFS prevented dampening of IL-17A but not of IFN-γ. Even though lactobacilli-CFS potently induced IL-10-production in PBMC, blocking of IL-10 did not prevent lactobacilli-mediated dampening. Addition of lactic acid to *S. aureus*-stimulated PBMC cultures selectively reduced the frequency of IFN-γ^+ unconventional T-cells and NK-cells in a dose dependent manner.

We conclude that lactobacilli-CFS induce and modulate both pro- and anti-inflammatory cytokine-responses in PBMC. Lactobacilli mediate dampening of IFN-γ by an IL-10-independent mechanism through the release of multiple molecules. Finally, lactic acid production may have a contributing role in the dampening of pro-inflammatory cytokine responses.

P306

Molecular trafficking of antigen in B cells

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B cell receptor mediated antigen recognition is followed by antigen internalization and intracellular processing along endo-lysosomal vesicular pathway. Although antigen processing and presentation to T helper cells is indispensable for efficient adaptive immune responses, molecular mechanisms of the pathway from antigen – B cell receptor intake to the peptide-antigen – MHCII presentation remain elusive, and the key regulator proteins unknown. Important for vesicle traffic are Rab proteins, small GTPases involved in budding, docking and fusion of vesicles in many cell types but little is known of their role in antigen trafficking in B cells.

Here, we spatiotemporally characterize the antigen processing pathway through various Rab protein-marked vesicular compartments. By spinning disk confocal imaging of both living cells and immunofluorescence samples we show that antigen first co-compartmentalizes with early endosomal marker Rab5 and subsequently with late endosomal and lysosomal markers Rab7 and Rab9. Notably, Rab9 also transiently associates with the antigen also shortly after the internalization. Simultaneous tracking of antigen and transferrin reveals fast diversion of these two molecules endocytosed by clathrin-coated pits.

Intracellular processing of antigen from the BCR to the peptide-MHCII is vital for efficient humoral immune response. Characterization of the responsible vesicular pathway leads the way towards understanding the molecular mechanisms behind efficient antigen processing in B cells.

P307

Circulating CXCR5$^+$ PD-1$^+$ ICOS$^+$ follicular T helper cells are increased near the onset of type 1 diabetes in subjects with multiple autoantibodies

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Type 1 diabetes (T1D) is thought to be a primarily T cell-driven autoimmune disease. However, autoantibodies produced by B cells are the best available biomarker for

early islet autoimmunity and increased disease risk. These antibodies are produced by autoreactive B cells, the activation of which is largely dependent on the function of $CD4^{+}$ $CXCR5^{+}$ follicular T helper cells (Tfh).
In the present study, we extensively characterized circulating Tfh cells in a clinical cohort of 55 children with recent-onset T1D, 68 at-risk children positive for multiple autoantibodies and 142 age-matched healthy controls. Markers associated with Tfh phenotype and function (PD-1, ICOS, CCR6, CXCR3, CCR7, IL-21) were analyzed by multi-color flow cytometry.
The frequency of activated $CD4^{+}$ $CXCR5^{+}$ $PD\text{-}1^{+}$ $ICOS^{+}$ Tfh cells was markedly increased in the peripheral blood of patients with recent-onset T1D. A similar increase was observed only in the subset of autoantibody-positive risk subjects who displayed impaired glucose tolerance (IGT), demonstrating that Tfh activation is associated with advanced beta cell-autoimmunity and dysglycemia. Intriguingly, the increase of activated Tfh cells was evident only in subjects with T1D or IGT that were positive for $\geq$ 2 biochemical autoantibodies.
Together, our findings demonstrate that alterations in the circulating Tfh compartment can be observed near the onset of T1D. Moreover, positivity for multiple autoantibodies appears to define a subgroup of T1D patients with pronounced peripheral blood Tfh activation. Our observations have important implications for both the potential use of Tfh cells as biomarkers of disease progression as well as for stratifying patients for future immunotherapy trials.

O401

Differential regulatory T-cell suppressive programs are controlled by FOXP3 isoforms

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Atherosclerosis, a chronic inflammatory disease of arteries, is the leading cause of cardiovascular diseases. Innate as well as adaptive immune responses contribute to atherogenesis, with components of cholesterol carrying low-density lipoprotein triggering inflammation, T-cell activation and antibody production during the course of disease. Regulatory T cells (TRegs), known to suppress immune responses and regulate peripheral tolerance, have been shown to attenuate atherosclerosis in experimental models. The transcription factor Forkhead box P3 (FOXP3) protein is the lineage specification factor of TRegs and plays a critical role in upholding the suppressive activity of TRegs. Three splice forms of FOXP3 mRNA exist in human given birth to isoforms with different functional properties. Full length FOXP3 and FOXP3Δ2 confer a suppressive phenotype to TRegs whereas FOXP3Δ2Δ7 inhibits the function of the other FOXP3 isoforms in a dominant negative manner. Herein we correlated the expression of FOXP3 isoforms with occurrence of symptoms prior endarterectomy. Moreover, transduction of a single FOXP3 isoform in naïve T-cells allowed us to decipher the role of each isoform for TReg functions.

O402

The endothelial protein PLVAP controls antigen and lymphocyte entry into lymph node

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The entry of lymph-borne antigens and lymphocytes to the lymph nodes occurs via the afferent lymphatic vessels. Antigens and lymphocytes enter the subcapsular sinus and must cross a lymphatic endothelial cell layer to reach the parenchyma of the lymph node. Antigens enter through the sinus floor in a size-dependent manner into a unique conduit system and lymphocytes gain access to the parenchyma by migrating through the floor. However, the molecular mechanisms regulating this cell and antigen traffic through the sinus floor are unknown. We have shown that the endothelium specific Plasmalemma vesicle-associated protein (PLVAP) is unexpectedly expressed in the lymphatic endothelial cells. PLVAP-deficient mice show enhanced entry of both small and large antigens into the conduit system and the transmigration of lymphocytes through the sinus floor is augmented. Mechanistically, PLVAP forms a physical sieve on the transendothelial channels that traverse the subcapsular sinus floor, controlling the entry of soluble antigens and lymphocytes into the conduit network and lymph node parenchyma.

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O403

Brahma complex modulates immune signalling in Drosophila melanogaster

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Drosophila melanogaster is a widely used model in immunology. *Drosophila* defence against yeast and bacteria is mainly mediated by two evolutionarily conserved NF-kappaB signalling pathways, the Toll and the Immune deficiency (Imd) pathway. Although the core Toll signalling is well known, the negative regulation of Toll signalling is poorly understood. In our genome-wide RNAi *in vitro* screen in S2 cells, we identified 14 genes that negatively regulate Toll pathway activity. Among these genes there were four components of the so-called Brahma complex that is involved in chromatin remodelling. We studied the *in vivo* significance of the Brahma complex in immune signalling using the *UAS-GAL4* method in *D. melanogaster*. The RNAi flies were crossed to the *C564-GAL4* driver, to silence the expression of two members of the Brahma complex (*brahma* and *osa*) in the fat body (equivalent to mammalian liver). The progeny flies were first infected with Gram-positive bacteria, *Micrococcus luteus*, to activate the Toll pathway. 24 h post infection, the flies were further infected with more virulent Gram-positive bacteria, *Enterococcus faecalis*. The infection experiments demonstrate that silencing of components of the chromatin remodelling Brahma complex increases survival of the flies against *E. faecalis*. Quantitative PCR analysis of antimicrobial peptide gene expression further suggests that the Brahma complex negatively regulates the target genes of the Toll pathway. These results indicate that DNA modification plays a role in the regulation of the *Drosophila* Toll signalling. Further studies will elucidate specific function of the Brahma complex in the *Drosophila* immune response.

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The role of extracellular vesicles (EVs) and adenosine in immunomodulation by human mesenchymal stromal cells (MSCs)

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Introduction: Mesenchymal stromal cells (MSCs) can counteract excessive inflammatory responses. MSCs employ a range of immunomodulatory mechanisms in response to signals in a particular environment. One immunosuppressive mechanism, not so well-known in MSCs, is mediated via adenosinergic pathway by ectonucleotidases CD39 and CD73, a known immunophenotypic marker of MSCs.
Methods: EVs were collected from serum free MSC conditioned medium by ultracentrifugation. Production of EVs was characterized by nanoparticle tracking analysis. The expression of CD39 and CD73 was analyzed using immune fluorescence and flow cytometry. The measurement of ectonuclease activity of the MSCs, T cells and MSC-EVs was performed using high performance liquid chromatography. The immunosuppressive effect of MSCs and MSC-EVs was analyzed by T cell proliferation assay and flow cytometry.
Results: Adenosine is actively produced from adenosine 5′-monophosphate (AMP) by CD73 on MSCs and MSC-EVs. Activated T cells highly express CD39 and produce AMP from adenosine 5′-triphosphate (ATP). The most efficient adenosine production from ATP requires co-operation of MSCs and activated T cells. MSCs and MSC-EVs suppress T cell proliferation *in vitro*, in assay conditions mostly utilizing indoleamine 2,3-dioxygenase (IDO). Still, adenosinergic signaling plays a role in immunosuppression by MSCs when ATP is present in the assay.
Conclusion: We suggest that adenosinergic signaling is an important immunoregulatory mechanism of MSCs, especially in situations where ATP is present in the extracellular environment, like in tissue injury. An efficient production of immunosuppressive adenosine is dependent on the concerted action of CD39-positive immune cells with CD73-positive cells such as MSCs or MSC-EVs.

O405

Conflicting HLA assignment by three different typing methods due to the apparent loss of heterozygosity in the MHC region

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Loss of heterozygocity (LOH) of the HLA region of chromosome 6 is common in solid tumours. However, the incidence of LOH in hematologic malignancies and severe aplastic anaemia is less well known. In these patients the LOH phenomenon causes apparent HLA homozygosity in patients who in fact are heterozygous. This is a challenge for histocompatibility testing as a stem cell transplantation from a genuinely HLA homozygous donor to a mistyped HLA heterozygous patient can increase the risk for acute life threatening graft versus host disease in transplanted patients.

We have observed LOH of the HLA region in five patients out of about 950 patients with hematologic malignancies in the last 3 years. The LOH was observed either in the HLA class I or II or both.

The ability of the three commonly used HLA typing methods SSP, SSO and SBT to detect heterozygocity was compared. In straight forward DNA dilution study, where the other allele was underrepresented, the detection levels were 2.5%, 17% and 33%. SSP seems to be the best method for detecting the heterozygocity and SBT loses the heterozygous signal easiest.

In a retrospective study we retyped 65 apparently HLA homozygous hematologic patients using SSP to confirm the HLA type but no undetected LOH cases were found.

The results underline the importance for an HLA laboratory to recognize and understand the characteristics and potential differences between the typing methods they are using. A protocol for confirming homozygocity in patients with hematologic diseases must be established in the laboratory.

O406

NOX2-derived ROS are not necessary during disease priming to protect from experimental autoimmune arthritis

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Neutrophil cytosolic factor 1 (NCF1) is the key regulatory component of the phagocytic NADPH oxidase (NOX2) complex which produces reactive oxygen species (ROS). NCF1 has been linked to rheumatoid arthritis, and loss-of-function mutations in *Ncf1* gene increase arthritis severity in rodents. In this study, we generated targeted *Ncf1* knock-in mice with inducible *Ncf1* expression and aimed at determining the critical time window during which the NOX2-derived ROS protect the mice from arthritis.

Targeted *Ncf1* knock-in mice lacked NOX2-derived ROS as intended. Allelic conversion of *Ncf1* was induced *in vivo* by administration of tamoxifen to activate the CreER^{T2} recombinase. *In vivo Ncf1* activation led to full protein expression and ROS production by the blood granulocytic cells in 10 days.

Collagen-induced arthritis was utilized to study the effect of ROS during the development of autoimmune disease. Mice with activated *Ncf1* developed only mild clinical symptoms whereas the ROS-deficient littermates had severe arthritis. During the priming phase, functional *Ncf1* limited expansion of T cells specific for the immunodominant type II collagen (CII) peptide and NCF1 seemed to diminish CII autoantibody production. When ROS-deficient mice were immunized and *Ncf1* gene activated only after the priming phase of the disease, *Ncf1*-dependent protection from autoimmune arthritis was still observed. Both CII autoantibody production and gamma-interferon response of T cells seemed to reflect disease severity.

The results point to a regulatory role of NOX2-derived ROS not only during priming but also during the effector phase of collagen-induced arthritis.

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The impact of fibrinogen carbamylation on fibrin clot formation and the inflammatory response

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Introduction: Carbamylation of lysine residues and protein N-termini is a ubiquitous, non-enzymatic post-translational modification that irreversibly changes protein charge, structure and function. It has been shown that carbamylated proteins increase in plasma and tissues during chronic kidney disease, and are an important biomarker of clinical outcome. The main objective of our study was to identify specific carbamylation patterns of fibrinogen and how they relate to malfunction of the coagulation cascade in dialysis patients. Moreover, we investigated the impact of carbamylation on immunological properties of the fibrinopeptides.

Methods: Fibrinogen was isolated from plasma and subjected to HPLC-MS/MS to identify carbamylation levels and modified lysine residues. Fibrin polymerization kinetics were measured based on turbidity changes while scanning electron microscopy was used to visualize fibrin clot structure. Chemotaxis of neutrophils in response to the fibrinopeptides was tested in the Insall chamber and detected by video microscopy.

Results: Fibrinopeptide release by thrombin was not impaired by carbamylation, but fibrin polymerization and the architecture of the fibrin clots were greatly affected. Interestingly, neutrophils displayed chemotactic activity towards carbamylated fibrinopeptide A but not towards its unmodified form.

Conclusions: Herein we showed that fibrinogen carbamylation might be one of the important factors underlying aberrant blood clot formation in chronic kidney disease. Identification of carbamylation patterns permitting prediction of coagulation abnormalities will help towards timely recognition of therapeutic requirements. Additionally, the chemotactic capacity of fibrinopeptide A might have profound impact on leukocyte infiltration, a histopathologic hallmark of chronic inflammation, enhancing leukocyte recruitment to sites of fibrin(ogen) turnover.

P402

Natural killer cells play an essential role in resolution of antigen-induced inflammation in mice

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NK cells have recently been implicated in resolution of allergic airway inflammation. This study examined the role of NK cells in resolution of inflammation in an antigen-induced peritonitis model. Mice were injected intravenously with an NK cell depleting antibody (anti-asialo GM1, αASGM1) or a control antibody 24 h prior to peritonitis-induction. Spleen, draining lymph nodes and peritoneal exudates were collected and cryosections stained by immuno fluorescence and peritoneal cells and soluble mediators analyzed by flow cytometry, ELISA and LC-MS/MS.

The number of peritoneal neutrophils 12 h after induction of inflammation was much higher in the αASGM1-injected mice than in the control mice. The number of neutrophils still remained high at 48 h in the αASGM1-injected mice contrary to the control mice in which the number of neutrophils had returned to baseline. Peritoneal concentrations of the neutrophil regulators G-CSF and IL-12p40 were higher at 12 h in the αASGM1-injected mice than in the control mice. Reduced apoptosis was detected in draining lymph nodes and spleens from the αASGM1-injected mice compared with the control mice and lower numbers of peritoneal NK cells expressing NKp46 and NKG2D, receptors implicated in NK cell-induced neutrophil apoptosis. Concentrations of the resolution type lipid mediators LXA_4 and PGE_2 were lower in the αASGM1-injected mice than in the control mice. These results indicate that NK cells are important for tempering neutrophil recruitment and maintaining neutrophil apoptosis as well as production of lipid mediators important for optimal resolution of inflammation.

P403

Variation in immune cell composition of stem cell grafts is associated with clinical outcome of allogeneic haematopoietic stem cell transplantation in patients with acute myeloid leukemia

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Several complications of allogeneic hematopoietic stem cell transplantation (HSCT) have been attributed to immune cells transferred into the patient with the graft. Numbers of $CD34^+$ or nucleated cells are routinely calculated, but immune cell composition of the graft is not evaluated. Our aim was to estimate the variation in percentages of immune cell subpopulations between individual clinical HSCT grafts and whether these variations associate with the clinical outcome of HSCT.
Composition of immune cells (natural killer, dendritic, T- and B-cells, $CD34^+$ hematopoietic stem cells) of 51 clinical grafts from single HSCT Centre were analyzed using FACS. Cell levels were statistically compared with clinical outcomes of HSCT. Overall survival, acute and chronic graft versus host disease (a/cGVHD) and relapse were used as the primary endpoints.
The results showed considerable variation between individual HSCT grafts in their numbers of different immune cell subsets, such as $CD123^+$ dendritic cells or $CD34^+$ cells. AML patients who developed aGVHD were transplanted with higher levels of $CD3^+$, $CD4^+$ and $CD8^+$ T cells as well as $CD19^+$ B and $CD123^+$ dendritic cells than AML patients without aGVHD. Grafts with a high $CD34^+$ content protected against aGVHD. Lower level of monocytes and higher proportion of $CD34^+$ cells associated to the incidence of cGVHD among AML patients.
There is considerable variation in the levels of different immune cell populations in the HSCT grafts which might affect the outcome of HSCT at least in AML patients. A detailed graft cell analysis could be used in risk assessment of HSCT.

P404

Disparity of the MHC class III and I regions between HLA matched unrelated donors in allogeneic HSCT

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Matching HLA alleles between the recipient and the donor is prerequisite for haematopoietic stem cell transplantation (HSCT). Five classical HLA loci are routinely screened prior to HSCT. However, matching these loci covers only minor part of the whole MHC area leaving out non-HLA or non-classical HLA regions. Therefore a HLA-matched donor can be haplotype mismatched especially in unrelated HSCT.
In this study, matching of genetic variation in the MHC class III region and in non-classical HLA G gene was compared between HLA-A-B-C-DRB1-DQB1 matched patient/donor-candidate pairs who both were Finnish (40 patients, 73 donor candidates) and those in which the patient was Finnish but donor candidate was from a non-Finnish registry (40 patients, 81 donor candidates). 25 SNPs of the complement gene 4 in the MHC class III were detected by GammatypeTM kit. No SNP differences between the potential transplantation pair was considered as match. An insertion/deletion polymorphism in HLA-G (14 bp indel 3′UTR region) was genotyped by PCR.
There was no statistical difference in the HLA-G match between the two study populations, obviously due to the strong linkage disequilibrium between HLA-A and HLA-G alleles. In contrast, statistically significant difference in the class III match was observed between Finnish/Finnish and Finnish/non-Finnish pairs ($P < 0.0001$). An average number of mismatched SNPs in Finnish/Finnish group was lower than in that Finnish/non-Finnish group. For Finnish patients, the HLA matched donors from the Finnish donor registry were more likely to result in entire haplotype match as compared to HLA-matched donors from non-Finnish donor registry.

P405

Analysis of C3 gene reveals haplotypes predisposing to pre-eclampsia

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Pre-eclampsia is a serious disease of the placenta and vascular endothelium that affects 3% of pregnancies. There

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is no known cure to the disease and the etiology is not known, although immunological factors and the complement system in particular are known to affect the development of pre-eclampsia. C3 is the central activating component of the complement system. A custom-made SNP genotyping chip covering 72 SNPs in complement genes revealed nominal association to *C3* variants in 271 pre-eclamptic and 449 non-pre-eclamptic women. *C3* exome and intron-exon boundaries were sequenced in 32 women with severe pre-eclampsia and 95 controls.
A haplotype was discovered in the middle of the gene that could associate to pre-eclampsia. From the 45 SNPs discovered by sequencing, 17 were selected and 15 were successfully replicated by Sequenom massArray genotyping in 1456 pre-eclamptic women and 704 controls. Genotyping revealed an intronic SNP (rs2241391) with possible loss of function effect by intron retaining with modest association to pre-eclampsia (OR = 1.743, CI95: 1.014–2.995). This SNP was observed in 20% of pre-eclamptic women versus 12% of controls. Furthermore, a rare predisposing haplotype spanning eight of the tested variants was observed in the middle of the gene (OR = 2.118, CI95: 1.176–3.817) in 2.1% of cases but only in 1% of controls. A second predisposing area of three SNP was found at the end of the gene (OR 1.9294, CI95: 1.0424–3.5714). These results suggest that altered regulatory regions of *C3* increase the risk of pre-eclampsia in a subgroup of pre-eclamptic women.

P406

Myeloid cell expressed proprotein convertase FURIN attenuates inflammation

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Proprotein convertase Furin converts immature proproteins into functional products. Previously we have shown that Furin is a key regulator of T cell mediated peripheral immune tolerance and it is upregulated upon the activation of monocytes with LPS. However, Furin's functional role in myeloid cells remains unknown.
Mice with a conditional deletion for Furin in myeloid cells (LysMcre-furf/f) did not reveal marked differences concerning macrophage and neutrophil populations in the bone marrow, spleen and peritoneal cavity. Interestingly, steady state IL-1β production was elevated in the serum of LysMcre-furf/f mice and Genome-Wide analysis of gene expression revealed the overexpression of several proinflammatory genes in unstimulated Furin deficient peritoneal macrophages. Moreover, LysMcre-furf/f mice intraperitoneally injected with a lethal dose of LPS showed higher production of proinflammatory cytokines and increased mortality.
Remarkably, unstimulated Furin deficient peritoneal macrophages produced lower levels of bioactive TGF-β1 and hyper-activation of TACE and Caspase-1 p20 upon LPS stimulus.
We conclude that deletion of Furin in macrophages results in a proinflammatory immune phenotype, which is partially due to the deficiency in TGF-β1 production and hyperactivation of TACE and Caspase-1.

P407

Extend of chromosome 6 matching in HLA identical sibling stem cell transplantation

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Matching of HLA alleles between donor and recipient is the golden rule for reducing risk for graft-versus-host disease (GvHD) after hematopoietic stem cell transplantation (HSCT). The HLA genes are located within a genomic DNA segment of approximately 4 Mbp in length usually inherited en bloc. Hence, HLA identical siblings have inherited, except for possible recombination, identical HLA gene segments from parents.
We here demonstrate by high density analysis of genetic variation in the HLA segment and chromosome 6p that six of 255 HSCTs between HLA A, B, DRB1 matched siblings had additional mismatches in HLA loci not typed. Five had HLA DPB1 mismatches and one had mismatches in DPB1 plus DQA1. The matching of HLA alleles in fact resulted in matching of segments of chromosome 6 substantially longer than the mere HLA genes. The segment of chromosome 6p that HLA identical siblings shared encompassed on average 1037 genes (range 6–1597, median 1120), in addition to the genes of HLA complex. Many of the genes are known to show genetic variation that

may either regulate the GvHD or act as a mismatched minor histocompatibility antigen. The results indicate that in transplantations when matching the HLA genes between siblings we in fact match a high number of other genetically variable genes that may influence to clinical outcome of HSCT. However, in this limited set of HSCTs the number of shared genes was not associated with the occurrence of acute or chronic GvHD.

O501

Early childhood CMV infections decelerate the progression from beta-cell autoimmunity to clinical type 1 diabetes

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Introduction: Type 1 diabetes (T1D) is considered to arise from gene-environment interactions. Cytomegalovirus (CMV) infections have been implicated to play a role in the development of T1D. However, no clear risk effect has been demonstrated. In contrast, signs of a possible protective effect of early childhood CMV infections on disease progression were observed by a recent study. Here, we set out to further delineate the possible impact of early childhood CMV infections in children with genetic T1D susceptibility.

Methods: A total of 543 children from the prospective DIPP study were analyzed for CMV-specific IgG antibodies at the age of 18 months. All children carried HLA class II genotypes conferring increased T1D risk. The effect of CMV infections on the appearance of T1D-associated autoantibodies (IAA, GADA and IA2A, n = 194) and on the progression rate from autoantibody seroconversion to clinical T1D (n = 111) were analyzed.

Results: Early childhood CMV infections were not associated with the appearance of T1D-associated autoantibodies. However, the infection was negatively correlated with the progression rate from the initiation of beta-cell autoimmunity to clinical disease, which was slower among subjects with early childhood CMV infection (P = 0.016, Kaplan-Meier log-rank test). T1D was diagnosed during the follow-up in 42.6% of the CMV-positive children compared to 60.5% of the subjects without an early CMV infection.

Conclusion: These results suggest that an early childhood CMV infection may decelerate the progression rate of beta-cell destruction after the initiation of autoimmunity and may thus protect children from developing T1D during childhood.

O502

Interleukin-26 and its role in inflammatory conditions of the skin

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Interleukin-26 (IL-26) is a member of the IL-10 family and located on chromosome 12q15. IL-26 was initially identified in herpesvirus saimiri-transformed T cells and later IL-26 mRNA expression was detected in T_H17 cells, T_H1 cells, and NK cells. IL-26 signals through a heterodimeric receptor complex consisting of IL-20R1 and IL-10R2. This receptor combination is exclusively expressed on epithelial cells suggesting a role in cutaneous and mucosal immunity. As psoriasis is a common chronic inflammatory skin disease mediated by T_H17 cells, we wanted to investigate the role of IL-26 in health and disease in more detail.

Using homology modeling and comparing IL-26 to IL-22, we found that IL-26 has a prominent amphipathic structure with clusters of cationic amino acids. As IL-26 has a remarkable high cationic charge, microbroth dilution assays were performed to where IL-26 was to be capable of killing bacteria in similar concentrations as LL37. Furthermore, the cationic IL-26 binds nucleic acids such as human DNA, which was investigated using a PicoGreenAssay and microscale thermophoresis. These IL-26/DNA complexes stimulate pDCs via TLR9 to secrete IFN-alpha. Looking at psoriatic lesions, we found that IL-26 gene expression is highly up-regulated which correlates with the significant increased amount of IL-26 protein in psoriasis. In conclusion, we found that IL-26 elicits a role in inflammation as well as autoimmunity. IL-26 efficiently kills gram-negative and gram-positive bacteria. On the other hand due its cationicity IL-26 binds nucleic acids, which are then prevented from degradation. These IL-26/DNA complexes in turn activate pDCs.

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Characterization of regulatory T cells in patients with type 1 diabetes and autoantibody-positive at-risk subjects

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$CD4^+$ $CD25^+$ $FOXP3^+$ regulatory T cells (Tregs) play a critical role in the maintenance of self tolerance. Reduction in the number or functional capacity of Tregs may be important in the pathogenenesis of autoimmune diseases, such as type 1 diabetes (T1D).

Here, we extensively characterized the Treg compartment in a clinical cohort of 44 children with recent-onset T1D, 68 at-risk children positive for multiple autoantibodies and 124 age-matched healthy controls. Using multi-color flow cytometry, we analyzed the frequencies of total, naive and memory Tregs, as well as memory Treg subsets defined by the expression of the chemokine receptors CCR6 and CXCR3. We also analyzed the frequencies of Tregs with proinflammatory potential as determined by the expression of CD161 and production of IFN-γ and IL-17A.

We detected an increased frequency of naive but not memory Tregs in T1D patients compared to autoantibody-positive subjects and healthy donors. Moreover, the frequency of Th1-type (CCR6-$CXCR3^+$) memory Tregs was increased and Th17-type ($CCR6^+$ $CXCR3^-$) Tregs was decreased in T1D patients. Again, no changes were observed in autoantibody-positive subjects compared to healthy donors. Finally, no differences in the frequency of cells expressing CD161 or producing IFN-γ and IL-17A within the memory Treg compartment was observed between the three study groups.

In conclusion, minor alterations in the Treg compartment were detectable in patients with recent-onset T1D. However, no alterations were observed in autoantibody-positive subjects, suggesting that the observed changes are likely associated with the clinical manifestation of the disease rather than being genetically-driven or manifestations of early autoimmunity.

O504

Dominant Autoimmune Regulator mutations associated with common organ-specific autoimmune diseases

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The autoimmune regulator (AIRE) gene is crucial for development of normal central immunological tolerance and prevention of autoimmunity. Mutations in AIRE cause a rare autosomal recessive disease, autoimmune polyendocrine syndrome type 1 (APS-1), where the patients display a variety of endocrine and ectodermal manifestations. The majority develops at least two of the three main components of adrenocortical insufficiency (Addison's disease), hypoparathyroidism and chronic mucocutaneous candidiasis.

We report a subset of specific, heterozygous AIRE mutations that directly causes common organ-specific autoimmunity with propensity for pernicious anemia and vitiligo. Multiple single cases and families with heterozygous mutations in the first plant homeodomain (PHD1) zinc finger presented with dominant inheritance, later presentation, milder phenotypes, and reduced penetrance compared to classical APS-1. Unlike mutations involved in recessive inheritance, missense PHD1-mutations suppressed gene expression driven by wild type AIRE in a dominant negative manner and localize to the cell nuclei in co-transfection experiments. In addition, exploring different mutations within the second PHD-finger of AIRE suggest certain amino acids to be of particular importance. Since AIRE mutations can cause common autoimmune diseases, we propose to redefine APS-1 into classical recessive and non-classical dominant forms, and to modify diagnostic criteria and clinical care accordingly.

O505

Protective roles for microglia in neuroinflammation

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Microglia are central nervous system (CNS)-resident immune cells. They are often referred to as brain macrophages and indeed share many phenotypic features with them. However, it is now recognized that they are of a separate lineage and are not replaced from blood-derived precursors, at least under normal circumstances. Microglia are implicated in neuroinflammatory and neurodegenerative diseases including multiple sclerosis (MS). We have shown that in a mouse model for MS- experimental autoimmune encephalomyelitis (EAE) numbers of microglia expressing CD11c significantly increase. These CD11c$^+$ microglia are effective antigen presenting cells, but poor inducers of Th1 or Th17 responses. Interestingly, CD11c$^+$ microglia express neuroprotective insulin-like growth factor 1 which suggests a neuroprotective rather than proinflammatory role. Here we show that CD11c$^+$ microglia predominated in the neonatal brain and expressed genes governing neuronal and glial survival, migration and differentiation. These cells were localized in sites of primary myelination such as cerebellum and corpus callosum. They expanded rapidly after birth and then contracted to become almost undetectable in the adult CNS. Neonatal and microglia from EAE CNS differed in their gene expression profiles, showing neurogenic and immune response gene signatures, respectively. Moreover, neonatal microglia showed a transient stem-cell like phenotype that was partially recapitulated by cells repopulating the adult CNS after microglia ablation. Interestingly, transplantation of neonatal microglia suppressed EAE. CD11c$^+$ microglia therefore can deliver signals necessary for neurogenesis and myelination.

O506

Investigation of patients with multiple sclerosis using bronchoalveolar lavage indicates T-cell activation in the lung and HLA-regulated response to smoke

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Cigarette smoking has been associated with an increased risk for a number of common autoimmune diseases, including multiple sclerosis (MS) but the immune cells and underlying biological mechanisms that contribute to this risk increase remains to be identified. To characterize the impact of smoke exposure on pulmonary immune cells and its role in the development of MS, we investigated a total of 84 individuals, including MS-patients (see table) by bronchoscopy with bronchoalveolar lavage (BAL).

Characterization of the cellular content of BAL revealed that cigarette smoking is associated with increased number alveolar macrophages, correlated with cumulative smoke exposure. The number of alveolar macrophages was further associated with specific HLA-II alleles. In particular, smokers carrying the MS risk allele HLA-DRB1*15 show a significant attenuation of the number of macrophages, compared to non-carriers.

Subsequent analysis of BAL cells using flow cytometry revealed that cigarette smoking is associated with a skewed distribution of CD4$^+$ and CD8$^+$ T-cells in the lung, and an increased proliferation of T-cells in the lung. In addition, intracellular stores of preformed CD40L in CD4$^+$ cells are significantly increased in MS non-smokers, suggesting an altered activation of T-cells in the lung.

This study confirms that smoke-exposure is a major regulator of immune cell activation in the lung, and indicates mechanisms that may be related to the development of MS. Further investigation of alveolar macrophages and pulmonary T-cells are currently undertaken, to define the role of these smoke-regulated mechanisms in the development of autoimmune disease.

	Healthy volunteers		Multiple Sclerosis	
	Non-smokers	Smokers	Non-smokers	Smokers
Total number	34	21	15	11
Gender (M/F)	16/18	6/15	5/10	2/9

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P501

Targeted proteomics to quantitate changes in protein levels during the development of Type 1 Diabetes

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Introduction: From the study of longitudinal serum samples, prospectively collected in the Finnish Diabetes Prediction and Prevention study, we have detected temporal differences in protein abundance between children that developed type 1 diabetes and matched controls with the same conferred genetic risk. In order to further study the levels of this panel of proteins in additional cohorts, and co-determine functionally related proteins, we have developed selected reaction monitoring (SRM) mass spectrometry method for their targeted detection. The method provides improvements in both sensitivity and selectivity, such that we have been able to target in the order of a 60 proteins in a single 1 h analysis.

Methods: Serum proteins were analysed using tandem mass spectrometry (LC-MS/MS). A Q Exactive Orbitrap and a TSQ triple quadrupole mass spectrometer were used for spectral library generation and targeted analysis, respectively. After analysis of the discovery data, the peptides associated with the differentially abundant proteins were evaluated for their suitability for SRM analysis. From these data, together with the analysis of isotopically labelled synthetic analogues, transition lists were developed for the validation assays.

Results: A method was developed to achieve interference free SRM mass spectrometry analysis from un-depleted serum. With the inclusion of functionally related proteins to the original targets, we have developed a method to profile 61 proteins in a single analytical run (~60 min). This method will enable us to quantify changes in protein levels during the development of Type 1 Diabetes as well as in other disorders.

P502

Autoimmune polyendocrine syndrome type 1 in Norway – a longitudinal follow-up

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Context: Autoimmune polyendocrine syndrome (APS-1) is a rare monogenic disease defined by the presence of two of the three major components hypoparathyrodism, primary adrenocortical insufficiency (PAI) and chronic mucocutanous candidiasis (CMC).

Objective: Long-term follow-up of all Norwegian APS-1 patients, describe their phenotype and correlate the clinical features to their autoantibody profiles and *Autoimmune Regulator (AIRE)* mutations.

Patients: All known patients with APS-1 recruited through the nation-wide patient registry.

Results: Fifty-two patients from 34 families were identified and included in the period 1996–2015. The majority presented with one of the major disease components during childhood and the diagnostic triad was present in the minority (40%). A mild phenotype was associated with the splice mutation c.879 + 1G > A. Enamel hypoplasia, hypoparathyroidism and CMC were most common. With age, most patients presented 3–5 disease manifestations, but some had milder phenotypes, and delayed diagnosis until adulthood. All patients except three had interferon-ω (IFN-ω) autoantibodies and all had organ specific autoantibodies. The most common *AIRE* mutation was c.967_979del13 found in homozygosity in 16 patients. Fourteen of the patients died during follow-up (median age at death 32.5 years) or were diseased siblings with a high

probability of undisclosed APS-1. The major causes of death were APS-1-related.
Conclusions: The APS-1 diagnosis should be considered in all patients presenting one of the major clinical manifestations, especially when it first presents in childhood. Antibodies against IFN-ω are a useful diagnostic tool and when a new patient is diagnosed; all siblings should be offered an investigation.

P503

Development and characterization of Th17-related U-PLEX® assays

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Th17-related cytokines mediate a host's defense mechanisms against various infections and play a crucial role in crosstalk between the immune system and affected tissues. Here we describe the development and characterization of multiplexed Th17-related immunoassays on MSD's flexible U-PLEX platform.
Monoclonal and polyclonal antibodies were conjugated with biotin and/or SULFO-TAGTM and screened as capture and/or detection reagents. A number of analytical parameters were used to select antibody pairs, and assays were developed by optimizing antibody concentrations, calibrator curves, specificity, matrix tolerance, and assay robustness. Assay performance was verified to be compatible with other U-PLEX assays by running Th17-related multiplexes on the U-PLEX platform with controls and samples.
Calibration curves showed expected signals, sensitivity, precision, and accuracy. Control samples for the assays had CVs <10% within runs and <25% between runs. Sensitivities were <1 pg/mL for the majority of the assays. Dilution linearity and spike recovery studies demonstrated acceptable matrix tolerance and accurate quantification for most of the assays tested across all matrices (typically between 75% and 125%). Cross-reactivity between assays was shown to be typically <0.5%. Results demonstrated a strong correlation between samples measured on U-PLEX multiplex and streptavidin singleplex panels with r^2 values >0.95 and slopes between 0.8 and 1.2.
Over 20 Th17-related assays for human and mouse were developed for the U-PLEX platform. These assays enable the measurement of multiple proteins on biological matrices that are relevant to a wide range of life science applications and pre-clinical or clinical studies.

P504

Class II HLA genotype association with FPIR reflects the islet autoantibody status

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Introduction: The association between HLA class II genotypes, autoantibodies and type 1 diabetes (T1D) risk is well characterized. However, limited data exist on the associations between genetic factors and first phase insulin response (FPIR), which, if reduced, is characteristic the disease process leading to clinical T1D.
Methods: A total of 471 HLA DR-DQ genotyped subjects in the prospective Finnish Type 1 Diabetes Prediction and Prevention (DIPP) Study were analyzed for FPIR after positivity for ≥1 diabetes-associated autoantibody. The association between log-transformed FPIR and four class II HLA-risk categories (high, moderate, slightly increased, neutral/protective) and number of biochemical autoantibodies at the time of the first FPIR (three groups: 0, 1, ≥2) was assessed with multi-way anova with age as a covariate.
Results: There was a strong correlation between HLA class II genotype and autoantibody group (χ^2, $P < 0.0001$). When the autoantibody-negative group was compared to the group with one or multiple autoantibodies, a clear positive trend was seen in Cochran Armitage Trend Test ($P < 0.0001$ for both) but not when comparing the one autoantibody group to the multipositive group ($P = 0.28$). There was a significant difference in the first FPIR between the autoantibody groups ($P = 0.0059$) and HLA risk groups ($P = 0.038$) whereas HLA* autoantibody group interaction term was not significant ($P = 0.43$). Islet autoimmunity was strongly associated with declined FPIR.
Discussion: The effect of the HLA class II genotype on the insulin response is mediated through the number of autoantibodies.

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P505

Effects of the Blue Lagoon psoriasis treatment on CD8, IL-17 and IL-22 staining in skin

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Introduction: Psoriasis is a chronic inflammatory skin disease, characterized by infiltration of T cells and epidermal thickening. In recent years the focus has shifted from a Th1 to a Th17 mediated immune response, with $CD4^+$ T cells secreting IL-17 and IL-22 being the main players in the pathology of psoriasis. Tc17 cells, $CD8^+$ T cells that have the same cytokine secretion panel as the Th17 cells, have previously been shown to be associated with the pathogenesis of psoriasis as well.
Materials and Methods: Skin biopsies were taken from patients taking part in a treatment study looking at the effects of the Blue Lagoon on psoriasis. Samples were taken before the treatment started (0 week) and at the end of treatment (6 weeks). The samples were cryosectioned and double-stained by immunofluorescence for CD8 and either IL-17 or IL-22. Stained cells were counted and graded.
Results: IL-17 was widely expressed in psoriatic skin, with high staining in keratinocytes in the lower layer of the epidermis and the upper layer of the dermis. IL-22 was less widely expressed in psoriatic skin than IL-17. Only few $CD8^+$ cells co-expressed IL-17. Six weeks of treatment led to reduced number of $CD8^+$ cells in epidermis but had no effect on the number and grade of IL-17 positive cells in epidermis and dermis.
Conclusions: Number of $CD8^+$ cells corresponds with the severity of the psoriasis but surprisingly the IL-17 staining is unaffected by the psoriasis treatment.

O601

Biosimilar agents in treatment of psoriasis – a comparison of Remicade and Remsima

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Psoriasis is a chronic, inflammatory skin disease characterized by a dysregulated cutaneous immune response. Biological treatment against TNF has been shown to be beneficial. However, even though this treatment is effective, it is very expensive. Biosimilars have therefore been taken into the clinic. We here compare signaling responses in peripheral blood mononuclear cells of psoriasis patients treated with the original agent infliximab (Remicade) and the biosimilar Remsima. We will use phosphoflow to investigate possible differences in cellular responses against the original drug and the biosimilar. The signaling signatures will be analyzed regarding phosphorylation of ERK, p38 and NFkB. The results will give important insights into the usage of biosimilars as cost effective treatment of psoriasis.

O602

Msr1 – a new player in leukocyte trafficking via the lymphatics

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Lymphocytes enter peripheral lymph nodes in a constant search for foreign antigens. Specialized blood vessels called high endothelial venules and the afferent lymphatics provide two main routes for lymphocytes to enter lymph nodes. Under physiological conditions lymphocytes are the only cells able to sequentially leave the lymph node via efferent lymphatics. Furthermore, the lymphatic system is a common route for migrating, metastasizing cancer cells. Selective leukocyte trafficking and cancer cell migration have been extensively studied, yet the underlying mechanisms have not been fully described. We have recently

discovered macrophage scavenger receptor 1 (Msr1), to be expressed by lymph node subcapsular sinus lymphatic endothelial cells (LEC), whereas it is not expressed by the lymphatic sinus LECs. Regarding leukocyte trafficking, we have studied the role of Msr1 in the binding and migration of lymphocytes to lymph nodes. The binding of lymphocytes to subcapsular sinus LECs was inhibited in Msr1 deficient mice by roughly 60%, and a similar result was obtained using human samples with Msr1 blocking antibodies in frozen section assays. In *in vivo* migration studies, lack of Msr1 allowed lymphocytes to enter further into the lymph node parenchyma in comparison to wild type mice. These results indicate a new role for Msr1 in leukocyte trafficking, and provide a prospect for further studies in relation to cancer cell migration.

O603

Gliadin-containing, poly(lactide-co-glycolide) nanoparticles reverse gluten-induced enteropathy in a celiac disease mouse model

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Background: In celiac disease (CD), immune tolerance to dietary gluten is lost, resulting in enteropathy and systemic disease manifestations. Therapeutic approaches rendering T cells tolerant to gluten could potentially cure CD, thus eliminating burdens associated with lifetime gluten-free diet (GFD) and preventing complications. Poly(lactide-co-glycolide) particles containing epitopes recognized by autoreactive T cells can induce antigen-specific tolerance in autoimmune disease models (Getts DR, et al. *Trends Immunol* 2015;**36**:419). The identification of gliadins as primary epitopes in CD permitted the development of poly (lactide-co-glycolide) particles containing gliadin (COUR-NP-GLI; Cour Pharmaceutical, Inc.) for tolerance induction.

Methods/Results: We tested the treatment effect of COUR-NP-GLI in a gliadin memory T cell transfer model of CD in $Rag1^{-/-}$ mice (Freitag TL, et al. *Am J Physiol Gastrointest Liver Physiol* 2014;**306**:G526). Treatment of mice maintained on gluten-containing diet with 2.5 mg of COUR-NP-GLI i.v. (containing 7.5 μg gliadin) on days 10 and 24 after adoptive T cell transfer diminished IFNγ and Il-17 secretion by gliadin-specific T cells at week 8, versus mice on GLUTEN diet alone (positive control; n = 14–16). Treatment also reduced histological duodenitis (histoscore: 5.25 median, 3.00–7.00 interquartile range versus 8.00, 7.00–8.00; scale 0.00–9.00, *$P < 0.05$) and prevented body weight loss, similar to the effect of GFD (negative control). Control treatment with similar particles containing irrelevant antigen (NP-LYSOZYME) showed minor effects only.

Conclusions: The results in this *in vivo* disease model support the concept of gliadin-specific immune tolerance induction using gliadin-containing nanoparticles in celiac patients. Additional mechanistic studies are ongoing.

O604

G-CSF regulates macrophage phenotype and associates with poor overall survival in human triple-negative breast cancer

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Tumor-associated macrophages (TAMs) have been implicated in the promotion of breast cancer growth and metastasis, and a strong infiltration by TAMs has been associated with estrogen receptor-negative tumors and poor prognosis. However, the molecular mechanisms behind these observations are unclear. We investigated macrophage activation in response to co-culture with several breast cancer cell lines (T47D, MCF-7, BT-474, SKBR-3, Cal-51 and MDA-MB-231) and found that high granulocyte colony-stimulating factor (G-CSF) secretion by the triple-negative breast cancer (TNBC) cell line MDA-MB-231 gave rise to immunosuppressive $HLA\text{-}DR^{lo}$ macrophages that promoted migration of breast cancer cells via secretion of TGF-α. In human breast cancer samples (n = 548), G-CSF was highly expressed in TNBC ($P < 0.001$) and associated with $CD163^{+}$ macrophages ($P < 0.0001$), poorer overall survival ($P = 0.021$) and significantly increased numbers of TGF-α^{+} cells. While G-CSF blockade in the 4T1 mammary tumor model promoted maturation of $MHCII^{hi}$ blood monocytes and TAMs and significantly reduced lung metastasis, anti-CSF-1R treatment promoted $MHCII^{lo}F4/80^{hi}MR^{hi}$ anti-inflammatory TAMs and enhanced lung metastasis in the presence of high G-CSF levels. Combined anti-G-CSF and anti-CSF-1R therapy significantly increased lymph node metastases, possibly via depletion of the so-called 'gate-keeper' subcapsular sinus macrophages. These results indicate that G-CSF promotes the anti-inflammatory

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phenotype of tumor-induced macrophages when CSF-1R is inhibited and therefore caution against the use of M-CSF/CSF-1R targeting agents in tumors with high G-CSF expression.

O605

Impact of starting material and expansion conditions on cell yield and composition of CAR T cell products

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Generation of effective T cell based therapeutics requires balance between cell number and quality of cells. Since T cell proliferation is coupled to cell activation, it may lead to cell exhaustion yielding less potent cells.
The aim was to develop an effective CAR T cell expansion protocol that should allow straightforward translation into a clinical-grade manufacturing process.
Lymphocytes were transduced lentivirally with CD19/CD28/4-1BB/z-CAR gene and expanded using CD3/CD28 Dynabeads and varying IL-2 concentrations. Cells were characterised from the starting material and at days 10 and 20 during expansion. Functional studies (e.g., cytotoxicity) were performed to demonstrate the significance of these results for adoptive cell therapy.
T cell expansion with low IL-2 yielded both CD4 and CD8 T cells with 32% T_{MSC} (CD45RO$^-$ CD27$^+$ CD95$^+$), 31% T_{CM} (CD45RO$^+$ CD27$^+$), 12% T_{EM} (CD45RA$^-$ CD27$^-$) and 23% terminal effectors (CD45RA$^+$ CD27$^-$). Higher IL-2 concentrations (100–300 IU/ml) and longer expansion time yielded high cell numbers but preferred T cell effectors over earlier memory cells. Further, IL-2 favoured CD4 but longer expansions favoured CD8 cells. The static cell culture protocol yielded a 60-fold T cell expansion. Expansion was as effective for both healthy volunteers and leukemia patients, however, transduction efficiency was lower for patients (38 versus 73%).
In conclusion, we show that cell culture parameters impact critically on T cell phenotype and will likely have a major influence on therapeutic effectiveness. Best balance between cell number, early T memory cells (SCM/CM versus EM/TE) and retained CD8 and CD4 populations was achieved using low IL-2 concentration and short expansion time.

O606

Druggable genome-wide siRNA screen to study the regulation of CD73 expression

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CD73, also known as ecto-5′-nucleotidase, is a cell-surface expressed ectoenzyme that generates adenosine by dephosphorylating extracellular adenosine monophosphate. Adenosine downregulates escalation of inflammation and improves vascular barrier function in several disease models. The objective of this study was to investigate the regulation of CD73 expression using human druggable genome wide siRNA lysate array (LMA) screen. CD73 expressing breast adenocarcinoma cell line (MDA-MB-231) was used in the screen. We obtained 380 hits (4.4% hit rate) from the screen and four hits were chosen for further validation. The validation was conducted by using both MDA-MB-231 cells and primary human umbilical vein endothelial cells (HUVEC) to study the regulation of CD73 and endothelial permeability. The hit siRNA's were transfected into the cells by using lipofection based transfection and the changes in CD73 expression were analyzed at the protein level with flow cytometry and at the RNA level with quantitative PCR. The efficiency of siRNA silencing was confirmed at the RNA level as well. Using this secondary screen two previously unknown hit molecules were found to regulate CD73 expression on cancer cells and also on endothelial cells. The functional significance of the hits will be confirmed by using functional experiments to study endothelial permeability *in vitro* and *in vivo*.

P601

Immunomonitoring of patients treated with mesenchymal stromal cells for graft versus host disease: a case study

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Graft-versus-host disease (GVHD) is a life-threatening complication after allogeneic hematopoietic stem cell

transplantation. T cells play a fundamental role in the development of GVHD. Mesenchymal stromal cells (MSC) can clearly modulate immune responses *in vitro*, and are currently used as salvage therapy to treat steroid-refractory GVHD. The aim of the present study is to assess GVHD patients' response to therapy with *ex vivo* expanded third-party MSCs. The bone marrow-derived MSCs were produced with a national ATMP hospital exemption license and in compliance with GMP and were expanded in platelet lysate. Whole blood samples for immune cell profiling and serum analysis were collected from patients during the course of the MSC treatment. Lymphocyte subpopulations, with special emphasis on CD4-positive T cells secreting IFN-γ (Th1), IL-4 (Th2) and IL-17 (Th17), as well as T cells expressing the transcription factor FoxP3 (regulatory T cells) were measured from the cell samples. A panel of serum proteins including Reg3alpha, cytokeratin-18 fragments and elafin, all described previously as markers of acute GVHD, were also analyzed. Our initial results from one case study indicate high initial levels of GVHD-associated markers in patient serum and alterations during the course of MSC treatment. Sequential monitoring of Th cell populations also revealed alterations during the follow-up period. The results of our on-going work will help in defining the usefulness of immune cell and serum protein profiling in the follow-up of MSC therapy.

P602

Imiquimod induces endoplasmic reticulum-stress in keratinocytes and melanoma cells independently of TLR7/8

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Endoplasmic reticulum (ER)-stress is a physiological response to protein overload or misfolded proteins in the ER. Certain anti-cancer drugs, e.g., bortezomib and nelfinavir, induce ER-stress implying that this could be a successful therapeutic strategy against several forms of cancer. To find novel ER-stress inducers we screened a panel of natural and synthetic TLR-agonists against human keratinocytes and identified the anti-cancer drug imiquimod and the imidazoquinoline 3M-011 as potent inducers of ER-stress. Other TLR7/8-agonists, including imidazoquinolines resiquimod and gardiquimod, did not induce ER-stress, demonstrating that imiquimod and 3M-011 induces ER-stress independently of TLR7/8. We further confirmed this by showing that imiquimod could still induce ER-stress in *Tlr7*$^{-/-}$ cells. Imiquimod also induced a rapid and transient influx of extracellular Ca^{2+} together with the release of Ca^{2+} from internal stores. Depletion of Ca^{2+} from the ER is a known cause of ER-stress suggesting that IMQ induces ER-stress via depletion of Ca^{2+}. The ER-stress inducing property of imiquimod is likely of importance for its efficacy in treating basal cell carcinoma, *in situ* melanoma, and squamous cell carcinoma. To identify a putative receptor for IMQ induced ER-stress and significant signalling transducers, we have introduced genome wide gene-perturbations to A375 melanoma cells using CRISPR/Cas9. The pool of mutated cells will be treated with IMQ and the surviving population of cells deep-sequenced to reveal genes that are essential for IMQ induced cell death in A375 melanoma cells. Our data could be harnessed for a rational design of even more potent ER-stress inducers and new anti-cancer drugs.

P603

Surfactant protein D deficiency aggravates cigarette smoke-induced inflammation

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Introduction: Surfactant protein D (SP-D) is a pulmonary collectin with established anti-inflammatory functions. SP-D promotes lung innate immune responses by opsonizing pathogens and apoptotic cells, enhancing phagocytic properties of alveolar macrophages, and modulating local cytokine secretion. SP-D-deficient mice (SP-D KO) exhibit pulmonary emphysema together with lipid and alveolar macrophage accumulation. SP-D has been linked to chronic obstructive pulmonary disease (COPD), while exposure to cigarette smoke (CS), main COPD risk factor, upregulates pulmonary SP-D in mice.
Aim: In the current study we hypothesized that SP-D deficiency leads to aggravation of CS-induced lung inflammation.
Methods: C57BL/6N male WT and SP-D KO mice were subjected to whole body CS exposure 5 days/week for 12 weeks (chronic experiment) or daily for 3 days (acute experiment). Recombinant fragment of human SP-D

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(rfhSP-D) was administered intranasally 1 h prior to each CS exposure in the acute regimen.
Results: CS exposure resulted in macrophage- and neutrophil-rich pulmonary inflammation. SP-D KO mice exhibited increased total cell and macrophage numbers in bronchoalveolar lavage (BAL) compared to WT littermates. In addition, BAL levels of macrophage inflammatory protein-1 alpha were significantly higher in SP-D KO mice than in WT animals after chronic CS exposure. Furthermore, CS-treated SP-D KO mice had increased BAL content of long-chain ceramides, pro-apoptotic lipid mediators, compared to WT littermates. Finally, the macrophage increase induced by 3-day CS exposure could be reversed by rfhSP-D treatment.
Conclusion: Our results indicate that SP-D protects from CS-induced pulmonary inflammation and that SP-D-based therapy might be a future potential treatment of COPD.

P604

Immune cell composition in human non-small cell lung cancer

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Lung cancer is the fourth most prevalent cancer in Norway, holding first place in mortality rate. Non-Small Cell Lung Cancer (NSCLC) is the most frequent type, representing about 85% of all lung cancer patients. Presently, the TNM staging, which is based on tumor size and localization, is used for diagnosis and prognosis in NSCLC. However, previous reports suggest that the analysis of tumor-infiltrating immune cells may represent a more accurate prognostic tool. The aim of this project was to perform comprehensive analysis of tumor-infiltrating immune cells in NSCLC using flow cytometry, as a first step to understanding the relationship between tumor-infiltrating immune cells and clinicopathological parameters. We used 10-color flow cytometry to investigate immune cells in tumors, distant lung tissue, lymph node, and peripheral blood, from 67 patients with primary NSCLC. The following populations of tumor-infiltrating immune cells were identified: $CD4^+$ T cells, $CD8^+$ T cells, each with memory and naive phenotypes; $CD19^+$ B cells, with naive, memory, germinal center and plasma cell subsets; $CD14^+$ macrophages, $CD123^+$ plasmacytoid dendritic cells, $CD11c^+$ $CD1c^+$ dendritic cells (DCs), and $CD11c^+$ $CD141^+$ DCs, $CD3^+$ $CD56^+$ natural killer T cells, $CD56^+$ natural killer (NK) cells, with $CD16^+$ and $CD16^-$ subsets, and all four granulocyte populations. Statistical analysis revealed increased percentage of $CD45^+$ leukocytes within tumors compared to distant lung tissue. Among leukocytes, B cells showed increased frequency in tumor compared to the distant lung ($P = 0.0001$). This suggests that the tumor microenvironment of NSCLC recruits immune cells and has a different immunological structure compared to normal lung tissue.

O701

Human regulatory T cells rapidly rewire the phosphoproteome of suppressed conventional T cells

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Regulatory T cells (Tregs) critically control key events of immunity in the pathogenesis of autoimmunity, allergy and cancer, primarily by suppression of effector T cells. We previously revealed that human Tregs rapidly suppress T cell receptor (TCR)-induced calcium, NFAT and NF-κB pathways in conventional T cells (Tcons) causing inhibition of effector cytokine transcription. However, the exact molecular mechanism initiating this suppression remains unknown. Due to the short time period required to induce suppression, it is probable that Tregs alter protein modifications or localizations in suppressed Tcons rather than *de novo* induction of repressive proteins. Attempts at deciphering the phosphoproteomic change upon suppression are limited to targeting limited number of phosphoproteins so far.
To analyze our hypothesis, we have performed unbiased global profiling of the phosphoproteome of primary human TCR-stimulated and suppressed Tcons. We observed that TCR stimulation rapidly alters the phosphoproteome of Tcons and Tregs revert the activation induced phosphorylation state of Tcons towards steady state. Analysis of three independent donors upon suppression revealed 68 significantly changing phosphoproteins, which have been reported to be involved in TCR signaling, cytoskeletal remodeling, protein shuttling, RNA processing and proliferation. We have demonstrated a novel role of a

phosphatase inhibitor in mediating the suppression of Tcons by Tregs.

We believe the insights into the phosphoproteome of suppressed T cells from our study may significantly contribute to improve treatment of autoimmunity and cancer, particularly considering the frequently observed resistance of target Tcons to Treg-suppression in human autoimmune disease patients.

O702

Resolving T helper cell fate decisions using single-cell RNA-sequencing

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Following activation by cognate antigen, T helper cells differentiate into functionally distinct effector cell subsets, such as interferon-gamma secreting Th1 cells, and follicular T helper cells (Tfh) specialized in promoting antibody-mediated immune responses. Differentiation of naïve T cells into these subsets is crucial for the orchestration of immune responses. Due to multiple levels of heterogeneity and overlap in differentiating T cell populations, this process has remained a challenge for systematic dissection *in vivo*. Single-cell RNA-sequencing has provided a powerful tool for identifying such transitional cellular states and elucidating their developmental relationships. Using an antigen-specific transgenic TCR mouse strain, we have dissected the $CD4^+$ T cell response to blood-stage Plasmodium chabaudi infection, during which both Th1 and Tfh populations emerge and contribute to the successful resolution of the infection. By using a novel computational approach, we have reconstructed the developmental trajectories of Th1 and Tfh cell populations at single-cell resolution in an unbiased way. On a genomic scale, these cell fates emerged from a common, highly proliferative and metabolically active precursor. Moreover, by tracking clonality from T cell receptor sequences, we infer that sibling cells derived from the same naïve $CD4^+$ T cell can concurrently populate both Th1 and Tfh subsets. We further found that precursor T cells were coached towards a Th1 but not a Tfh fate by inflammatory monocytes.

O703

Systems biology analysis of neonatal immune system development

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Newborn children are highly susceptible to infectious and inflammatory diseases, but the underlying biology is poorly understood. In addition, things like mode of delivery, acquisition of a microbial gut flora, and type of diet early in life have been shown to have long-term consequences and possibly affect the probability to acquire a number of diseases such as asthma and diabetes. However, the immunological basis for this is not known. In general, we have only a rudimentary understanding of how the immune system develops early in life and we know even less of how the different components of the immune system interact with each other and the environment to perform their function during development.

We take advantage of several recent technical developments increasing the number of measureable parameters such as mass cytometry and serum protein profiling, combined with novel sampling techniques developed in our lab.

In this way, we are able to quantify a large number of immune cell populations and serum proteins from small blood samples drawn during the first months of life in children born at various gestational ages.

Here we will present our experimental approach and our most recent results on neonatal immune system development and the imprint of some early life environmental exposures on this process.

O704

High prevalence of isotype-switched antibody responses against carbohydrate structures

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Despite the paradigm that carbohydrates are T cell-independent antigens, isotype-switched glycan-specific IgG antibodies and polysaccharide-specific T cells are

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found in humans. We employed a systems level approach combined with glycan array technology to decipher the repertoire of carbohydrate-specific antibodies. A strikingly universal architecture of this repertoire with modular organization among different donor populations revealed an association between immunogenicity or tolerance and particular structural features of glycans. Antibodies were identified with specificity not only for microbial antigens, but for a broad spectrum of host glycans that serve as attachment sites for viral and bacterial pathogens and/or exotoxins. The comparison of IgG with IgA and IgM further reveals novel insights into the isotype-specific glycan-recognition in human sera, breast milk, saliva and in the gastrointestinal tract. Our study highlights the power of systems biology approaches to analyze immune responses and reveals potential glycan antigen determinants that are relevant to vaccine design, diagnostic assays, and antibody-based therapies.

O705

Exosomes carrying biomarkers in pulmonary Sarcoidosis

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Exosomes represent a new scientific field with great potential in diagnostic, prognostic and therapeutic contexts of immunology. Sarcoidosis is an inflammatory granulomatous disorder characterized by an accumulation of polarized Th-1 type $CD4^+$ T cells and immune-effector cells within the affected organs, most frequently the lungs. We here aimed at further understanding the proinflammatory role of bronchoalveolar lavage fluid (BALF) exosomes in sarcoidosis, and to search for disease biomarkers. We performed a mass spectrometric characterization of BALF exosomes from 15 sarcoidosis patients and 5 healthy controls, and verified the most interesting results with flow cytometry, ELISA or western blot analysis in additionally 39 patients and 22 controls. More than 690 proteins were identified in the BALF exosomes, some of which displayed significant upregulation in patients, including inflammation-associated proteins such as Leukotriene A_4 Hydrolase and most complement activating factors, while the complement-regulatory CD55 was lower in patients compared to healthy controls. In addition, we detected for the first time the presence of Vitamin D binding protein (VDBP) in BALF exosomes, which was more abundant in patients. To further evaluate exosome-associated VDBP as a biomarker for sarcoidosis, we isolated exosomes from plasma of 23 patients and 11 healthy controls and found significantly higher expression in patients. Together, these data contribute to understanding the role of exosomes in sarcoidosis, and provides suggestions for highly warranted sarcoidosis biomarkers. Further, the validation of an exosome-associated biomarker in the blood of patients opens up for novel, and less invasive, support of disease diagnosis.

P701

RNAseq based method for HLA typing and allele specific expression studies

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The highly polymorphic human leukocyte antigens (HLA) are crucial in presentation of self, non-self and tumor antigens to T cells, and play a role in autoimmunity, infection responses and transplantation. Expression levels of certain HLA alleles have recently revealed to differ but this has not been studied systematically throughout all HLA genes, cell types and disease conditions.

We have developed a targeted RNA-based HLA sequencing method over exons 2 and 3 showing the highest allelic variation. Reverse transcription of whole transcriptome is first performed with oligo-dT primer with universal tail, along with template switching oligo with molecular barcode and another universal primer site for the 5′ end. After full length cDNA amplification and bead purification the targeted HLA transcripts are further enriched with the 5′ universal primer and HLA gene specific reverse primers. Sample barcodes and Illumina adapters are incorporated by PCR to both ends of the amplicon. After purification and quantification the pooled dual index paired end libraries are sequenced by Illumina MiSeq. Classical HLA alleles are called and their expression levels quantified with in-house and public analysis tools (e.g., HLAforest and Seq2HLA). We are currently applying the method to study systematically the allele specific expression level differences between HLA types in PBMCs of healthy individuals, as well as in different tissues and cell types in human autoimmune models. In future the method will be expanded also to non-classical HLA-genes, minor histocompatibility antigens and to KIR locus.

P702

Identification of global regulators of T-helper cell lineage specification

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Activation and differentiation of T-helper (Th) cells into Th1 and Th2 types is a complex process orchestrated by distinct gene activation programs engaging a number of genes. This process is crucial for a robust immune response and an imbalance might lead to disease states such as autoimmune diseases or allergy. Therefore, identification of genes involved in this process is paramount to further understand the pathogenesis of, and design interventions for, immune-mediated diseases. We aimed at identifying protein-coding genes and long non-coding RNAs (lncRNAs) involved in early differentiation of T-helper cells by transcriptome analysis of cord blood-derived naïve precursor, primary and polarized cells. Here, we identified a high confidence list of genes as well as a list of novel genes involved in early differentiation of Th1 and Th2 subsets by integrating transcriptional profiling data from multiple platforms. We show that the density of lineage-specific epigenetic marks is higher around lineage-specific genes than anywhere else in the genome. Based on next-generation sequencing data we identified lineage-specific lncRNAs involved in early Th1 and Th2 differentiation and predicted their expected functions through Gene Ontology analysis. We also found out that there is an enrichment of disease SNPs around a number of lncRNAs identified, suggesting that these lncRNAs might play a role in the etiology of autoimmune diseases. The results presented here show the involvement of several new actors in the early differentiation of T-helper cells and will be a valuable resource for better understanding of autoimmune processes.

P703

Comparison of NGS HLA-typing algorithm accuracies and improvement via ensemble prediction

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The human leukocyte antigen (HLA) genes code for proteins having a central role in the function of immune system by presenting peptide antigens to T cells. HLA genes show extremely high genetic polymorphism and exact determination of HLA alleles is based on DNA sequencing. Several HLA alleles have been associated with susceptibility to autoimmune diseases, and HLA-matching of donors and patients is vital both in hematopoietic stem cell and organ transplantation.

Traditional HLA-typing methods have relied on more low-throughput methods with specific primers, but next generation sequencing (NGS) technologies should enable more accurate and extensive HLA-typing, also from whole-genome and whole-exome sequencing datasets. However, as the HLA/MHC region contains duplicated genes with very similar sequences, shows variation in gene numbers and has very high numbers of alleles, accurate determination of HLA alleles is demanding.

We present here a comparison of the typing accuracies of several published NGS-based HLA-typing algorithms. Accuracies are assessed using different datasets, consisting of NGS data produced by various targeting panels (exome, genome, MHC), including the full 4 Mbp MHC sequencing from 90 Finnish stem cell transplantation patient/donor pairs and 1000 Genomes data. None of the methods works consistently well for all the genes and samples, but we find that an ensemble prediction of the results can be used to gain improvements in accuracies.

P704

Qualitative and quantitative profiling of the serum complement system using targeted mass spectrometry

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Introduction: Abundance differences of complement proteins are frequently detected in comparisons of sera from diseased and healthy subjects, suggesting that a

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detailed overview of the status of these proteins would be useful and might provide clinically important insight. In recent years, the use of selected reaction monitoring (SRM) has gained popularity as an efficient method to validate observations from mass spectrometry based proteomics experiments. The method provides improvements in both sensitivity and selectivity, and can be used to target in the order of a hundred proteins in a single analytical run.

Methods: Digested serum proteins were analysed using tandem mass spectrometry (LC-MS/MS). An Oribitrap Velos Pro and a TSQ triple quadrupole mass spectrometer were used for profiling and targeted analysis, respectively. After analysis of the profiling data, the frequently detected peptides were evaluated for their suitability for SRM analysis and transition lists were developed using Skyline software together with the analysis of isotopically labelled synthetic analogues.

Results: LC-MS/MS analysis of depleted serum consistently characterized 36 proteins associated with the Alternative and Classical Complement pathway. These were all detected with two or more peptides (excluding missed cleavages and methionine containing sequences) and represent a manageable panel for SRM targets that can be selectively determined in a single LC-MS/MS separation. In related work we have developed an SRM assay for 12 of these targets such that they may be simply determined in un-depleted serum. Further method development of the full panel is ongoing.

P705

Comparative analysis of human and mouse transcriptomes of Th17 cell priming

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Th17 cells have been associated with cancer, autoimmune and inflammatory diseases. In this study we compared transcriptional regulation of the early stages of Th17 cell differentiation in human and mouse. Importantly, the comparison was done with RNA sequencing enabling quantification of transcripts without species-specific probe biases. We identified cross-species conserved gene expression containing several transcripts not previously linked to Th17 cell differentiation. In addition, we identified the long non-coding RNAs differentially regulated during human Th17 cell priming.

The kinetic RNA expression data was used to predict the key transcriptional regulators and co-ordinately controlled genes. Furthermore, the genes identified to belong to the Th17 cell-specific transcriptome were overlaid with the single nucleotide polymorphisms (SNP) associated with human diseases. When the most strongly regulated genes in mouse were used as an input in the analysis the overlap between the regulated genes and the disease-associated SNPs was lower than among the top genes regulated both in human and mouse. Therefore, our study suggests that identification of similarly regulated genes between human and mouse pinpoints signalling pathways predisposing to these diseases which can be studied with mouse models.

In summary, our study provides new candidate regulators of Th17 cell differentiation. Furthermore, identification of conserved transcriptional signature of Th17 cell priming enables generation of hypotheses valid for human and testable in mouse models, important for translational research.

O801

Microbe–host interplay in atopic dermatitis and psoriasis

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Human skin represents a primary interface between the body and the environment, functioning as a physical barrier against the invasion of microbial pathogens, while concurrently providing a home to numerous commensals. Despite recent advances in understanding the role of microorganisms in tissue homeostasis and local immunity, the potential role of microbial dysbiosis in inflammatory disease is poorly understood. Here we characterize the skin microbial communities coupled to global patterns of cutaneous gene expression. Atopic dermatitis (AD, n = 88) and psoriasis (PSO, n = 129) were classified by distinct microbes, which differed from healthy volunteer (HV, n = 117) microbiome in composition and relative abundance. AD was overgrown with a single microbe

(*S. aureus*), associated with a specific disease relevant transcriptomic signature. In contrast, PSO was characterized by multiple communities of microbes associated with psoriasis core gene transcripts. Our work highlights the importance of a distinct composition of microbial communities in skin inflammation and provides a basis for the discovery of biomarkers and targeted therapies.

O802

Enterovirus-associated changes in plasma proteome of children with genetic susceptibility to type 1 diabetes

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Viral infections and especially human enteroviruses (EV) have been associated with type 1 diabetes (T1D) in several studies. However, despite increasing amounts of evidence indicating associations between EV infections and T1D, we still lack proper understanding of EV-induced responses and their potential contribution to the development of T1D. Our aim was to study the associations between EV infections and T1D by analyzing EV^+ and EV^- plasma samples of children at risk for developing T1D with label-free quantitative proteomics.

The plasma samples in this project have been collected as part of the Finnish Type 1 Diabetes Prediction and Prevention (DIPP) study. All DIPP children have an increased genetic risk for developing T1D, and they have been followed-up regularly until the age of 15 years or until the development of T1D. For this study, we have analyzed 54 samples from 28 children (13 cases who have later developed T1D or are positive for T1D autoantibodies and 15 autoantibody negative controls). The plasma samples were depleted of the most abundant serum proteins, digested with trypsin and analyzed with mass spectrometer.

The proteomics analyses resulted in the identification and relative quantitation of approximately 300 proteins. Based on our analyses EV infection is associated with changes in the expression levels of multiple plasma proteins. Interestingly, EV-associated expression level changes of a few plasma proteins seemed to differ between the cases and the controls. This indicates that the children who remain healthy and children who will later develop T1D might respond differently to EV infection.

O803

Protective immunity to tuberculosis

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Tuberculosis is still one of the top killers worldwide. Disease control suffers from lack of effective vaccination, antibiotics and reliable diagnostic methods. Currently, it is unknown what kind of immune response is needed for the bacterial elimination at any state of the mycobacterial infection even though heavily exposed humans with clearing response have been reported. We have previously noticed that individuals of adult zebrafish population with low mycobacterial load or bacterial clearance have more type-2 T helper cell (Th2)-orientated immune response whereas individuals sensitive to mycobacterial infection have low Th2/Th1 ratio. In this project, the protective immune response is studied in the Mycobacterium marinum-zebrafish-infection model.

We have screened different kind of immunoactivators including known TLR ligands and vaccine adjuvants in induction of protective immune response. All activators were given to adult zebrafish 1 day prior the infection. We have found one immunoactivator that can induce beneficial immune response and leads to restricted growth or elimination of the mycobacteria in 70% of the adult zebrafish. Now, our aim is to look the details of protective immunity against mycobacteria by measuring changes in gene expressions and by imaging early events of the infection with fluorescent mycobacteria. These results can give insights into protective immune response against tuberculosis and help to understand tuberculosis pathogenesis.

O804

Reactivation of a latent mycobacterial infection in adult zebrafish

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An estimated one third of human population have a latent *Mycobacterium tuberculosis* infection, and therefore

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have a 5–10% lifetime risk of reactivation to active tuberculosis. Often reactivation is associated with immunosuppression, but the mechanisms of reactivation are poorly understood, hence reactivation cannot be prevented. Lack of adequate animal models has hampered tuberculosis research. The zebrafish (*Danio rerio*) has emerged to be a promising model organism to study tuberculosis. Like humans, adult zebrafish has both innate and adaptive immunity. In addition, a natural fish pathogen and a close relative of *M. tuberculosis*, *Mycobacterium marinum,* causes a disease that closely resembles human tuberculosis, having both a natural latency with granuloma structures, and a reactivation phase. Our aim was to develop a reliable method to reactivate latent mycobacterial infection in adult zebrafish. Fish were infected with a low dose of mycobacteria, and the following latent infections were reactivated by suppressing their immune system by feeding with cortisone containing food. Bacterial counts of each fish was determined by qPCR in different time points. Granuloma structures and dissemination of the bacteria were also visualized histologically by Ziehl-Neelsen staining. Reactivation of latent infection was seen as gradually increasing bacterial burdens: after 6 weeks, the bacterial counts per fish had increased 50–450-fold. Histological staining confirmed the increased bacterial counts, and in addition, the amount of granulomas seems to increase as the reactivation progresses. This method provides a new means to study reactivation of mycobacterial infection in an animal model.

O805

Analyzing internal antigens to be included in a universal adenovector-based influenza A vaccine

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Current influenza vaccines are directed towards the surface proteins hemagglutinin and neuroaminidase providing an antibody-mediated immunity. High variability of these proteins results in costly annual evaluation and production of new vaccines without providing protection against heterosubtypic strains or upcoming pandemic variants. Consequently, there is a need for a new universal influenza vaccine strategy that can provide a basal protection when the conventional vaccines fail. In contrast to the surface proteins, the internal proteins of influenza A are highly conserved. Using one of these, nucleoprotein, encoded in an adenovirus construct (AdNP) we have induced a CD8 T-cell response, which provide heterosubtypic protection. Further, by vaccinating both systemically and locally mice were completely protected against challenge from influenza for at least 8 months post vaccination.

To increase the breadth of the vaccine we have investigated the potential of other conserved influenza proteins as vaccine targets with the intention of making a 'vaccine cocktail' by combining the constructs in one vaccination. PB1 expressed from an adenovirus (AdPB1) elicits about the same number of antigen-specific CD8 T cells in mice as AdNP. However, the PB1 directed T cell response protects only 25% of vaccinated mice. We found that one of the underlying reasons for this lack of protection from AdPB1 vaccination relates to a significantly lower *in vivo* cytotoxicity of these cells compared T cells induced by AdNP. It is important to consider these results and to further evaluate why some epitopes induce a more protective response than others in future vaccine design.

P801

Adequate Th2-type response associates with restricted bacterial growth in latent mycobacterial infection of zebrafish

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Tuberculosis is still a major health problem worldwide. Currently it is not known what kind of immune responses lead to successful control and clearance of *Mycobacterium tuberculosis*. This gap in knowledge is reflected by the inability to develop sufficient diagnostic and therapeutic tools to fight tuberculosis. We have used the *Mycobacterium marinum* infection model in the adult zebrafish and taken advantage of heterogeneity of zebrafish population to dissect the characteristics of adaptive immune responses, some of which are associated with well-controlled latency or bacterial clearance while others with progressive infection. Differences in T cell responses between subpopulations were measured at the transcriptional level. It was discovered that a high total T cell level was usually associated with lower bacterial loads alongside with a T helper 2 (Th2)-type gene expression signature. At late time points, spontaneous reactivation with apparent symptoms was characterized by a low Th2/Th1 marker ratio and a substantial induction of *foxp3* reflecting the level of regulatory T cells. Characteristic *gata3*/*tbx21* has potential as a biomarker for the status of mycobacterial disease.

P802

Genes affecting mycobacterial infection in zebrafish

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In 2014, 1.5 million people died of tuberculosis and 9.6 million developed the disease. *Mycobacterium marinum,* a natural zebrafish pathogen, causes a systemic infection in zebrafish. This infection is partly similar to tuberculosis. Thus *M. marinum* infection in zebrafish is a potential model for tuberculosis. The aim of this study is to identify genes underlying defence mechanisms against *M. marinum* infection in zebrafish and to study their role during this infection. To achieve our aim we carry out a gene-breaking transposon (GBT)-based forward genetic screen. For the genetic screen, we have injected GBT RP2 construct with synthetic mRNA of Tol2 transposase into fertilized wild type zebrafish eggs to induce random mutations. We have raised and crossed further the successfully injected embryos. In F3 generation we have infected 128 zebrafish mutant lines and controls to screen for resistance against *M. marinum*. We have identified five lines which have altered resistance against *M. marinum* infection compared to the wild type. Currently, we locate the mutation sites using targeted sequencing and inverse PCR. We expect this study to provide novel information about the host mechanisms involved in resistance against mycobacterial infection.

P803

Genes affecting host response against mycobacteria in zebrafish (Danio rerio)

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Human tuberculosis (TB) is an epidemic disease caused by *Mycobacterium tuberculosis* (Mtb). Susceptibility to TB depends on the Mtb strain and on a number of host factors; environmental conditions, personal health status as well as genetic variation. Today, increasing amount of data are available on genetic polymorphisms related to TB immunity. To study the complex effects of host genetics in a mycobacterial infection, a suitable animal model is required. In this study, we used *Mycobacterium marinum* infection in zebrafish to model TB. Agilent microarray analysis was performed for wild-type fish after low-dose (~50 CFU) *M. marinum* infection at 3 and 14 days post infection (dpi). 35 and 187 genes were induced at least 5-fold at 3 and 14 dpi, respectively. Counts of down-regulated (<0.3-fold expression) genes in infections were 221 at 3 dpi and 130 at 14 dpi. Genes involved in several different processes, such as muscle contraction and immune response, showed induction. From these genes, we found 11, which also associate with the risk of human TB, and chose seven to investigate their relevance for mycobacterial resistance. Knock-out (KO) zebrafish for these genes are being produced with a CRISPR-Cas9-based mutagenesis. Subsequently, the outcome of *M. marinum* infection will be determined by measuring bacterial burden and investigating granuloma formation in both KO embryos and adults. In addition, host response is monitored by analysing cytokine expression profiles and blood cell composition. We believe that our results will bring important insights into pathogenesis of TB.

P804

Cytokine profiling of the cerebrospinal fluid of Lyme neuroborreliosis patients

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Objectives: Lyme borreliosis is a tick-borne infectious disease caused by Borrelia burgdorferi *sensu* lato spirochetes which are able to disseminate from the initial tick bite site into distant organs like the nervous system. Previously, it has been established that chemokine CXCL13 levels in cerebrospinal fluid (CSF) of patients suffering from Lyme neuroborreliosis (LNB) are extremely high and it can be used as a marker for the disease. Our objective in this study was to further characterize cytokine/chemokine profiles of the CSF of LNB patients to discover new cytokines/chemokines, in addition to CXCL13, which could potentially be used as markers for the disease.

Methods: CSF samples obtained from retrospectively identified patients suffering from LNB were analyzed with BioRad multiplex kits (Human Cytokine 21-plex, Human Cytokine 27-plex) with Luminex equipment. As a com-

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parison, CSF samples collected from non-LNB patients were also assayed. In addition, the cytokine profiles of LNB patients' CSF samples obtained before and after antibiotic treatment were compared.

Results: The levels of many cytokines analyzed were elevated in LNB patients when compared to the reference group. Cytokines/chemokines IL-1ra, IL-10, IP-10, RANTES, TNF-α, MIP-1α, MIG, and IFN-γ appeared to be the most relevant and their levels decreased significantly after the antibiotic treatment.

Conclusion: In this study, we found several cytokines from the CSF samples of the LNB patients that were elevated when compared to non-LNB patients' CSF. In the future, the applicability of these cytokines as markers for LNB, and as indicators of antibiotic treatment response in LNB, can be evaluated.

P805

A forward genetic screen for zebrafish genes involved in pneumococcal infection

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Streptococcus pneumoniae (pneumococcus) is a major human pathogen and one of the leading causes of pneumonia, septicemia, and meningitis. The complex interactions occurring between the pneumococcus and the immune system are only partly understood and, therefore, the optimal treatment and prevention methods are lacking. Previously, we showed that zebrafish (*Danio rerio*) are valuable hosts in the study of the innate immune response against pneumococcus. In the present study, we have employed the zebrafish embryo model for the forward genetic screen to identify novel host genes involved in pneumococcal infection. Using the gene-breaking transposon based mutagenesis method, we generated 150 zebrafish families with unknown mutations and screened these families for the altered susceptibility for systemic pneumococcal infection. With this screen, we were able to reveal 21 mutant families that are hypersusceptible to pneumococcal infection and, recently, we identified the insertion sites in these families by Next Generation Sequencing (NGS). NGS revealed several genes and intergenic regions with a potential, previously unknown, role in pneumococcal infection. Now, the role of these genomic sites is assessed in zebrafish embryos using the CRISPR/Cas9 site-directed mutagenesis and the role of these sites in the defense against pneumococcus is characterized further. Eventually, the screen is likely to expand our understanding of the innate immune response associated with pneumococcal diseases.

P806

Leishmania major infected bone marrow dendritic cells direct Th2/Treg response through arginase 1 and indoleamine 2, 3 dioxygenase activities

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Introduction: *Leishmania major* is an obligate intracellular parasite hosted by phagocytes, including dendritic cells (DCs). The aim of this study was to investigate the immune-modulatory effect of *Leishmania major* in BALB/c mouse-derived DCs infected by *L. major* promastigotes, *in vitro*.

Methods: The metacyclic promastigotes were collected at stationary phase and used for DCs infection. BALB/c derived-DCs were differentiated in complete medium with 20 ng/ml GM–CSF for 8 days. Thereafter, immature DCs were infected for 6 h or 24 h with *L. major* promastigotes (parasites: DC ratio, 10:1). The immune-modulatory effect of *Leishmania major* on DCs immune response was determined by measuring the supernatant concentration of cytokines and infection index. The capacity of infected DCs to induce T cells polarization (Th2 and Treg response) was assessed by arginase 1 and indoleamine 2,3 dioxygenase (IDO) activity.

Results: Our results showed sustained DCs infection giving an infection index of 70.30 ± 6.04 at 24 h post-infection. *Leishmania* infected- DCs enhanced IL-10 secretion (45%), 24 h post infection. In addition, *Leishmania* potentiated both kynurenine production by enhanced IDO activity 50% and arginase 1 activity 47%, 24 h post-infection.

Conclusion: Taken together, our results highlight some biochemical events bypassing the protective Th1 response and sustained arginase 1 and IDO activities which direct the differentiation of T cells into a Th2/Treg pathway.

P807

CRISPR-Cas9 mutated zebrafish to study chemokine mediated immunity against tuberculosis

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Tuberculosis is a major global challenge, with 9.6 million new cases and 1.5 million deaths in 2014. The currently available BCG-vaccine only offers limited protection against the *Mycobacterium tuberculosis* infection, and improved vaccines are needed to combat the spread of the disease. In order to develop better vaccines, it is essential to understand the mechanisms of host protective immunity. Since chemokines are important mediators of immunity, they could serve as Th cell response enhancing adjuvants and improve efficiency of the vaccination against mycobacterial infection. Zebrafish (*Danio* rerio) is an important model organism in immunological research. To identify chemokines which could be used as vaccination adjuvants, we will knock-out chosen chemokine receptor genes using CRISPR-Cas9 mutagenesis. We will test the role of these receptors in the progression of mycobacterial infection in zebrafish. The mosaic mutants generated with CRISRP-Cas9 will be bred into homozygous knockout fish lines. The knockout fish will then be infected with *Mycobacterium marinum*, which is closely related to *M. tuberculosis*. To investigate the roles of the target genes before adaptive immunity is activated, we will also perform the infection experiments with knockout fish larvae. The survival of the mutant fish will be monitored after infection and compared to that of wild type fish to establish the importance of chemokines in protective immunity against tuberculosis. We are hoping to identify adjuvants that enhance the Th cell response to improve the efficiency of vaccination against tuberculosis.

P808

Increase IL-10 and TGF-beta levels in relapsing patients with hydatidosis: their involvement in the persistent infection control

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Introduction: Human hydatidosis is a widely endemic helminthic disease, caused by the larval stage of metacestode *Echinococcus granulosus*. It constitutes a serious public health problem in various parts of the world, particularly in Algeria. We have previously shown the role of cytokines Th1, Th17 and Th2 in human Hydatidosis. This study aims to investigate the role of IL-10 and TGF-β in the immune response against *Echinococcus granulosus* infection.
Materials and Methods: We evaluated GF-β, IL-10, IFN-γ, IL-17A and NO production in sera from Algerian hydatic patients and in supernatants culture of peripheral blood mononuclear cells (PBMCs) from the same patients stimulated by a major parasitic antigen. In the same way, the expression of inducible NOS (NOS2) was measured in PBMCs of patients. We also studied NO modulation by TGF-β and IL-10 in PBMC and monocytes cultures from patients in presence of *Echinococcus granulosus* protoscoleces (larval form of parasite).
Results: Analysis of cytokines and NO production revealed that the levels of TGF-β and IL-10 were elevated in all sera and PBMC culture supernatants from patients who relapsed after surgical removal of cysts and did not display any immune response against parasitic antigen. However, IFN-γ, IL-17A and NO activity was very low in the same patients. We observed with interest that NOS2 expression was down regulated in PBMCs. We noted with interest that co-cultures treatment with TGF-β caused a decrease NO production and an increased in the percentage of viable protoscoleces.
Conclusion: Collectively, our results indicate that chronic infection with *Echinococcus granulosus* appears to be established via multiple suppressive factors.

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