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Allergy/asthma

T.64. Immune Memory is Established at the Incipient, Preclinical Stages of Food Allergy

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Most patients with food allergy experience clinical symptoms at their first known exposure. A pre-requisite for the expression of such symptoms is the development of allergen-specific IgE that facilitates mast cell and basophil degranulation upon subsequent allergen exposure. Here, we searched for immunological events that might precede the generation of allergen-specific IgE. As these potential events are silent, we used a murine system. Mice were orally exposed only once to a food allergen (peanut or ovalbumin) with cholera toxin (CT), a classical Th2 adjuvant. Under these conditions, mice lacked allergen-specific germinal centres in the lamina propria (LP) of the small intestine and mesenteric lymph nodes (mLNs). They also had undetectable levels of allergen-specific IgE in serum and allergen-specific plasma cells in the bone marrow. Accordingly, these mice did not experience anaphylaxis on systemic allergen challenge. However, we detected allergen-specific $IgG1^+$ B cells in LP, spleen and mLNs by flow cytometry. In addition, stimulation of splenocytes with allergen *in vitro* induced $CD4^+$ T-cell proliferation and Th2-associated cytokine secretion. When these mice were orally re-exposed to allergen without adjuvant, they developed allergen-specific IgE and underwent anaphylaxis on systemic challenge, even when allergen re-exposures were done 9 months after the initial, single exposure to allergen + CT. These findings indicate that long-lived allergen-specific memory is established after a single, clinically and humorally silent, immunogenic exposure to allergen, which is activated by inherently innocuous allergen exposures. These findings encourage further research to identify tools for the early prediction of food allergy.

T.65. GSDMB, a Gene Located in the 17q21 Asthma-susceptibility Locus Induces an Asthma Phenotype Mediated by Airway Remodeling Without Lung Inflammation

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BACKGROUND: Chromosome 17q21 contains a cluster of genes that may either individually, or in combination, be responsible for its strong genetic linkage to asthma. In this study, we focused on GSDMB as its biological function in asthma is unknown.

METHODS: GSDMB expression was evaluated in human lung sections from asthmatic patients and normal individuals. As the SNP linking chromosome 17q21 to asthma is associated with increased GSDMB expression, we generated transgenic mice expressing increased levels of the human GSDMB transgene ($hGSDMB^{Zp3\text{-}Cre}$). The levels of Th2 cytokines, remodeling genes and chemokines were assessed by qPCR, immunohistochemistry and ELISA.

RESULTS: We identified that GSDMB is highly expressed in lung bronchial epithelium in human asthma. Overexpression of GSDMB in primary human bronchial epithelium increased the expression of genes important to both airway remodeling (TGF-β1, 5-LO) and airway-hyperresponsiveness (AHR) (5-LO). Interestingly, unchallenged $hGSDMB^{Zp3\text{-}Cre}$ mice showed a significant spontaneous increase in airway remodeling, with increased smooth muscle mass and increased fibrosis as early as 8 weeks of age, which persisted until 24 weeks of age. In addition, $hGSDMB^{Zp3\text{-}Cre}$ mice also showed a significant

spontaneous increase in AHR in the absence of airway inflammation, with increases in the same remodeling and AHR mediators (TGF-β1, 5-LO) observed *in vitro* in GSDMB-overexpressing epithelial cells.

CONCLUSION: Our studies demonstrate that GSDMB, a gene highly linked to asthma but whose function in asthma is previously unknown, regulates AHR and airway remodeling without airway inflammation through a previously unrecognized pathway in which GSDMB induces 5-LO to induce TGF-β1 in bronchial epithelium.

T.66. Gabapentin Therapy for Biphasic Anaphylaxis

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Idiopathic Anaphylaxis is a constellation of symptoms that include one or more of the following: angioedema, urticaria, gastrointestinal abnormalities, and decreased blood pressure without any recognized external trigger. Gabapentin, an anticonvulsant drug, has been used in different conditions associated with chronic neuropathic pain and pruritic disorders. Because neuropathic pain and pruritus share similar pathogenic mechanisms, neuropathic analgesics like gabapentin have been shown to be effective therapeutic options.

We present a 29-year-old female patient with biphasic idiopathic anaphylactic reactions, with accompanying angioedema, urticaria, severe abdominal pain, and diarrhea. The angioedema attacks occurred in laryngeal, facial, and upper extremity regions. Her lab results show normal complement, tryptase levels, and stool culture, and bone marrow biopsy was negative for systemic mastocytosis. Frequency of attacks ranged from 1 to over 20 times a day. Frequency and unpredictability of attacks prompted her to switch to a liquid elemental diet. She was unresponsive to cromoglicic acid, extended-release morphine, hydromorphone, and fentanyl, and only responded to IM/IV epinephrine during the attacks.

We placed the patient on gabapentin 300 mg 4 times daily and within 2 days, she reported 90% resolution of gastrointestinal pain, and returned to a normal diet without anaphylactic episodes.

Gabapentin therapy appears to have been beneficial in the treatment of idiopathic anaphylaxis, including abdominal pain and angioedema. Additional studies investigating the mechanism by which gabapentin mediates pain, autonomic and mast cell responses are needed.

Autoimmune neurologic diseases

F.1. Suppression of Regulatory T Cells by Exosomes In Multiple Sclerosis

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Exosomes are extracellular vesicles which are involved in intercellular communications by delivering a variety of molecules such as miRNAs. MiRNAs are involved in differentiation and function of helper T cells, which play a pivotal pathogenic role in multiple sclerosis (MS). In this study, exosomes were collected from the plasma of patients with MS and healthy controls (MS-exo and HC-exo). We performed comprehensive analysis of exosomal miRNAs in MS, for the first time to our knowledge. The miRNA profiles clearly differentiated MS-exo from HC-exo. RT-qPCR validated four miRNAs that were increased in MS-exo. After co-culture with T cells, MS-exo decreased the frequency of $IFNg^{-}IL17A^{-}Foxp3^{+}$ Treg cells compared with HC-exo. Among the upregulated miRNAs, the amount of *let-7i* in the added exosomes was negatively correlated with the frequency of Treg cells in this experiment. Transfection of *let-7i* reduced the frequency of Treg cells,

and further experiments suggested that this was via suppression of insulin-like growth factor 1 receptor (IGF1R) and transforming growth factor-beta receptor 1 (TGFBR1). Knockdown of these receptors were shown to inhibit differentiation of Treg cells. Furthermore, there was reduced expression of these receptors on naïve $CD4^+$ T cells in the peripheral blood of MS patients. The frequency of Treg cells in the peripheral blood positively correlated with TGFBR1 expression on naïve $CD4^+$ T cells. In summary, this study suggests that exosomes play a pathological role in MS by suppressing Treg cells via *let-7i-IGF1R*/*TGFBR1* axis.

F.2. Single-cell Phenotypic Analysis of Clonally Expanded CD8+ T Cells Reveals a Specific Clustering in Patients with Multiple Sclerosis

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Clonally expanded memory $CD8^+$T cells are involved in multiple sclerosis (MS). We have shown that up to 87% of expanded $CD8^+$T cells in brain-lesions of MS patients can be found in the cerebrospinal fluid (CSF) and 37% in the blood of the patients. We now aim to study $CD8^+$T cells in the blood of MS patients at single-cell level to identify a molecular signature of pathogenic $CD8^+$T cells and more precisely of the clonally expanded $CD8^+$T cells.

To analyze single-cells from MS patients and controls, we used the C1 Single-Cell Auto-Prep System (Fluidigm) with subsequent microfluidic qPCR of 96 well-chosen genes and TCR Vβ-chain genes followed by sequencing. In parallel, we performed β-chainTCR immunosequencing on pooled memory $CD8^+$T cells to analyze the repertoire and identify expanded clones.

We detected clear clustering and increased expression of more than 20 genes in MS patients compared to healthy volunteers (HV). Most of those genes are associated with $CD8^+$T cell activation or MS. We also identified clonally expanded $CD8^+$T for which we can compare the phenotype to the non-expanded $CD8^+$T cells.

Our data are the first to describe a phenotypic analysis of $CD8^+$T cells from MS patients at single-cell level and to link this phenotype to the TCR clonality. We detected in MS a gene expression profile which is clearly different to that of HVs. The gene expression differences and the clustering can serve as the basis to develop a biomarker to detect MS specific pathogenic cells in the peripheral compartments.

F.3. T-cell Antigens in Pediatric-Onset Multiple Sclerosis: A Unique Window Into Early Disease Mechanisms

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Multiple sclerosis (MS) involves immune attacks on the CNS, leading to demyelination, axonal injury and increasing neurological dysfunction. Though both CD4+ and CD8+ T cells are implicated, the particular subsets and their antigenic targets remain largely unknown. In adult-onset MS, distinguishing immune responses that are consequences of, rather than cause of, injury, is difficult. In contrast, pediatric-onset MS offers a unique early window into disease mechanisms given the narrower gap from biological onset. We aim to identify and characterize disease-relevant antigen-specific effector T cell responses to traditional and novel antigenic targets involved early in the MS disease process.

Our group has implicated putative target antigens and T cell subsets in pediatric-onset MS, by following patients from time of an initial presentation with acquired demyelinating syndrome (ADS) and comparing those confirmed to have MS with those who remain monophasic. A CSF proteomic study implicated novel axo-glial apparatus molecules as early injury targets, rather than traditional compact myelin antigens. A series of multiparameter flow-cytometry panels applied to pediatric peripheral blood mononuclear cells (PBMC) revealed that MS children harbor abnormally increased frequencies and pro-inflammatory cytokine responses of particular effector T cell subsets compared to controls.

Our strategy involves developing assays, initially in fresh PBMC samples from adult MS and controls, then miniaturizing the approach and validating it for use in the small numbers of available cryopreserved pediatric PBMC samples to quantify antigen-specific responses including proliferation and cytokine profiles of distinct disease-implicated T cell subsets to both traditionally and newly implicated antigens.

F.4. The Hygiene Hypothesis in MS: Inducing TLR2 Tolerance Treats the Myelin Repair Component as well as the Inflammatory Component of MS

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Multiple sclerosis (MS) is a CNS autoimmune disease characterized by both an inflammatory/demyelinating component and a defective myelin repair (re-myelinating) component. Re-myelination defects, which may underlie progressive forms of MS, remain poorly understood. Recent studies implicate toll-like receptor 2 (TLR2) signaling as contributing to both the inflammatory component and defective re-myelination of MS. We previously reported evidence that the "Hygiene Hypothesis," as represented by a systemic deficiency in microbiome-derived "tolerizing" TLR2 ligands, may be involved in MS. In proof-of-concept studies, we reported that inducing TLR2 tolerance with low doses of microbiome-derived TLR2 ligands significantly attenuates adoptively transferred EAE. Here we ask if TLR2 tolerance also enhances the re-myelinating component of MS.

Utilizing the cuprizone-induced murine model of demyelination, we report that inducing TLR2 tolerance significantly improves myelin thickness during both the demyelination and re-myelination phases of myelin damage. Systemic TLR2 tolerance was induced by administering low dose TLR2 ligands for either 4 weeks during cuprizone administration (demyelinating phase) or 16 days after halting cuprizone administration (re-myelinating phase). Evaluation of myelin integrity via Black-Gold staining and electron microscopy (g-ratios) revealed significantly enhanced myelin recovery in mice tolerized either during ($p=0.0012$) or after cuprizone treatment ($p=0.0006$).

These results indicate that TLR2 tolerance enhances myelin repair via mechanisms independent of modulating systemic immune function. Given that defective re-myelination may represent an important component of the pathophysiology in progressive MS, our results suggest that inducing TLR2 tolerance may represent a two-pronged approach for treating both the inflammatory and myelin repair components of MS.

F.5. BAFF Expands Circulating Transitional B cells in Multiple Sclerosis Patients Treated with Fingolimod

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Background: Fingolimod is a sphingosine-1-phosphate receptor agonist used as a disease-modifying drug for multiple sclerosis (MS). We have previously shown that MS patients treated with fingolimod have an increased proportion of circulating transitional B cells, but the underlying mechanism remains unknown. B cell-activating factor of the TNF family (BAFF) is a cytokine involved in the differentiation and proliferation of B cells. Mice overexpressing BAFF have been shown to have increased numbers of transitional B cells; therefore, we hypothesized that BAFF is involved in an increase

in transitional B cells in MS patients treated with fingolimod. Subjects and methods: Serum samples were collected from 25 healthy subjects, 32 untreated MS patients, and 30 MS patients treated with fingolimod. Concentrations of BAFF in serum were quantified by ELISA. In addition, alterations in serum BAFF levels after starting fingolimod were analyzed in three treatment-naïve MS patients. The proportions of B cell subsets in 14 MS patients treated with fingolimod were determined by flow cytometry, and the relationships between serum BAFF concentrations and each B cell subset were analyzed. Results: Serum concentrations of BAFF were significantly higher in fingolimod-treated MS patients than in healthy subjects or untreated MS patients ($p < 0.001$). Serum levels of BAFF rose significantly after starting fingolimod treatment in the longitudinal study ($p=0.014$). A significant positive correlation was found between the serum concentration of BAFF and the percentage of transitional B cells ($p=0.0244$). Conclusion: BAFF is involved in the increase in circulating transitional B cells in MS patients treated with fingolimod.

F.6. Development of Eomes+ Helper T cells in Association with CNS-derived Antigen-presenting Cells

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We have identified a previously unappreciated pathogenic CD4+ T cell subset expressing Eomes (Eomes+ Th cells) in a late/chronic course of experimental autoimmune encephalomyelitis (EAE) that presumably represent a secondary progressive multiple sclerosis (SPMS), a refractory subtype of MS. In the current study, we evaluated the role of antigen-presenting cells (APCs) accumulated in central nervous system (CNS) for induction or functional modification of Eomes+ Th cells in late/chronic EAE. First, we demonstrate that CNS APCs isolated from late EAE animals, including B cells and non-B/MHC class II+ cells, clearly promote Eomes expression in naÃ¯ve splenic CD4+ T cells associated with enhanced cytotoxicity. Gene chip analysis reveals that numerous genes are differentially expressed in CNS APCs between early and late EAE. Among those differentially expressed genes, prolactin is upregulated only at late disease. Interestingly, exogenous prolactin induces Eomes expression in naÃ¯ve splenic CD4+ T cells *in vitro*. Moreover, *in vivo* administration of an inhibitor for prolactin, Bromocriptin (BM), could effectively deley the onset and ameliorate severity of late EAE. In line with the clinical outcome, Eomes+ CD4+ T cells in CNS of late/chronic EAE are decreased in BM-treated mice and CNS APCs from the BM-treated mice show a defect in inducing Eomes expression in CD4+ T cells. Therefore, CNS APCs accumulated in CNS during late EAE facilitate the progression of chronic disease by means of promoting Eomes+ Th cells through the expression of prolactin. Collectively, treatments targeting prolactin may have a benefit for treating late/chronic disease of EAE and SPMS.

F.7. Gene Expression Profiles as Biomarkers for Secondary Progressive Multiple Sclerosis

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Multiple sclerosis (MS) is an autoimmune disease that targets the central nervous system. We and others have investigated for various biomarkers to predict the disease course in order to personalize treatment. There are some promising biomarkers for predicting relapses in relapsing remitting MS (RRMS) the most common type of MS, but very few for secondary progressive MS (SPMS). In contrast to SPMS, benign MS (BMS) is diagnosed primarily retrospectively and largely based on motor progression using the Expanded Disability Status Scale (EDSS). This study aims to identify gene expression changes between SPMS and BMS, as biomarkers for disease progression using blood samples from 21 SPMS and 13 BMS patients for RNA isolation. Microarray analysis was performed using Affymetrix GeneChip. Differential gene expression analysis was performed using limma package, and Gene Ontology was used for gene enrichment analysis comparing SPMS with BMS. Our results indicate that SPMS has a different gene expression profile than BMS. Using iPathwayGuide for pathway analysis, there were 12 of 115 differentially expressed genes identified in humoral immune response. Most interestingly, in the analysis of molecular function with iron ion binding, 7 out of all 79 genes were differentially expressed in SPMS versus BMS. Iron is essential for myelin formation and oxidative phosphorylation. Iron

imbalance is associated with pro-inflammatory cytokines and oxidative stress. Therefore, our data suggest the role of humoral immunity and iron dysregulation in the pathogenesis of MS disease progression.

F.8. B cell Subsets as Potential Biomarkers for Fingolimod Treatment in Multiple Sclerosis (MS)

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Several S1P-receptor functional antagonists are being pursued for the treatment of MS, with fingolimod approved as first-in-class in 2010. While many patients become free of new brain lesions and clinical relapses (Stable), a proportion of patients continue to develop new disease activity (Active) in spite of ongoing treatment. In this study, we aimed to identify a potential predictive biomarker to distinguish such Active versus Stable patients in a prospectively followed cohort of 33 RRMS patients receiving clinically-indicated fingolimod treatment. Patients were ascertained as either Active (n=17) or Stable (n=16) based on whether they did, or did not, develop new T2 brain MRI lesions and/or clinical relapses over 2 years of treatment. High quality PBMC were collected pre- and post-fingolimod and interrogated in-batch using 18-colour flow cytometry panels to functionally immune-phenotype over 80 immune-cell subsets. As expected, circulating counts of all major lymphocyte populations decreased substantially on-treatment, in both groups. While counts of T cell, myeloid cell and NK cell subsets did not differ pre-fingolimod between the two groups, B cell counts were significantly higher in the Active group at baseline (p=0.0066), mainly reflecting increased numbers of mature (p=0.0061) but not transitional (p=0.6233) B cells. Among mature B cells, both pro- and anti-inflammatory cytokine-defined B cell subsets were increased in the Active group, suggesting a more general active phenotype of these B cells in patients destined to experience continued disease activity on-treatment. Our data identify mature B-cell counts as a potential predictive biomarker of fingolimod treatment-response in RRMS patients.

T.1. Dysregulation of B cells in Myalgic encephalomyelitis/Chronic Fatigue Syndrome

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[Objective] Myalgic encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) is a severely debilitating disease of unknown etiology. The core symptoms of ME/CFS are profound fatigue, post-exertional malaise, cognitive impairment, sleep abnormalities and orthostatic intolerance. Various immune impairments have been described and remarkably, about two thirds of ME/CFS patients receiving B cell depletion therapy by rituximab had a clinical response. However, the underlying mechanism of the therapeutic effect of rituximab on ME/CFS patients is still unclear. The aim of this study is to evaluate immune abnormalities in ME/CFS patients.

T.2. GPR15 Expression is Increased in Smokers and Patients with Multiple Sclerosis and is Associated with a CCR6+ T Helper Cell Phenotype

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Background: Multiple sclerosis (MS) is an immune-mediated disease of multifactorial origin, targeting the central nervous system. Smoking is established as a risk factor associated with an increased risk of development and progression of MS. Circulating immune cells are believed to be involved in the pathogenesis of MS, but to what extent smoking affects circulating immune cells in MS is still unknown.

Objective and methods: With the present study, we aimed to characterize differences in gene expression due to smoking in circulating immune cells of MS patients and healthy controls (HC). We used Affymetrix microarray and real-time qPCR gene expression analyses to identify genes associated with smoking in cohorts of MS patients and healthy controls. Flow cytometry analyses were used to further characterize immune cell phenotypes.

Results: Two genes, *GPR15* and *LRRN3*, were upregulated in smokers in both MS patients and HC. *GPR15* gene expression was also increased in MS patients compared with HC among non-smokers. *GPR15* gene expression was further associated with increased frequencies of circulating $CCD6^{+}CXCR3^{-}$ $CD4^{+}$ T cells in smokers. In CSF of patients with relapsing-remitting MS, GPR15-expressing $CD4^{+}$ T cells were enriched in CSF compared with blood.

Conclusion: Smokers and MS patients have increased *GPR15* gene expression in blood which is associated with increased levels of Th17-like cells. In CSF from MS patients $GPR15^{+}$ $CD4^{+}$ T cells are enriched, suggesting a role of GPR15, an orphan chemokine receptor-like molecule, in pathogenic T cell recruitment in MS.

T.3. Cross-talk between MS disease-relevant B cell subsets and Microglia/macrophage: implication in MS disease progression.

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B cell depleting therapies efficiently decrease new multiple sclerosis (MS) relapses. Previously, we demonstrated that MS patients harbor abnormally higher proportions, as well as greater activation propensity, of pro-inflammatory effector B cells (Beff), producing high IL-6, TNF and GM-CSF levels compared to matching controls. B cells are also known to be fostered within the MS central nervous system (CNS), persisting within lesions and meningeal aggregates. The latter are adjacent to an important subpial cortical injury, involving microglia/macrophage activation and neuronal loss, which has now been strongly associated with progressive MS. However, how distinct B cells persist and interact with underlying CNS cells to potentially propagate to disease progression (a major unmet need) remains unknown. First, we demonstrated that M1 human microglia supernatants increase B cell activation (CD86 & CD95), while M2c-derived microglia supernatants were cytotoxic to B cells. In turn, Beff down-regulated IL-10 production by both microglia and macrophage through soluble products, and substantially enhanced the myeloid pro-inflammatory cytokine (IL-12, TNF & IL-6) responses, effects that were not seen by soluble products of anti-inflammatory (IL-10 expressing) B cells. Initial data also suggest that activated B cell soluble factors also increase myelin phagocytosis by microglia. These results indicate a potential bi-directional interaction between disease-relevant human B cell subsets and both resident CNS microglia and infiltrating myeloid cells, which may influence the propagation of MS-CNS compartmentalized inflammation associated with MS disease progression.

T.4. Modulating IL-6/STAT3 Signaling Pathway for Multiple Sclerosis Therapy

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Multiple Sclerosis (MS) is an immune-mediated chronic CNS disease and IL-6/STAT3 pathway plays a critical role in MS pathogenesis. We have previously demonstrated that IL-6 induces the development of highly encephalitogenic Th17-cells. Meanwhile, IL-6/STAT3 pathway is a key signaling pathway blocking the development of inducible T-regulatory cells (iTreg); critical for dampening pathogenic inflammatory T-effector (Teff) responses. Therefore, dysregulated IL-6/STAT3 signaling skews Teff/Treg balance toward an enhanced Teff response; favoring the development of CNS autoimmunity. The dysregulation of IL-6/STAT3 signaling pathway has been observed in MS patients, suggesting IL-6/STAT3 signaling

pathway may serve as an innovative target for reversing pathogenesis in patients. In support, both IL-6-/- and STAT3-/- mice are completely resistant to the EAE model of MS. Therefore, we developed small-molecule lead compounds targeting IL-6/STAT3 pathway. MDL-analogs bind to the D1 domain of GP130, preventing the IL-6/GP130 interaction; while LLL12-analogs bind to the SH2 domain of STAT3, preventing STAT3 phosphorylation. Both inhibit IL-6 induced IL-17 production in myelin-specific CD4 T-cells in a dose dependent manner without significant toxicity. Furthermore, adoptive transfer of LLL12 treated myelin-specific CD4 T-cells into naïve recipient mice leads to a significant reduction in EAE severity compared to control group, suggesting LLL12 analogs suppress the encephalitogenicity of myelin-specific CD4-T cells. More importantly, EAE mice treated with LLL12 analogs *in vivo* demonstrate reduced disease severity compared to control mice, suggesting LLL12 analog treatment *in vivo* suppresses EAE development. Together, our data suggest that these lead compounds have great potential to serve as an innovative therapy for MS.

T.5. Clinical Significance of Intrathecal BAFF/APRIL Axis in MS

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B cell-activating factor (BAFF) and a proliferation-inducing ligands (APRIL) are TNF superfamily ligands involved in differentiation and maturation of peripheral B cells. Both ligands can promote survival of autoreactive B cell clones and have been associated with numerous autoimmune conditions. In fact, increased level of BAFF in blood has been previously reported during MS relapse; however, its clinical significance remains controversial. We have explored clinical features of relapsing-remitting MS patients, currently not on DMT, by focusing on the balance of CNS-produced BAFF/APRIL at the time of their clinical relapse. We have found that the ratio of BAFF CFS-to-serum index against that of APRIL (herein referred to as BAFF/APRIL index ratio) is indeed significantly elevated in patients with increased intrathecal IgG production and oligoclonal band (OCB) presence. Furthermore, BAFF/APRIL index ratio is positively correlated with CNS lesion numbers, but not with duration of the disease, nor severity of the attack, nor disease activity leading to the attack, as represented by EDSS and prior annual relapse rate (ARR). Interestingly, patients with higher BAFF/APRIL index ratio seem to experience more frequent relapses within the following year despite the treatment. Our study implies that patients with activated B cell status during their attack, via BAFF skewed signaling pathway in CNS, may experience difficulty in controlling disease activity. Although further study is required to determine whether our findings reflect a particular phase or a distinctive subtype of this disease, BAFF/APRIL index ratio may be an effective marker for patients benefitting from B cell-directed immunotherapy.

T.6. Regulation of Myeloid Cells Function by Nanoparticles

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Antigen-presenting cells (APCs) makeup a significant proportion of the cells found within lesions of multiple sclerosis (MS) and its animal model, experimental autoimmune encephalomyelitis (EAE). These APCs have the ability to capture myelin debris and to promote either an inflammatory response or a tolerogenic response from the effector lymphocytes. In MS patients and EAE animals, APCs induce an inflammatory response.

Recently, the Miller laboratory showed that intravenous (i.v.) injection of myelin antigen-coupled nanoparticles (Ag-NPs) made from the copolymers of lactic and glycolic acid (PLG) could prevent the onset of EAE and, more importantly, attenuate active disease. Our goal was to understand by which mechanism these NPs modulate the immune response and induce T cell tolerance.

We found that all $CD11b^+$ myeloid cells are taken up NPs, as early as 30 minutes after i.v. injection, with mDCs and inflammatory monocytes being the most efficient cells. Interestingly, microglia are unable to engulf NPs *in vitro*. Moreover, immunofluorescence analysis shows that NPs accumulated at 3h in the spleen, but were largely undetectable 24h post-

injection. After ingestion of NPs, myeloid cells decrease their expression level of costimulatory molecules CD80, CD86 and CD40, as well as the cytokines IL-12, IL-23 and TNF. In addition, $CD4^+$ T cells co-cultured in presence of APCs, which have uptaken NPs, produce reduced levels of IL-17 and GM-CSF, and exhibit increased expression of TGF-beta, CTLA-4 and PD-1.

Collectively our data demonstrate that NPs are captured by APCs, which subsequently change their phenotype and function to an immune regulatory profile.

T.7. Evidence of a Pathogenic Role of the Immune System in **Huntington's** Disease

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Huntington's disease (HD) is a progressive, neurodegenerative and autosomal dominant disorder caused by a CAG repeat expansion in the huntingtin gene (htt), which leads to the expression of a mutated huntingtin protein (mHtt). Htt is ubiquitously expressed in the central nervous system (CNS) and mHtt is hypothesized to contribute to disruption of postsynaptic signaling, transcriptional regulation, and apoptosis induction leading to damage of the CNS. Htt is also expressed in cells of the immune system, and it is currently debated whether a pathogenic immune response is implicated in HD pathology. To investigate if the immune system plays a role in the pathogenesis of HD, we measured 30 cytokines in the CSF of 38 htt gene expansion carriers, thereof 19 premanifest and 19 manifest, compared to 19 healthy non-carriers (HC). Intriguingly, this analysis showed clear lymphocyte activity (e.g. decreased IL-7 and increased IL-17 and LT-a) in the CSF of htt gene expansion carriers already at pre-manifest disease stages. Furthermore, we found that the htt gene expansion carriers expressing at least one of the CSF neuroinflammatory biomarkers IgG, MMP-9, and CXCL13 were associated with a highly inflammatory profile; the expression level of 13 of the 30 analyzed cytokines was increased in this group as compared to non-inflammatory htt gene expansion carriers. Altogether, these observations demonstrate early lymphocyte activity in htt gene expansion carriers and suggest that the immune system plays a pathogenic role in Huntington's disease.

T.8. Deep Learning Predicts Gut Microbiota Associated with Untreated Multiple Sclerosis.

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The gut microbiota influences its host's immune system, both systemically and in the CNS, and influences blood brain barrier permeability and demyelination. Thus, it may be associated with Multiple Sclerosis (MS). Several studies have found differential abundances of bacterial taxa among relapse-remitting MS (RRMS) patients. However, some of the patients Have been treated and confound the analysis. Machine learning could be used to predict a microbiota associated with MS as well as screen for biomarkers for MS. However, the nature of microbiota sequencing studies makes this approach challenging; often the number of variables in each sample far exceeds the number of samples available, making the most powerful deep machine learning algorithms, support vector machines (SVM) and artificial neural networks, incompatible. Sparse SVM provides a solution to this problem by selecting only the most informative variables to use in its algorithms; thus, it may also be used to screen for bacterial biomarkers. A Sparse SVM model was used to; (1) predict who has MS, (2) find operational taxanomic units (OUTs) and subspecies bacterial markers for untreated RRMS patients. V4 regions of 16S rRNA reads of untreated MS patient gut microbiota samples were retrieved from two previous studies. A Sparse SVM model was trained with gut bacterial OTUs or subspecies abundance profiles as input variables. The Sparse SVM model screened for the most informative bacterial taxa whose abundance predicted patients who have RRMS with the highest accuracy, above 90%. Some of the positively selected subspecies are known to regulate systemic immunity. Ã‚Â

T.9. Myelin-Specific CD8+ T cells Exacerbate CNS Autoimmunity and Increase Clinical Signs of Brain Inflammation.

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Multiple sclerosis (MS) is a chronic, inflammatory, demyelinating disease of the central nervous system (CNS). Although the pathogenic pathways of MS are not fully understood, myelin-specific CD4+ T cells are hypothesized to initiate disease. However, substantial data from MS patients indicate that CD8+ T cells also contribute to MS pathogenesis. We hypothesized that recruitment of myelin-specific CD8+ T cells to the site of inflammation initiated by CD4+ T cells will influence disease. To test our hypothesis, we use a mouse model of MS, experimental autoimmune encephalomyelitis (EAE) in which we initiate disease by adoptive transfer of CD4+ T cells specific for myelin oligodendrocyte glycoprotein (MOG). To determine the influence of CD8+ T cells, we induced disease in TCR-transgenic mice that express a TCR specific for a MHC class I-restricted epitope of myelin basic protein (MBP), or in wild-type mice that also received an injection of naïve CD8+ T cells from the TCR-transgenic mouse. We found that the recruitment of the MBP-specific but not control CD8+ T cells exacerbated EAE. Strikingly, MBP-specific CD8+ T cells increased symptoms associated with brain inflammation. Increased disease severity was associated with a higher number of MOG-specific CD4+ T cells within the brain and spinal cord. Furthermore, MBP-specific CD8+ T cells produced more TNFa and in the brain and spinal cord as compared to WT CD8+ T cells. These data suggest that the interplay between CD4+ and CD8+ T cells specific for two different myelin proteins is critical for determining the manifestation of CNS autoimmune disease.

T.10. In-depth Immunophenotyping of Newly Diagnosed Relapsing Remitting Multiple Sclerosis (RRMS) Patients and Patients treated with Dimethyl Fumarate

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There is limited information about the subpopulation dynamics in multiple sclerosis (MS) patients. Our aim is to assess the changes in peripheral immune responses of MS patients with and without treatments. We designed two multi-color panels (14 and 17 surface markers) to analyse cell surface expression of 41 various leukocyte subpopulations including B cells, monocytes, CD4+ and CD8+, NK cells and also dendritic cells by flow cytometry. To date, we have processed 12 healthy controls (HC), 53 relapsing remitting patients (32 treated with dimethyl fumarate (MS-DMF), and 21 newly diagnosed patients (MS-ND). In agreement with previous reports, MS-DMF had lower PBMC frequency than both HC and MS-ND (p values ≤0.003). T cell % was significantly different in MS-ND comparing to MS-DMF group (67.1% vs. 48.1%; p value 0.0001). Surprisingly, we observed lower CD8% in all MS patients comparing to HC (23.2% vs 34.1%; P value 0.0004). TCRγδ subpopulation was lower in the MS-DMF group vs. HC (1.28% vs. 3.2%; p value 0.0085). Also within CD4+ subpopulations, CD45RA- CD62L- was significantly lower in MS-DMF (6.6%) when compared to HC (17.0%) (P value 0.0001), Within B cell population, the B-cell subtype CD27+IgD- was 17.7% in HC, 7.9% in MS-DMF (P value 0.0017) and 16.1% in MS-ND (p value=NS versus HC or MS-DMF). The physiological importance of these observations are under investigation. Our data will shed new light on the mechanisms of inflammatory events in MS and also help to identify and predict patients' response to treatment.

Autoimmune rheumatologic diseases

F.9. The Epigenetic Landscape of T Cell Subsets in SLE Identifies Known and Potential Novel Drivers of the Autoimmune Response

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Super-enhancers (SEs) are large stretches of the genome characterized by permissive chromatin marks indicating active transcription. SEs recruit clusters of transcriptional regulators and regulate expression of genes important determining cell fate and immune cell functional state. We analyzed SEs from naïve, memory and regulatory CD4+ T cells purified from peripheral blood of systemic lupus erythematosus (SLE) patients to identify key drivers of SLE pathogenesis. We used chromatin immunoprecipitation sequencing to assess genome-wide regions enriched in histone H3K27 acetylation, a marker of active enhancer regions. SE regions were computationally linked to specific genes and Ingenuity Pathway Analysis was then used to map these into specific signaling pathways and identify upstream drivers of gene expression. Additionally, differential SEs between SLE patients and matched healthy donor T cells were determined. This analysis identified both known and unexpected novel differences in epigenetic landscape between SLE and healthy cells and delineated how critical pathways in T cell biology are differentially regulated in SLE. Specifically, we found that all T cell subpopulations in SLE display altered epigenetic programming at genes encoding key T cell effector molecules. Our data also suggests that T cells in SLE may not rely on survival pathways typically utilized by T cells in the absence of autoimmune disease (e.g. IL-7) and identifies key upstream transcription regulators in disease. Epigenetic profiling also confirms a deficiency in regulatory T cell function in SLE (e.g. loss of SE at CTLA4 in SLE). We are in the process of validating putative targets derived from this analysis.

F.10. Distinct Single Cell Gene Expression Signatures of Monocyte Subsets Differentiate Between TNF-Alpha Inhibitor Treatment Response Groups in Rheumatoid Arthritis

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Work from our group demonstrated that pre-treatment serum type I IFN-β/α ratio>1.3 predicts non-response to anti-TNFα therapy (TNFi) in RA patients. Mechanisms that underlie the IFN-β/α ratio that predicts response are unknown. Effects of IFN on uncommon cell populations may be masked in whole blood or mixed cell populations. We used single cell analysis to investigate whether monocyte gene expression differs significantly between RA patients according to their pre-TNFi serum IFN-β/α ratio. Single classical (CL) and non-classical (NC) blood-derived monocytes were isolated from seropositive RA subjects prior to biologic therapy. Subjects were grouped by pre-TNFi serum IFN-β/α ratio: IFN-β/α>1.3(n=6) and IFN-β/αJAK1,IL1A,TLR2,CD32A,CD36, CXCR3,IL8,IRAK1, and TYK2 expression were retained in the mixed monocyte gene expression model for differentiating between groups. JAK1 and IL1A were also retained in the models from monocyte subsets. TLR9,STAT1, and FCER were retained in the CL model. STAT2 and IFI27 were retained in the NC model. Regression models from the monocyte subsets provided increased discriminatory potential in comparison to the mixed monocyte model. Within-cell co-expression patterns demonstrate biological differences in monocyte subsets of RA patients with an IFN-β/α>1.3, the ratio of type I IFNs which predicts non-response to TNFi.When monocyte subsets were analyzed separately, differentiation by gene expression was strongest and distinct expression signatures were identified, suggesting that further study of monocyte subsets will illuminate molecular differences that determine treatment response to TNFi in RA.

F.11. Rs9268832 Risk Allele Enhances Antigen Presentation and Autoantibody Production in Systemic Lupus Erythematosus

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SNP rs9268832 (C>T variation) is located in HLA-DR locus and has been associated with higher risk of SLE and other autoimmune diseases. In this study, the association between the alleles of rs9268832 and the expression of antigen presentation genes as well as the levels of autoantibodies was accessed in a cohort of SLE patients and normal controls (NC). The frequency of T allele is significantly higher in SLE than NC (Odd=1.7, p=0.0003). SLE patients carrying TT allele of rs9268832 exhibited significantly higher DRB1 and DRB5 genes expression compared with CC allele carriers ($p < 0.01$). The SLE patients carrying TT allele also showed a dramatic hypomethylation on DRB1 and DRB5 genes compare with CC individuals. Autoantibody profiling revealed that the anti-DNA autoantibodies (anti-dsDNA, anti-ssDNA, anti-Chromatin, anti-nucleosome) were significantly higher in TT allele SLE patients compared with CC or CT allele SLEs ($p < 0.05$). The DNA fragment containing TT allele from SLE showed more than 2 times higher enhancer activity than the CC allele fragment by a luciferase activation assay. We further confirmed that the TT allele DNA fragment exhibited higher binding activity with transcription factor GABPα by gel electrophoresis mobility shift assay (EMSA). In conclusion, the rs9268832 is a potential functional variant which may enhance the expression of antigen presenting genes HLA-DRB1 and DRB5 by modulating the binding activity with transcription factor GABPα. The hyper expression of the antigen presenting genes could be associated with initial breach in immune tolerance to self-antigens in the risk population.

F.12. Increased Levels of Sputum Antibodies to a Subset of Citrullinated Peptide Antigens Correlate with Sputum Neutrophil Extracellular Trap Levels in Subjects At-Risk for Future RA

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Background: Our previous studies have identified anti-citrullinated protein/peptide antibodies (ACPA) generated in the lung in individuals with established RA and individiuals who are at-risk for RA suggesting that the lung could be a site of initiation of RA-related autoimmunity. Neutrophil extracellular trap (NET) formation is one potential mechanism that could trigger ACPAs because NETs externalize citrullinated proteins and release peptidylarginine deiminase. Herein, we explored associations between NETs and individual ACPAs in the lungs from subjects At-Risk for RA.

Methods: We studied 24 subjects At-Risk for future RA based on familial or serologic risk factors. Blood and induced sputum were tested for ACPAs using a bead-based array with 29 individual citrullinated proteins/peptides. Levels of NET complexes were measured by a DNA-myeloperoxidase (MPO) and DNA-neutrophil elastase (NE) sandwich ELISA. Using Bonferonni's correction, results were significant if $p < 0.002$ for DNA-MPO and DNA-NE.

Results: In At-Risk subjecs, increasing sputum NET levels significantly correlated with 27/29 ACPA levels. The strongest associations ($p \leq 0.001$) were cit-H2A/a2$_{1\text{-}20}$, cit-vimentin$_{58\text{-}77\text{cyclic}}$, cit-alpha-enolase$_{5\text{-}21}$, cit-fibrinogen$_{27\text{-}43}$, cit-fibrinogen$_{211\text{-}230\text{cyclic}}$, cit-fibrinogen$_{616\text{-}635\text{cyclic}}$, cit-fibrinogenB$_{54\text{-}72}$, and cit-apolipoprotein E$_{277\text{-}269\text{cyclic}}$. No significant correlation was seen between NET and individual ACPA levels in the blood.

Conclusions: In subjects At-Risk for RA, we identified a strong correlation between levels of sputum NET complexes and multiple ACPAs. Importantly, these associations were not present in serum. Therefore, these findings suggest that NET formation in the lung may be associated with the local mucosal production of multiple ACPA reactivities. Additional studies

are needed to determine if NET-associated cit-proteins are an initial trigger or a self-perpetuating stimulus of sputum ACPA generation.

F.13. In active Juvenile Idiopathic Arthritis, the Synovial Microenvironment May Shape a Discordant Transcriptome Profile in Pathogenic Immune Subsets from a Common Precursor

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We have previously identified T cell subsets in both Teff (CPLs) and Treg (iaTreg) compartments that are HLA-DR positive, antigen experienced, pro-inflammatory, correlating with disease activity and sharing strong TCR oligoclonality with synovial T cells. Their phenotype and association with clinical fate inspired out current hypothesis that these functionally discordant cells may originate from a common precursor. Next generation RNA sequencing was performed on sorted CPLs ($CD3^+$ $CD4^+$ $CD14^-$ $HLADR^+$ CD25/CD127 Teff gate) and iaTregs ($CD3^+$ $CD4^+$ $CD14^-$ $CD25^{hi}$ $CD127^{lo}$ Treg gate) from 8 active disease JIA patients. Comparative analysis between CPLs vs iaTreg and that of the common pool of Teff vs Treg, indicate a dramatic reduction from 916 to only 143 unique gene regulation despite functional lineage differences, suggesting strong microenvironment influences. TCR sequence oligoclonality in CPLs/iaTregs versus that of the common Teff/Treg pool reinforce the possibility of a common precursor. Deep immuno-phenotyping revealed commonality in the dysregulation exhibited in both CPLs and iaTreg, notably perturbations in TCR/PD1 signalling and IFN-g pathways. The retention of 143 conserved differentially regulated genes (immune response, apoptosis, intracellular signalling and metabolic processes) between CPLs and iaTregs provide insights to the pathways pertinent to pathogenesis and may identify targets of therapeutic value. Intriguingly, as both subsets transit in the inflamed synovium, these data underscore a potential role of the microenvironment in shaping two functionally dichotomic populations from a common precursor.

F.14. The Effect of Functional Human TLR8 on Autoimmunity and Renal Inflammation in Systemic Lupus Erythematosus

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Objective: Lupus nephritis (LN) causes significant morbidity and mortality in patients with systemic lupus erythematosus (SLE). Using a systems biology approach we found that renal macrophage functional pathways, linking phagocytosis with activation of TLR pathways, are shared between mice and humans with LN. Surprisingly, we observed overexpression of TLR8, but not other endosomal TLRs, in resident renal macrophages from nephritic mice as well as in human LN kidneys. Our goal was to evaluate the effect of functional human TLR8 (huTLR8) on autoimmunity and renal inflammation.

Methods: Because mouse TLR8 does not recognize ssRNA, NZW/B6.Yaa mice expressing huTLR8 as a BAC transgene (huTR8tg) were generated and followed clinically. huTLR8 mRNA was confirmed by qPCR. 24-week-old male mice were administered TL-506 (TLR8-agonist) subcutaneously for 4weeks and spleens and kidneys were harvested for analysis after 8weeks. Mitochondrial respiration of TLR8-stimulated bone marrow-derived macrophages (BMDMs) was assessed using the Seahorse XF Analyzer.

Results: A single copy of huTLR8 in NZW/B6.Yaa mice did not exacerbate/accelerate disease however TLR8-agonist administration appeared to enhance germinal center formation and plasma cell generation in male huTLR8tg mice. TL-

506-stimulated BMDMs from huTLR8tg mice had a significant decrease in mitochondrial respiration and increased glycolysis compared to wt BMDMs.

Conclusions: One copy of huTLR8 does not initiate/exacerbate lupus in NZW/B6.Yaa mice. We are now following lupus mice with 2 copies of huTLR8 to determine effects on lupus phenotype. Further elucidating how TLR8 influences macrophage metabolism and phenotype will improve our understanding of the role of this TLR in inflammation and autoimmunity.

F.15. A Dual Immune Regulatory Mechanism is Associated with Quiescent Disease in Juvenile Systemic Lupus Erythematosus

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Introduction:
Systemic Lupus Erythematosus (SLE) is a multi-factorial autoimmune disease and the conventional investigative approach involving one cell type at a time is inadequate for its study. The ability to interrogate different immune cell types simultaneously is a critical unmet need. We hypothesise that abnormalities within the immune-regulatory mechanism contributes to lupus pathogenesis. To address such unmet needs and hypotheses, we employed a multi-dimensional approach using mass cytometry to study the immunome of juvenile SLE (jSLE) patients.

Methods:
Peripheral blood mononuclear cells from 12 jSLE patients stratified clinically into active and inactive disease, were interrogated with mass cytometry and subsequent analysis done using an unbiased, unsupervised machine learning approach with dimensional reductions followed by automated cell classification, clustering and visualisation.

Results:
A statistically significant enrichment of T and B regulatory cells with high CXCR5 expression were found in inactive jSLE patients. CXCR5 is important as it can direct these regulatory cells to the lymph node germinal centre, a critical lupus related microenvironment. Secondly, distinct B cell differences exist with an absence of the IL10 secreting B regulatory cells and a lower expression of CD32B (an inhibitory immune receptor) associated with active disease.

Conclusion:
A dual regulatory mechanism, involving both the T and B regulatory cells, is significantly associated with lupus inactivity. In addition, dichotomisation of the B cells into two distinct signatures with the different disease states were also found, underpinning its potential for its utilisation as a predictor of clinical fate or future therapeutic target.

F.16. Sequencing the Plasmablast Antibody Repertoire at Serial Time Points Reveals Affinity Maturation that Drives Epitope Spreading in Rheumatoid Arthritis

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Rheumatoid arthritis (RA) is characterized by autoantibodies targeting citrullinated antigens, nevertheless the precise specificity and functional properties of anti-citrullinated protein antibodies (ACPAs) remain poorly understood. We sequenced the blood plasmablast antibody repertoire at serial time points to characterize the affinity maturation of key ACPA and gain insight into pathogenic mechanisms underlying RA. To profile the autoantibody repertoire, we used a cell barcoding technology that enables sequencing of the full-length, paired heavy and light chain genes expressed by

individual B cells. This enables direct linkage of sequence information with antibody specificity through recombinant expression. We sorted and sequenced plasmablasts from peripheral blood sampled at up to four time points from eight individuals with established RA and used a citrullinated peptide-based sort reagent to isolate plasmablasts expressing ACPA. Bioinformatics analysis identified clonal lineages that persisted across serial time points, some of which share similar CDR3 sequences across patients, and characterized their expansion and contraction. Recombinant antibodies representative of the plasmablast clonal families were expressed and characterized using *in vitro* assays to define their antigen targets and compare immune complex stimulation of TNF production from macrophages. Certain clonal family members from later time points possessed higher levels of somatic hypermutation and demonstrated binding to an expanded set of antigen targets as compared to earlier family members. Our work provides evidence that affinity maturation of ACPA-encoding B cell lineages drives epitope spreading in individuals with established RA.

F.17. Local Administration of Anti-**High Mobility Group Box 1 Attenuates Dry eyes in a Mouse Model of Sjögren's** Syndrome

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Purpose: Extracellular high mobility group box 1 (HMGB1) acts as a damage associated molecular pattern (DAMP) molecule, which may be involved in the pathogenesis of Sjögren syndrome. Therefore, we aimed to investigate effect of subconjunctival administration of anti-HMGB1 on dry eyes in a mouse model of Sjögren's syndrome.

Methods: 0.02 to 2 μg of anti-HMGB1 antibodies or PBS were injected subconjunctivally into 10-week-old NOD.B10.H2b mice twice a week for 2 weeks. Tear volume and corneal staining scores were measured after treatment. Goblet cell density was counted in PAS stained forniceal conjunctiva and inflammatory foci score was measured in extraorbital glands. The changes of $BrdU^+$ cells, IL17-, IL10- or IFNγ-secreting cells, functional B cells, and IL-22 secreting innate lymphoid cells (ILCs) were evaluated in cervical lymph nodes. The level of IL-22 was measured in intraorbital glands.

Results: Injection of 2 μg anti-HMGB1 attenuated corneal epithelial erosions and increased tear secretion and goblet cell density. However, the inflammatory foci score, and the number of $BrdU^+$ T or B cells, IL-17-, IL-10-, IFNγ-secreting cells, and functional B cells were not changed. On the other hands, the percentages of IL-22 secreting ILCs was significantly increased in draining lymph nodes and the expression of IL-22 was significantly increased in intraorbital glands with 2 μg of anti-HMGB1 administration.

Conclusion: This study suggests that subconjunctival administration of anti-HMGB1 attenuate clinical manifestations of dry eye by increasing IL-22-secreting ILCs, rather than modulating B or plasma cells, in a mouse model of Sjögren's syndrome.

F.18. Multi Dimensional Analysis of Immune Cells in Systemic Sclerosis Reveals Altered Frequencies and Function of MAIT Cells

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Systemic sclerosis (SSc) is an autoimmune disease characterised by excessive fibrosis of skin and internal organs, and vascular dysfunction. Association of T and B cell subsets have been reported in SSc, however there is a lack of systematic study of immune cells in this disease. Mucosal associated invariant T(MAIT) cells represent a novel class of innate like lymphocytes shown to be important in anti bacterial immunity. Recent reports have also highlighted the role of MAIT cells in autoimmune diseases. Here we report high dimensional mass cytometry based analysis of immune cells from peripheral blood of SSc patients. Mononuclear cells from blood of 10 diffused SSc (DcSSc), 10 limited SSc (LcSSc) and 10 healthy

controls were analysed by mass cytometry using a 36 marker panel. Unsupervised clustering analysis was performed using Accense, to identify differentially expressed cell clusters in patients with systemic sclerosis. Clusters representing CD8+ T cells expressing TCR Valpha7.2 showed altered frequencies in patients. Particularly, T cells coexpressing TCR Valpha7.2 and CD161, representing MAIT cells, were significantly decreased in peripheral blood of patients with DcSSc (median=1.480%, p=0.0003) and LcSSc (median=1.2%, p=0.0047) as compared to healthy controls (median=9.245%). Upon activation, MAIT cells from patients showed reduced IFN-gamma and TNF-alpha production.
Our high-dimensional mass cytometry analysis reveals altered frequencies and function of MAIT cells in peripheral blood of SSc patients. This may be due to recruitment of MAIT cells to tissues or death mediated by chronic stimulation through possible microbial or yet undiscovered ligands.

T.11. CD11b Activation Protects Against Lupus Nephritis by Suppressing TLR-dependent IFN Responses

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Elevated serum level of IFN I is a heritable risk factor for systemic lupus erythematosus (SLE) and play a pathogenic role. If single-nucleotide polymorphisms (SNPs) in the *ITGAM* gene (coding for CD11b) are linked to high IFN I and whether CD11b activation could be a therapeutic strategy is studied here. We measured serum IFN I activity in 171 SLE patients and determined their *ITGAM* genotype to test for a direct link between *ITGAM* SNPs and the IFN I pathway. We show that three *ITGAM* SNPs significantly associate with elevated levels of IFN I in SLE. We tested whether partial CD11b activation with small molecule agonist, leukadherin-1 (LA1), reduces IFN I responses and determined the underlying mechanistic pathways. To test the efficacy of LA1 *in vivo*, we used the MRL/*lpr* mice that develop IFN I-dependent multi-organ pathology with renal injury. LA1-treated mice had reduced proteinuria, IgG renal immune complex deposition, and glomerular damage as compared to vehicle-treated controls. CD11b activation reduced TLR-dependent pro-inflammatory signaling in leukocytes and suppressed IFN I signaling, via an AKT-FOXO3-IRF7 pathway. TLR-stimulated macrophages from CD11b SNP carriers showed increased basal expression of IRF7 and IFNB and increased nuclear exclusion of FOXO3, which was suppressed by LA1. LA1 suppresses TLR-stimulated overproduction of cytokines *in vivo*, which have been directly linked to exacerbation of SLE. These findings suggest that pharmacological CD11b activation is a potential novel therapeutic target in SLE, particularly in patients identified as carriers of specific genetic polymorphisms.

T.12. Variability in ICAP (International Consensus on ANA Patterns) Pattern Reporting in Testing for Antinuclear Antibodies (ANA) by Indirect Immunofluorescence Assay (IFA)

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ANA IFA pattern may guide clinical evaluation by directing specific antibody testing. ICAP has defined consensus ANA IFA patterns and the level of competency required to identify and interpret them. Laboratories participating in CAP proficiency testing for ANA in 2016 were queried to identify current practices in this interpretation and reporting. Of 638 performing ANA by IFA and reporting a pattern, nearly 100% reported nucleolar, 99% homogeneous and speckled, and 96% centromere, all competent-level ICAP patterns. Only 42% reported nuclear dots (competent-level). 53% reporting nucleolar pattern further described expert-level subpatterns. Of 519 reporting speckled patterns, only 29% reported dense fine speckles, a competent-level pattern reportedly found in normals. "Other" speckled was reported by 44%. 4% did not report speckled pattern at all. Of those reporting nuclear dots, 86% differentiated *many* nuclear dots and 84% *few* nuclear dots. Nuclear envelope (expert-level) was reported by 18%. Competent-level cytoplasmic patterns were reported: golgi 69%, mitochondrial 65%, speckles 30%, 17% rods and rings, reticular 12% and polar 10%. Expert-level cytoplasmic patterns were reported: spindle apparatus 59%, centriole 55%, mid body 45%, and lysosomal 32%. Only 54% used an internal fluorescence intensity standard. Pattern reporting practice is variable. Cytoplasmic pattern reporting is limited, possibly

reflecting a lack of consensus that cytoplasmic patterns should be reported in an "antinuclear" antibody test. Failure to use an internal fluorescence intensity standard by nearly half of the laboratories may increase inter-assay and inter-observer variation in the threshold for staining positivity and in titer determination.

T.13. Characterizing the Plasmablast Antibody Repertoire in Patients with AIRE Deficiency

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Autoimmune polyendocrinopathy candidiasis ectodermal dystrophy (APECED) is a monogenic autoimmune disease caused by mutations in *AIRE*, a transcription factor necessary for central tolerance through the expression of tissue specific antigens in the thymus. APECED patients are known to produce high-affinity neutralizing autoantibodies against a pool of shared auto-antigens that may contribute to some of the hallmark clinical features of the disorder, along with additional private autoantibodies that may reflect patient-specific disease manifestations. To further characterize the impact of AIRE deficiency on the B cell response, we used a cell barcoding technology to sequence the paired heavy and light chains of individual plasmablasts derived from the blood of APECED patients. Through bioinformatic analysis of the sequence datasets, we identified clonally expanded lineages of affinity-matured plasmablasts, which we hypothesize to play a role in disease pathogenesis. To elucidate the roles of their encoded antibodies, we recombinantly expressed representatives from select highly-mutated clonal families. We are characterizing the binding properties of these antibodies using human cytokine and protein arrays, as well as evaluating their functional properties through *in vitro* immune cell assays. Our approach will allow us to gain further insight into the immune dysregulation that arises from AIRE deficiency and the pathogenic mechanisms that underlie APECED.

T.14. Shared and Endorgan Specific Transcriptional Networks in Skin versus Kidney Biopsies in Systemic Lupus

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Skin rash can often herald the onset of a systemic disease flare in systemic lupus. The subtype of skin lesion may confer a differential risk of renal involvement. We hypothesized that renal flares may exhibit crosstalk between skin and kidneys and that similar molecular mechanisms may underlie skin and renal disease. We used systems biology approaches to integrate the regulatory events occurring in subacute cutaneous lupus erythematosus (sCLE, n=43) and discoid lupus erythematosus (DLE, n=47) and compared with those in the ERCB lupus nephritis (LN) class II+IV cohort (n=22). Shared transcriptional networks in SLE skin lesions versus LN kidney biopsies reflect similar pathway regulation (p value below 0.05) including complement, B-cells, dendritic cells (DCs), IL4, IL8, and inflammasome signaling pathways. IL-12 signaling and production in macrophages, IL-3, IL-15 signaling pathways were regulated only in LN glomeruli and sCLE rashes, while there were metabolic pathways unique to DLE. CCL21 mRNA expression was specifically up-regulated in sCLE and LN tubulointerstitium and correlated with eGFR, which suggests it may play a role in cutaneous and renal lupus pathogenesis. SCLE, which is associated with a higher risk of systemic disease involvement compared with DLE, shares overlapping gene regulation with lupus nephritis. Dendritic cell pathways and associated upregulation of the CCR7 ligand CCL21, that is involved in recruitment of immune effector cells, may serve as a marker for sCLE patients at risk for LN. These data thus identify potentially important molecular targets for novel therapies in cutaneous and renal lupus.

T.15. A Role for IFNB in Systemic Lupus Erythematosus (SLE) Is Predicted by Analysis of Gene Expression Data Sets

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SLE patients overexpress interferon signature genes (ISG), but the major interferon (IFN) involved in lupus pathogenesis remains uncertain. To address this issue, Gene Set Variation Analysis (GSVA) was carried out on hundreds of SLE and control microarrays from B cell, T cell, myeloid cell, PBMC, WB, kidney, skin and synovium. The GSVA reference datasets used were the three previously reported IFN-Modules (IFN-M, Chiche, 2014) and microarray data from *in vitro* treatment of healthy human PBMC with IFNA2, IFNB1, IFNW, IFNG, IL12 and TNF (Waddel, 2010). GSVA using the *in vitro* PBMC data showed similar or better separation of SLE patients from controls than the IFN-M, and additionally identified patients with TNF and IL12 signatures lacking IFN signatures. Z score calculations to determine the most likely upstream regulator predicted IFNB1 and IFNW as the major IFNs inducing the IGS for all SLE tissues. Confirmation of the strong IFNB1 signal was carried out using published microarray data of the IFNB1 signature in Multiple Sclerosis (MS) Patients (MS IFNB1; Nickles, 2013). The MS IFNB1 had superior overlap to SLE datasets compared to both IFN-M and the *in vitro* PBMC IFN Signature. Z score calculations using the MS IFNB1 signature showed high Z scores for all active SLE cells and tissues. The data indicate that IFNB and IFNW are the most likely IFN family member upstream regulators accounting for the ISG in active SLE cells and tissues and suggest that IFNB1 and IFNW may represent novel therapeutic interventions for SLE.

T.16. Ultrasensitive Assays to Measure Saliva and Plasma Cytokines in Primary **Sjögren's Syndrome and Sicca** Control Donors - Association of Cytokines with Clinical Traits

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Primary Sjögrens' syndrome (pSS) is a chronic autoimmune disease characterized by inflammation and loss of secretory function of the exocrine glands.Both presence of Th17cells in salivary glands as well as elevated levels circulating IL-17 have been reported, indicating the involvement of this cell population in pSS.Although Tcells predominate in early lesions,B cells predominate later.Chronic Bcell activation plays an important role in the pathogenesis of pSS and leads to increased risk of lymphoma.Overexpression of BAFF on one hand and IL-21 on the other hand are thought to be critically involved in the enhanced plasmacell formation in pSS patients.

Therefore in this study we measured BAFF,IL-21 and IL-17A both in proximal (saliva) and peripheral (plasma) biofluids.Existing assays for these cytokines were not sensitive enough for saliva.We used novel ultrasensitive platforms to measure these cytokines in order to investigate the role of these biomarkers associated with these Bcell and Th17cell pathways in pSS and sicca patients.All cytokine assays were validated for sensitivity and specificity in their respective matrices.Saliva cytokine levels were normalized to flow rates.SalivaBAFF and plasmaIL-21 levels were significantly elevated in pSS vs. sicca patients. Plasma and saliva BAFF were significantly elevated in pSS patients who tested positive vs.negative for impaired salivary flow and lymphocytic infiltrates in salivary gland biopsies.PlasmaIL-17A was significantly higher in pSS patients positive versus negative for dry mouth and salivary gland lymphocytic infiltration.Cytokines in saliva and plasma were measurable at single femtogram concentrations offering hitherto unattainable detection sensitivities,particularly in saliva,enabling testing of patient stratification strategies in clinical trials.

T.17. The paracaspase MALT1 plays a Central Role in the Pathogenesis of Rheumatoid Arthritis

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The paracaspase MALT1 is a key player in the activation of lymphoid, myeloid and mast cells, indicating MALT1's crucial role in innate and adaptive signaling. Therefore, MALT1 is regarded a promising target for the treatment of autoimmune diseases and defining its role in the pathogenesis of rheumatoid arthritis (RA) is a critical first step.

To unravel MALT1's role in RA, we initially assessed MALT1-activation in mice challenged with collagen-induced arthritis (CIA), the prototype model for RA. We then sought to address MALT1's role in the pathogenesis of RA by subjecting MALT1-deficient mice to the CIA model. To determine the importance of MALT1 in T-cells, CIA was additionally induced in CD4-specific MALT1-deficient mice. Finally, the effect of MALT1-deletion on bone homeostasis was assessed by measuring bone density by μCT-analysis of the tibiae and by a three-point bending test of the femurs.

We provide evidence that MALT1 is activated in RA and plays a crucial role in its pathogenesis since MALT1-deficent mice were completely protected against CIA. This protection was additionally observed in CD4-specific MALT1-deficient mice, indicating that the selective ablation of MALT1 in CD4-positive cells is sufficient for the observed resistance. Paradoxically to the protective effect of MALT1-deletion on inflammation, we show that MALT1-deficiency negatively influences bone density at steady state.

Altogether, our data provide evidence for a dual role of MALT1 in arthritis, showing a protective effect of its deletion on the inflammatory aspect and a negative effect on bone homeostasis.

T.18. Identification and Functional Characterization of T cell Reactive to Citrullinated Tenascin-C in HLA-DRB1*0401-Positive Rheumatoid Arthritis Patients

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Anti-citrullinated protein antibodies (ACPAs) are a hallmark of rheumatoid arthritis (RA) and target a number of synovial and inflammation associated proteins. Antibodies against Tenascin-C, an extracellular matrix protein, have been observed in ACPA+ RA patients and a citrullinated peptide was recently identified as their major target. Our aim was to determine whether T cell responses against cit-tenascin-C are present in subjects with RA. We utilized an algorithm to predict 64 possible HLA-DRB1*0401 restricted epitopes within tenascin-C based on its binding motif. These peptides were tested in a binding assay, identifying 10 citrullinated peptides that bound with moderate to high affinity. We next performed *in vitro* assays, expanding PBMC obtained from HLA-DRB1*0401+ patients and staining with HLA class II tetramers, confirming 6 of these peptides as immunogenic. We utilized tetramers to directly stain cit-tenascin-C specific T cells and observed that tenascin-C specific cells were readily visualized in the peripheral blood of HLA-DR*0401+ patients. To further investigate the specificity of tenascin-C specific T cells, we isolated a T cell clone from an RA patient using *ex vivo* single cell sorting. The expanded clone remained tetramer positive and proliferated in response to a citrullinated tenascin-C peptide (1012-1026 modified at amino acids 1014 and 1016). These results demonstrate that T cells that recognize citrullinated tenascin-C peptides are present in HLA–DRB1*0401+ RA patients. We expect that further characterization of cit-tenascin-C specific T cells will indicate unique functional characteristics and others that are shared by T cells that recognize conventional RA autoantigens

T.19. Gut-derived TNF as Risk Factor for the Development of Sacroiliac Inflammation

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An intruiging link exists between gut and joint inflammation in spondyloarthritis (SpA), with about 50% of SpA patients having subclinical gut inflammation, which represents a risk factor for development of Crohn's disease, sacroiliitis and

evolution into ankylosing spondylitis. However, the underlying mechanisms are still relatively poorly understood. Our goal was to examine the relationship between TNF, microscopic gut inflammation and axial inflammation. Therefore we examined in situ expression of TNF, TNFR1 and TNFR2 using triple in situ hybridisation in gut biopsies of human SpA patients and found marked upregulation of TNF, TNFR1 and TNFR2 in inflamed versus non-inflamed biopsies. In line with this, we found that patients with gut inflammation had a higher need for anti-TNF therapy and their degree of clinical response after anti-TNF was also markedly higher. We speculated that TNF in the gut represents an important risk factor for disease severity and progression in SpA. To investigate this further we generated a mouse-model over-expressing human TNF in the ileum. These mice, together with wild type littermates, were evaluated for the development of arthritis up until the age of 13 weeks. Transgenic mice exhibit a runt phenotype and hallmarks of inflammatory bowel disease. While in peripheral joints no clear signs of arthritis were observed, the sacroiliac joints in transgenic mice, by contrast, showed marked signs of inflammation. These data propose a new paradigm that gut-derived TNF is sufficient to trigger sacroiliitis and provide an alternate explanation on the relationship between gut inflammation and axial inflammation in SpA.

T.20. Myositis from Mouse to Man: New Insight from a Unique Experimental Paradigm

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Myositides are characterized by muscle weakness, leading to bedridden state and possibly death. Pathophysiological studies and therapeutic advances have been hampered by the lack of appropriate mouse models. We report the first model of spontaneous autoimmune myositis not requiring active immunization.

Disease clinical evolution in both *Icos*$^{-/-}$ and *Icosl*$^{-/-}$ NOD mice was attested by significantly decreased muscle grip strength and locomotor disability (impaired cadence and print area). Pathological analysis revealed the presence of necrotic myofibers and important inflammatory infiltrates (CD4^{+} T cells, macrophages). Muscle lesions were objectifiable using small animal MRI, correlated with histopathology and regressed under steroid therapy. CD4^{+} T cells were Th1 biased. Myositis developed in CD8- but not CD4-deficient mice. Disease was conferred to NOD.*scid* recipients by *Icosl*$^{-/-}$ CD4^{+} T cell adoptive transfer. Promoting *in vivo* activated CD4^{+} effector T cells, administration of IL-2/anti-IL2 complexes exacerbated myopathy. Serum proteomic analysis revealed five potential autoantibody targets, among two were over-expressed in diseased muscle. Searching for corresponding auto-antibodies in patients, we developed a ALBIA (Luminex™ immunoassay) using human ortholog proteins. One of them revealed positivity in a minority of individuals from a ~700 patients cohort.

These results establish the *Icos*$^{-/-}$ and *Icosl*$^{-/-}$ NOD mice as a unique paradigm of myositis, useful for pathophysiological and therapeutic research. It justifies performing MRI-guided muscle biopsy in patients. Further analyses will determine if it allowed to discover a new myositis-specific autoantibody.

T.21. B cell Specific TLR9 Modulates Disease in Murine Lupus

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The importance of Toll-like receptor (TLR) signaling in lupus is well documented. Despite being a "pro-inflammatory" innate immune receptor, TLR9 deficiency in lupus prone mice exacerbates clinical manifestations including reduced lifespan and more severe nephritis, despite lacking anti-nucleosome (anti-DNA) antibodies; while TLR7 deficiency dominantly ameliorates disease.

The mechanisms by which TLR9 suppresses rather than promotes autoimmunity are unclear. We hypothesized that TLR9 has cell-specific functions. Thus, we created two novel murine strains: a conditional TLR9 knock-out (*Tlr9*^{flox})and a conditional TLR9 overexpression allele (*rosa26-flox-stop-Tlr9*). Both were crossed onto lupus prone backgrounds with a B cell specific (CD19) Cre allele, given the importance of TLR signaling in B cells in lupus, then aged and analyzed for clinical manifestations.

Strikingly, B-cell specific deletion of TLR9 exacerbated disease, similar to the complete knockout, exhibiting increased proteinuria and nephritis ($p < 0.05$) with loss of anti-nucleosome antibodies ($p < 0.001$). The B cell specific TLR9 overexpression construct resulted in a 1.8 fold overexpression of TLR9 ($p < 0.01$) with a concomitant increase in function. This modest TLR9 overexpressed in B cells ameliorated disease in two models of SLE, MRL.Fas^{lpr} and B6.*Fcgr2b*$^{-/-}$.*Yaa*,. Both strains exhibited reduced renal disease including proteinuria and nephritis ($p < 0.05$) without alterations in tested autoantibodies, suggesting that this response may be antibody independent. These data, in which we manipulate TLR9 expression in both directions, indicate B cell expression of TLR9 accounts for a substantial proportion of the known TLR9 regulatory effect. Given its significant ameliorative effect, TLR9 overexpression in B cells alone may represent a potential therapeutic strategy.

T.22. IL-6 Expression is Correlated with Increased T Cell Proliferation in Giant Cell Arteritis

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Giant cell arteritis (GCA) is the most common vasculitis in adults affecting large and medium-sized arteries. It can cause of blindness and stroke. IL-6 and T cell accumulation within the arterial wall contributes to the development of GCA, and blockade of IL-6 activity is efficacious in its treatment. We examined the relationship between levels of IL-6 and immunological processes that control the expansion of T cells within GCA lesions. The expression of IL-6 RNA was quantified by RT-qPCR in 14 GCA-positive temporal artery biopsies and examined in relation to the frequency of proliferating CD4 T cells (identified by immunohistochemistry [IHC] for CD4 and Ki67), T regs (identified by IHC for Foxp3), and T cells undergoing apoptotic cell death (identified by IHC for CD4 and cleaved caspase-3). There was a significant positive correlation between the expression of IL-6 and increased frequency of proliferating CD4 T cells ($r = 0.56$, $p < 0.05$). The expansion of T cells can be inhibited by T regs but IL-6 expression was not correlated with differences in T reg accumulation. IL-6 levels were also not significantly correlated with differences in apoptotic death of CD4 T cells, although there was a trend toward a negative correlation. In summary, IL-6 may contribute to the accumulation of CD4 T cells in GCA by supporting their proliferation within the arterial wall through mechanisms that are independent of effects on T reg expansion.

T.23. Plasma Mitochondrial DNA Increases and Levels of IL-7, MCP-1 and PDGF Decrease in RA Patients Treated with Biological Agents for >12 months.

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Mitochondrial DNA (mtDNA), released upon tissue damage is a danger signal and a potent inflammatory trigger. The abundance of mtDNA is correlated with aging, frailty, cardiovascular diseases, diabetes, SIRS, and suggested as a cancer biomarker. We proposed to study its levels by quantitative real-time PCR in plasma from 19 rheumatoid arthritis (RA) patients before and at least 12 months after starting biological therapy (11 anti-TNFα, 5 CTLA-Ig and 3 anti-IL6R); clinical status was assessed by DAS28, ESR and CRP, and concentration of 40 cytokines was measured by Quantibody Human Inflammatory Array 3 (RayBiotech,Inc. Norcross, GA). Plasma mtDNA was lower ($p < 0.05$) in RA patients before biologicals compared with 20 age-matched healthy controls (mean 63 and 62 yrs) but it increased after therapy by $1\log_{10}$on average ($p < 0.01$). We found no correlation between mtDNA levels and disease activity at baseline and follow-up; changes observed were unrelated to plasma values of cytokines studied, including TNFα and IL6 or IL6R. 33/40 cytokines decreased after biological therapy, with five (including TNFα and TNFRII) showing a significant reduction ($p < 0.001$). The others were MCP-1/CCL2, IL7 and PDGF-BB, produced by stromal cells, monocytes and synoviocytes. A correlation of these molecules with TNFα levels was observed at baseline but not after therapy. These growth factors may be markers of synovial hyperplasia and activity in RA, contributing to inflammatory damage, whereas the amount of plasma mtDNA seems not related to inflammation or disease activity in RA, as also found in SLE.

T.24. Serum Vitamin D Receptor Levels in Patients with Behçet's Disease

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Background: Behçet's disease (BD) is a chronic multisystem inflammatory disease of unknown aetiology. Recent studies suggest that several immunological abnormalities may play pathogenetic role in BD. In BD, increased release of proinflammatory cytokines and chemokines and, peptide hormones may play a role in inflammatory stages of the disease. Recent studies have suggested the potential role of vitamin D and vitamin D receptor (VDR) in modulating the immune response and VDR's inflammatory or anti-inflammatory effects. In this study, we analyzed the possible role of serum VDR levels in the pathogenesis of BD.

Materials: 57 patients with BD and 59 healthy controls (24 female,35 male; mean age 34.3 ± 4.7 years) were enrolled in this study. Twenty-three patients were in active stage (8 female, 15 male, mean age; 33,7 ± 4.2 years, mean disease duration 11.7±6,9 years) and 34 patients were in inactive stage (11 female,23 male, mean age;34,6 ± 4.3 years, mean disease duration; 10,5 ± 4,7 years). Serum VDR levels were determined by ELISA.

Results: The mean serum VDR levels were 65.1±25.9 ng/ml in healthy controls, 15.2±9.8 ng/ml in active BD patients and 33.5±13.5 ng/ml in inactive BD patients. Serum VDR levels were significantly low in patients with BD compared with healthy controls ($p < 0.01$). Serum VDR levels were significantly high in active BD patients compared with in inactive BD patients ($p < 0.001$).

In this study, it was observed that; the serum VDR level may be an indicator for activity of BD.

T.25. The Distinct New IFL Liver Staining HALIP (HMGCR-Associated Liver IFL Pattern) May Help Detection of HMGCR Autoantibodies in Clinical Laboratories and Lead to a Higher Rate of Statin Associated Autoimmune Myopathy Diagnosis

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Statin-Associated Autoimmune Myopathy (SAAM) with Hydroxyl-Methyl-Glut aryl-CoA Reductase Antibodies (HMGCRAb) has recently been described. Several specific immunoassays are in use to detect HMGCRAbs but require a high degree of clinical suspicion. In the course of systematic autoantibody screening a new and distinct IFL staining that regularly coincided with HMGCRAbs was detected. Here we investigated whether this new IFL pattern is specifically linked to SAAM and correlated with HMGCAb tests currently used. Twenty-three patients (14 SAAM) positive for HMGCRAb by two ELISAs from different sources and confirmed by immmunoblot, were tested by IFL on rat liver cryostat sections from four providers. Sera from autoimmune diseases (n=90) and non-autoimmune statin treated patients (n=45) were the controls. A characteristic IFL staining of sparse distinct hepatocytes was seen in 21/23 (91%) HMGCRAb+ sera on all the liver substrates thus generating a pattern designated as HALIP (HMGCR-Associated Liver IFL Pattern). Statistical concordance between HALIP and HMGCRAb ELISAs was 98.7%, kappa 0.95. Control sera from autoimmune or non-autoimmune statin treated patients did not produce the HALIP staining which was completely and specifically removed by absorption with human purified HMGCR. HALIP therefore is a new and distinct IFL staining pattern associated to HMGCR myopathy detectable in the routine screening for autoantibodies by IFL. Since the initial communication of HALIP in SAAM, it has already been confirmed by at least another group. Awareness of this new pattern can help to detect HMGCR autoantibodies in statin-treated-patients in which the associated mypathy is not suspected.

T.26. Complement C1q Limits Osteoarthritis Pathology by Regulating Macrophage Activation

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Osteoarthritis (OA) is the most common form of arthritis and a leading cause of disability. While it is clear that OA pathogenesis involves low-grade inflammation, the precise immune mechanisms underlying this inflammation remain unknown. The complement component C1q is known to regulate inflammation via mechanisms involving apoptotic cell clearance and macrophage activation. We tested the hypothesis that C1q is a negative regulator of inflammation in OA by surgically inducing osteoarthritis in C1q-deficient (C1qa-/-) and wildtype (WT) mice and found that C1qa-/- mice develop exacerbated cartilage damage relative to WT controls. qPCR analyses of bone marrow-derived macrophages from C1qa-/- or WT mice, differentiated in sera with or without C1q, revealed that while WT macrophages grown in C1q-containing sera mounted a balanced pro-inflammatory vs anti-inflammatory response to cartilage debris stimulation, those differentiated in C1qa-/- sera had an enhanced pro-inflammatory response. Further, we found that C1qa-deficient macrophages grown in C1qa-/- sera had a markedly exaggerated pro-inflammatory phenotype which was partially reversed by growing C1qa-deficient macrophages in WT sera. Similarly, *in vitro* analyses of human macrophages stimulated with cartilage debris showed that macrophages differentiated in C1q-depleted serum exhibited an enhanced pro-inflammatory phenotype compared to those differentiated in normal human serum containing C1q, suggesting that serum C1q limits macrophage activation *in vitro*. Furthermore, we found that C1q elicits its immunoregulatory role via phosphorylation/activation of the ITIM-containing receptor, LAIR1 and the downstream adaptor, SHP1. Together, our data suggest that the C1q/LAIR1/SHP1 axis regulates unrestrained macrophage activation and limits inflammation and cartilage damage in osteoarthritis.

T.27. Perturbations within the T cell Immunome in Polyarticular Juvenile Idiopathic Arthritis Patients Who Relapse Upon Withdrawal of Biologic Therapy

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Clinical management of polyarticular JIA with anti-TNF-alpha biologics has been met with significant success, with up to 80% of patients demonstrating clinically meaningful efficacy. However, concerns about drug toxicities and costs have driven the clinical need to find predictors for successful drug discontinuation. Previous published data suggests a pivotal role for T cells in disease progression. To distill this pathogenic signal located within the T cell immunome, a high dimensional single cell resolution platform, CyToF, was deployed. Patients treated with anti-TNF-alpha who demonstrated at least 6 months of clinical inactive disease on medication (Wallace criteria) were enrolled in the Understanding TNF-alpha trial and segregated into flare or no flare arms (evaluated based on 6 JIA core set parameters) after discontinuation of therapy. PBMCs from n=39 JIA patients (19 flare, 20 no flare) and n=20 healthy paediatric controls were stained with a 37 markers T cell CyToF panel. Cluster analysis reveal a dysregulated CD4 T cell memory compartment ($CD69^+CD28^+ICOS^+TNF\text{-}alpha^{hi}$) prior to therapy withdrawal that is predictive for flare ($p< 0.05$). Upon flare, this population of $TNF\text{-}alpha^+$ memory cells have expanded into a) $IL\text{-}6^+$, b) $IFN\text{-}g^+$, c) $CD152^+$ or purely retaining TNF-alpha expression ($p< 0.05$). Concomitantly this dysregulation extends into the CD8 memory compartment ($CD8\ IFN\text{-}g^+TNF\text{-}alpha^+$, $p < 0.05$). These results suggest that clinical fate is immunologically predetermined and patients who will realize different clinical fates can be identified from prior biologic sampling.

T.28. Autophagic Memory in Stress Experienced Human T cells

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Autophagy is required for memory T cell generation and maintenance. Previous studies from our group showed higher levels of autophagy in CD4+ T cells from RA patients. Here, we hypothesise that T cells which experience immunological encounters and activation attain, when compared to naïve cells, heightened autophagic levels when a similar stimulus is encountered again, a phenomenon, which we term "autophagic memory". This phenomenon may be pathogenically relevant. Flow cytometry analysis of T cells from healthy individual showed indeed higher autophagy levels in memory CD4 and CD8 cells after activation, compared to naïve T cells. However the basal level of autophagy was similar in both cell types, suggesting a memory for autophagy. To dissect the molecular mechanisms of autophagic memory, we trained human T cells in low serum medium, to elicit stress-induced activation, for 5 days. The cells were then cultured in 10% serum media for 100 generations. RNA-sequencing and methylome analysis of the trained and control cells at 5, 30,70 and 100 generations, identified clusters of genes stably up or down regulated in trained cells compared to control cells until the 30th generations, suggesting for role of these genes in retention of memory for autophagy.

In conclusion, we describe here epigenetic and trasciptional elements which determine and control persistence of autophagic memory in experienced T cells. We suggest that autophagic memory is an integral part of efficient activation of memory T cells, and it also contributes to persistence of T cell mediated inflammation in autoimmunity.

T.29. A Human Gut Commensal as a Cross-Reactive Trigger of Autoantigen-specific CD4+ Memory T Cells in Antiphospholipid Syndrome

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Antiphospholipid syndrome (APS) is an HLA-DR53-associated autoimmune clotting disorder targeting the plasma protein b_2-glycoprotein I (b_2GPI). The gut microbiota provides an enormous antigenic challenge to the mucosal adaptive immune system. We thus hypothesized that a homeostatic adaptive immune response to commensals can trigger and sustain

autoreactive T cells via cross-reactivity with autoantigens in an HLA-susceptible host. We have identified in silico a human gut commensal, *Roseburia intestinalis,* as a cross-reactive candidate with highly homologous peptide sequences to the immunodominant b_2GPI T and B cell epitopes. *R. intestinalis* is abundant in human microbiomes including APS patients based on a species-specific fecal PCR. HLA-DR53-positive APS PBMCs proliferated significantly more to *R. intestinalis* protein extracts compared to phylogenetically closely related *Eubacterium rectale*, lacking homologous sequences. We next synthesized an MHCII tetramer for a major T cell epitope in b_2GPI that is conserved in *R. intestinalis*. Tetramer-positive CD45R0+ CD25- CD4+ memory T cells from APS patients showed significant and dose-dependent proliferation to heat-killed *R. intestinalis* compared to controls. Cytokine measurements revealed a heterogeneous but pathogen-associated phenotype including GM-CSF, TNF-α, IL-21, and IL-9. We further cloned b_2GPI-specific memory CD4+ T cells using a T cell library assay, confirmed clonality by TCR Vbsequencing, and were able to also demonstrate cross-reactivity to the mimic sequence in *R. intestinalis*. In summary, we have identified human b_2GPI autoepitope-specific CD4+ memory T cells, which have a pathogen-associated phenotype and cross-react to *R. intestinalis*. We propose that ubiquitous gut commensals could represent persistent triggers for autoreactive lymphocytes in human autoimmunity.

T.30. Bacterial Dysbiosis Associates with Functional Intraepithelial Lymphocyte Changes in Inflammatory Bowel Disease and Spondyloarthritis

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Dysbiosis occurs in spondyloarthritis (SpA) and inflammatory bowel disease (IBD), subdivided into Crohn's Disease (CD) and Ulcerative Colitis (UC). The immunologic consequences of dysbiosis have not been defined. Intraepithelial lymphocytes (IELs) are T cells within the intestinal epithelium that are in close contact with bacteria, and as such, are likely to be modulated by dysbiosis. We correlated IELs with resident bacteria in SpA, IBD, and controls. Subjects with biopsy-proven IBD (N=10 with CD and 7 with UC), SpA fulfilling ASAS criteria (N=5), and healthy controls (N=15) were evaluated for fecal microbiome by 16S rRNA sequencing and IELs from colon biopsies analyzed by flow cytometry and ELSIA. Subjects with SpA had significantly lower numbers of IELs compared to controls ($p=0.03$). Subjects with CD had significantly increased IL-17A, ($p=0.03$) and IFN-γ ($p < 0.01$) whereas those with UC had higher IL-1β ($p=0.01$) compared to controls. Both subjects with CD and SpA had significantly increased secretion of TNF-α ($p=0.04$). Correlating cytokines to bacteria revealed an association between TNF-α and the Simpson Diversity Index in subjects with UC (Spearman's $r=0.943$, $p < 0.01$); in subjects with CD, Fusobacterium had a negative correlation with TNFα+IFNγ+IL-1β (Spearman's $r=-0.786$, $p=0.02$). Sequencing results from subjects with SpA are pending. Our data indicate differences in IEL function among subjects with SpA, CD, and UC compared to healthy controls. We hypothesize that the correlations between dysbiosis and IEL function are relevant to the pathogenesis of SpA and IBD. Future studies will be aimed at further understanding these mucosal T cell and microbial interactions.

T.31. Evaluating the Effects of Selective Estrogen Receptor Modulators (SERMs) in Bone Marrow Derived Dendritic Cells

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ERα plays a significant role in systemic lupus erythematosus (SLE) pathogenesis, an inflammatory disease with a profound sex bias affecting females 9:1 over males. Treatment options for SLE patients are limited and have significant side effects. The development of novel therapeutic targets is of great clinical importance. Lupus mice with a deletion of the ERα AF-1 activation domain have significantly prolonged survival and less renal disease. Ligand bound ERα impacts several immune cell types and affects transcription of inflammatory mediators. Selective estrogen receptor modulators (SERMs) were developed to selectively induce non-genomic effects of ERs in breast cancer yet retain metabolic and

vascular protection without impacting fertility or reproductive tissue. Their effects in immune cells have not been investigated. The effects of SERMs on bone marrow derived dendritic cells (BMDCs) isolated from lupus-prone mice prior to disease onset were tested. mRNA concentrations of proinflammatory cytokines important in SLE pathogenesis were measured. To assess the non-genomic effects of the SERMs on ERα in immune cells, phosphorylation of ERK kinase (a MAPK pathway marker) was analyzed. Our results demonstrate that these novel SERMs reduce mRNA expression of IL6 and MCP-1 in TLR-stimulated BMDCs after 24 hours, but not as potently as estrogen. Future results from this study will determine the potential role of SERMs in modulating the effects of ERα in immune cells, characterize the non-genomic effects of ERα in immune cells and lay the foundation for the potential use of these compounds in the treatment SLE and other immune mediated diseases.

T.32. Interrelationships of CR1 and CR2 in the clinical disease activity of Rheumatoid Arthritis (RA)

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Introduction: Rheumatoid Arthritis (RA) is a chronic inflammatory disease characterized by pain, swelling and progressive destruction of multiple joints, affecting approximately 1% of the human population. The complement proteins have been known to play complex roles in the pathogenesis of RA.

Objectives: Studies on animal models suggest disease modulating activity of Leukocyte CR1 (L-CD35) and CR2 (L-CD21) in autoimmune disorders. Therefore, we aimed at elucidating a case control study to explore the role of L-CR1 and L-CR2 in RA patients.

Methodology: The L-CR1 and L- CR2 expression in 50 healthy controls and 50 RA patients on different leukocyte subpopulations was observed by Flow cytometry and expression at mRNA level was monitored by using RT- PCR. The clinical parameters Circulating Immune Complexes (CIC), C3, C3d and Disease activity scores (DAS28) were determined and correlated with L-CR1 and L-CR2 expression in patients.

Results: The L-CR1 and L-CR2 transcripts declined significantly in patients. A significant negative correlation of CR1 and significant positive correlation of CR2 transcript were observed with C3d and CIC in patients. C3 was correlated positively with CR2 transcript in patients. CR1 and CR2 transcript were negatively correlated with Disease activity.

Conclusion: Decline in the L-CR1 level suggests failure in protective role of L-CR1 against complement mediated damage in RA patients. Follow-up study confirming L-CR1 and L-CR2 role as pivotal biomarker is warranted. In essence, our findings suggest a close relationship of CR1 and CR2 with the pathophysiology and disease activity of RA and their importance as putative disease marker.

W.1. Integrated, multi-cohort gene expression analysis identifies a signature associated with active systemic lupus erythematosus

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Systemic lupus erythematosus (SLE) is a complex autoimmune disease that follows an unpredictable disease course and affects multiple tissues. Despite decades of research and millions of dollars of R&D, therapeutic options for SLE treatment remain inadequate. We present an integrated, multi-cohort analysis of 4,325 gene expression microarray samples from 37 studies that leverages study heterogeneity to identify a highly persistent SLE signature. The SLE signature is significantly elevated in relatives of SLE patients compared to unrelated healthy volunteers, distinguishes SLE from other autoimmune, inflammatory, and infectious diseases, and persists across diverse tissues and cell types, including kidney, synovium, B

cells, and T cells. Additionally, the SLE signature is significantly correlated with disease activity (SLEDAI) and clinical measures of inflammation, and decreases in response to treatment. Interestingly, many genes contained in the signature are independent of the type I and II interferon pathways. In particular, we notice a significant enrichment in genes from pathways related to nucleic acid metabolism. We prospectively validate our analysis in an independent cohort of pediatric SLE patients using RT-qPCR. In conclusion, the robust SLE signature has potential to aid clinicians in the diagnosis and monitoring SLE, and implicates novel biological pathways in SLE pathogenesis.

W.2. Endothelial Progenitor Cell Number is not Decreased in 34 Children with Juvenile Dermatomyositis

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Conclusion: The EPCs for JDM were in the normal range, differing from a study of adults with DM/PM. These data suggest that the age of the patient contributes significantly to dermatomyositis pathophysiology.

W.3. Variability in Method of Testing for Antinuclear Antibodies (ANA): A Survey of Participants in the College of **American Pathologist's (CAP) Proficiency Testing Program**

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A 2010 American College of Rheumatology position paper designated indirect immunofluorescence assay (IFA) on HEp-2 cells the "gold standard" for ANA testing and that laboratories performing other methods should state the method used and describe its performance parameters. Laboratories participating in CAP proficiency testing for ANA answered supplemental questions in 2016. Of 5847 kits distributed, 1206 (21%) participants responded (942 in the US and 264 international). ANA screening method varied: 56% IFA, 21% ELISA, 12% multi-bead immunoassay, and 18% "other" methods. Ordering test name indicated method used in only 32%; only 39% stated method used on the report. Of 644 laboratories, 80% used HEp-2 substrate, 18% HEp-2000 (HEp-2 cell line engineered to overexpress SSA), and 2% "other." Slides were prepared manually (67%) or on an automated platform (33%), and examined by direct microscopy (84%) or images captured by an automated platform (16%). IFA patterns were interpreted by personnel in 95% of laboratories; < 1% used automated image capture and analysis solely; 4% interpreted images both by personnel and an automated platform. 97% of 641 laboratories reporting ANA by IFA provided a titer. Only 51% reported a positive result at the traditional 1:40 dilution. Titer was reported to endpoint routinely by 43%, only upon request by 23%, or never by 35%. 8% did not report dual patterns. Of those reporting multiple patterns, 24% did not report a titer with each pattern. In conclusion, only slightly more than half of testing laboratories utilize the ACR "gold standard" IFA method using HEp-2 cells.

W.4. Rituximab-induced Hypogammaglobulinemia in Pediatric Patients with Autoimmune Diseases

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Rationale. While as many as 40% of adults with lymphoma develop hypogammaglobulinemia after receiving rituximab, the prevalence of rituximab induced hypogammaglobulinemia in pediatric patients is less clear.

Methods. We retrospectively studied IgG levels in all pediatric rheumatology patients after being given rituximab, seen 2010-2017. Diagnoses were 12 with Autoimmune CNS diseases (AICNS), 9 with ANCA vasculitis, 11 with SLE and 5 with other autoimmune diseases (RF +ve JIA, Systemic Sclerosis and Overlap Syndrome).

Results. Of 37 patients (35 receiving immunosuppressive medications including corticosteroids, cyclophosphamide, azathioprine, mycophenylate mofetil), 22 patients (60%) were found to have hypogammaglobulinemia, including 4 at baseline. Nineteen patients developed hypogammaglobulinemia within 4 months of completing rituximab therapy. Occurrence ranged from 75% in patients with AICNS to 0% in the miscellaneous group (p=0.03, Chi-square test). Severity varied among the 4 groups, with AICNS patients having more severe hypogammaglobulinemia. Of note, 3 patients with AICNS and 2 with lupus were given IgG replacement therapy for recurrent infections.

Conclusions. The prevalence of hypogammaglobulinemia in patients studied is higher than published data for adults, especially for AICNS. Onset of hypogammaglobulinemia is usually early. We speculate that possible causes of increased prevalence include lower reserve of plasma cells in children, developmentally related susceptibility to rituximab, either administered by itself or following immunosuppressive medications. We recommend close monitoring for hypogammaglobulinemia after the use of rituximab in pediatric patients.

W.5. Mass Cytometry (CyTOF) Analysis of Trafficking Molecules in Ankylosing Spondylitis

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Background: Ankylosing spondylitis (AS) is an HLA-B27-associated, inflammatory arthritis of the axial skeleton. Gut inflammation is a common co-morbidity in AS; the majority of AS patients have subclinical gut inflammation, while some have clinical inflammatory bowel disease (IBD). We hypothesize that inter-tissue trafficking of immune cells through shared trafficking receptors mediates the gut-joint axis of inflammation in AS.

Methods: We have examined the expression of trafficking markers by mass cytometry (CyTOF). Blood mononuclear cells from 26 AS patients, 20 healthy controls (HCs) and 17 rheumatoid arthritis (RA) patients, were surface stained using a 36-marker antibody panel. Cells from 6 AS and 3 RA synovial fluid samples and 2 IBD gut samples were also examined. Data was acquired on the CyTOF2 and analyzed using viSNE and Citrus algorithms

Results: In the blood, T cells from AS patients had a significant downregulation of CXCR3 and upregulation of CXCR4 compared to HC. A distinct population of integrin manifesting CD8+CD45RO+ T cells was seen in AS synovial fluid. These cells were characterized as CD103(aE)+CD49a(a1)+CD29(b1)+CD18(b2)+b7+. A similar population was enriched in IBD gut tissue, but not RA synovial fluid. Current experiments are being undertaken in the SKG and IL-23 minicircle mouse models of AS to identify and further characterize the integrin manifesting CD8+ T cells. Ã‚Â Ã‚Â

Conclusions: Unbiased analyses using CyTOF revealed disease- and tissue-specific alterations in trafficking marker expression in AS patient T cells. This technology allows for the identification of novel therapeutic targets to interfere with recruitment of potentially pathogenic cell subsets in AS.

W.6. Quantitative Profile of Innate and Adaptive Immune Response Abnormalities Related to Disease Activity in Patients with Systemic Lupus erythematosus: Preliminary Results of a Prospective Cohort Study

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Introduction: Patients with systemic lupus erythematosus (SLE) show global immunologic abnormalities, some of them may be related to disease flare. The aim of this study was to correlate diverse innate and adaptive immunologic abnormalities with disease activity in a cohort of SLE patients.

Methods. Fifty-two patients with SLE were prospectively followed-up during a median time of 6 (1-9) months. Clinical features were assessed at baseline, one and three months later. We assessed disease activity with the SELENA/SLEDAI scale. PBMCs were isolated by density gradient and the percentage and absolute numbers of CD4+, CD8+, NK cells, monocytes subsets, Th subsets, Tregs and B lymphocytes as well as TLR2 mean fluorescence intensity and percentage and absolute number of monocytes subsets expressing TLR2 were assessed by means of multiparametric flow cytometry.

Results: At baseline, patients with constitutional symptoms had lower CD4+ cells, p=0.019, B lymphocytes, p=0.034 and higher percentage of NK cells, p=0.036. Subjects with hematologic activity had higher percentage of Th17 cells, p=0.006 and patients with SN involvement had higher Th1 percentage, p=0.06. Variables inversely correlated with severe flare at baseline and 3 months later were: percentage of CD4+, ρ -0.41, p=0.047, of NK ρ -0.52, p=0.028, of CD8+, ρ -0.56, p=0.015, of B lymphocytes ρ -0.55, p=0.021, and number of lymphocytes ρ -0.54, p=0.025 and percentage of TLR2 positive monocytes ρ -0.36, p=0.045.

Conclusions: Low CD4+, CD8+, NK and B cells at baseline and lower TLR2 positive monocytes in the following 3 months correlated with severe SLE flare according to the SELENA/SLEDAI scale.

W.7. ATF3 Upregulates the Long Non-Coding RNA NTT In Peripheral Blood Mononuclear Cells of Rheumatoid Arthritis Patients

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Long non-coding RNAs (lncRNAs) exert essential functions via multiple mechanisms. There is emerging evidence that dysregulation of lncRNAs in immune cells might contribute to the pathogenesis of autoimmune diseases. The regulation and function of lncRNAs in rheumatoid arthritis (RA), a systemic autoimmune disease, remained largely unknown. Non-coding transcript in T cells (NTT) is a nuclear lncRNA first discovered in activated T cells. In this study, we also found NTT expression in monocytes and macrophages, and NTT was highly upregulated in peripheral blood mononuclear cells (PBMCs) of untreated RA patients. Further DNA Chromatin Immunoprecipitation (ChIP) assay revealed the binding of the transcription factor ATF3 on NTT promoter. Knockdown of ATF3 in THP-1 cell line decreased NTT expression. Moreover, knockdown of NTT in THP-1 showed markedly decreased expression of Prostate and Breast Cancer Overexpressed 1 (*PBOV1*), a gene in proximity with the chromosomal location of NTT. Both *ATF3* and *PBOV1* were found to be upregulated in PBMCs of untreated RA as compared with healthy controls. The level of *PBOV1* in untreated RA even reached 5000~50000 fold higher than controls. Our study suggests that ATF3 mediates NTT and subsequent *PBOV1* activation in RA PBMCs. NTT and *PBOV1* expression levels could be sensitive diagnostic markers for new-onset RA.

W.8. Identification of Naturally Processed Immunodominant Topoisomerase I Epitopes in Patients with Systemic Sclerosis

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A subset of patients with systemic sclerosis (SSc) have autoantibodies and CD4+ T cells specific for the autoantigen topoisomerase-I (Topo-I), which are associated with the presence, progression and severity of lung fibrosis. Identification of immunodominant Topo-I epitopes is critical for understanding disease pathogenesis and developing autoantigen-

specific diagnostic and therapeutic tools. Existing data are limited by the low precision of in silico epitope prediction and the poor sensitivity of autoantigen-specific T cell detection assays. Thus, we developed a novel method which couples the specificity of natural antigen processing to the sensitivity of CD154 upregulation for measuring antigen-specific T cell responses. After pulsing with whole Topo-I protein monocyte-derived dendritic cells obtained from 6 anti-Topo-I antibody positive SSc patients, HLA-DR/peptide complexes were isolated by immunoprecipitation. Bound peptides were eluted and identified by mass spectrometry. The 10 Topo-I peptides detected were located mainly in the core (III) and linker domain of the molecule. Peptide overlap among patients existed despite differences in their HLA-DR haplotype, with 8 out of 10 epitopes being presented by two or more subjects. All Topo-I peptides were able to stimulate significant CD154 upregulation by CD4+ T cells from at least one anti-Topo-I positive patient when compared to subsequently tested HLA-matched healthy controls(n=14). In conclusion, our approach allowed successful identification of naturally processed autoreactive Topo-I peptides and showed that a restricted set of immunodominant Topo-I epitopes is shared by SSc patients carrying diverse HLA-DR alleles.

W.9. Novel Candidates for Genetic Control of Collagen Induced Arthritis are Involved in Transcriptional Regulation of B-Cell Proliferation

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Introduction: Rheumatoid Arthritis (RA) is the most common autoimmune disease, caused by a complex interplay of genetic and environmental factors. This project aims to investigate the role of proteins encoded by four genes in a 2 Mega base-pair fragment on mouse chromosome 5 (*Eae39r*), identified in genetic studies of Collagen Induced Arthritis (CIA), an experimental model for RA.

Methods: CIA is induced in mice by immunization with collagen type II. The whole genomes of the parental mouse strains have been sequenced by NGS. Congenic and control mice have been characterized by flow cytometry, *in vitro* lymphocyte activation assays and transcript level studies. Breeding of lymphocyte-specific knock-out mice and pathway studies are currently underway.

Results: Our recent CIA experiments in *Eae39r* congenic, sub-congenic and control mice have shown that a sub-locus is controlling the severity of arthritis, in addition to antibody titers. Differential expression of the genes located in this sub-locus has been observed in spleens and specifically B cells of naïve *Eae39r* congenic and control mice that can be attributed to various regulatory variations identified by NGS. *In vitro* activation experiments have shown increased B cell proliferation in response to anti-IgM antibody stimulation in congenic mice as compared to littermate controls, along with down-regulation of these genes upon stimulation.

Conclusion: The genes located in the *Eae39r* fragment are identified to have a role in lymphocyte proliferation and arthritis development. We expect that one or more of these proteins are important for disease mechanisms in RA and can be developed as potential drug targets.

W.10. Molecular and Functional Characterisation of IL-17+ CD8+ T cells in Psoriatic Arthritis

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Psoriatic arthritis (PsA) is a spondyloarthropathy affecting the joint and skin. Our lab previously showed high frequencies of IL-17+ CD8+ T-cells (Tc17) in PsA synovial fluid (SF), which correlate with clinical, serological and imaging measures of disease activity, and thus may contribute to PsA pathogenesis. Our aim is to functionally and molecularly characterise Tc17 cells to understand their role in PsA.

Since the frequency of Tc17 cells in human peripheral blood (PB) is very low (0.22±0.05%, n=16), we investigated Tc17 induction *in vitro*. Addition of rhIL-1β and IL-23 to healthy PB CD8+ T-cells cultured with α-CD3/28 beads for 3 days, led to a modest but significant increase in IL-17+ CD8+ T-cells (0.95±0.29%, p=0.0078, n=8). Addition of rhIL-6 or IL-2 did not lead to a further increase. We optimised an IL-17 secretion assay to sort these IL-17-secreting cells. Successful sorting was validated by ELISA (n=2). Sorted IL-17+ CD8+ T-cells were cultured for 24 hours and their supernatants added to synovial tissue fibroblasts. Addition of Tc17 (or Th17) cell culture supernatants enhanced IL-6 and IL-8 production by PsA synovial tissue fibroblasts compared to their IL-17-negative counterparts (n=2). These data indicate that *in vitro* generated IL-17+ CD8+ T-cells can exert pro-inflammatory activity.

We are currently translating these findings by investigating the pro-inflammatory capacity of synovial Tc17 cells from PsA patients, and determining their molecular phenotype. These data will provide novel insights into the contribution of Tc17 cells to the immunopathology of PsA.

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W.11. Autologous Hematopoietic Stem Cell Transplantation: Evolution in Treatment of Scleroderma

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Introduction: Systemic sclerosis (SSc) is a multisystem autoimmune disease with heterogeneous clinical manifestations. Deeper knowledge of pathogenesis of SSc has initiated a new era in treatment with autologous hematopoietic stem cell transplantation (HSCT). It prevents and even reverses damage from autoimmune diseases. The first successful HSCT for SSc was reported in 1997. Thereafter several trials were conducted which revealed significant improvement in outcome.

Discussion: HSCT is a multistep process including mobilization of HSCs from bone marrow or blood, conditioning with immunoablative therapy and reinfusion of HSCs. Inclusion criteria are as follows: diffuse SSc with organ involvement, early-stage of disease < 5 years, mRSS >15, age < 65, lung disease with FVC or DLCO between 45-80% of predicted. Since 2001, three prospective, controlled trials have been conducted to evaluate efficacy, safety and long-term side effects of Autologous HSCT compared to cyclophosphamide. ASSIST: phase II trial reported significant improvement in HSCT group compared to control group. ASTIS: phase III trial revealed a better long-term event-free survival in HSCT group compared to control group although early treatment-related mortality was 10.1%. SCOT: phase III trial which applied total body irradiation and equine anti-thymocyte globulin in conditioning phase which make it different from two other studies. This study follows similar endpoints and is still ongoing.

Conclusion: SSc is an autoimmune disease with high level of mortality and morbidity. HSCT might bring new hopes for patients with diffuse SSc, but more investigations are necessary to answer which patient will get more benefit of HSCT and how to decrease treatment-related mortality.

Bone marrow or stem cell transplantation

F.21. Use of a Bispecific anti-CD86 X monomeric-IL-10 ADAPTIRTM Molecule to Induce Alloantigen-specific Tr1 Cells

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IL-10 is a potent immunosuppressive cytokine that promotes the differentiation of tolerogenic dendritic cells (DC-10) and the induction of alloantigen-specific T regulatory type 1 (Tr1) cells *in vitro*. Use of IL-10 *in vivo* is limited by its effects on multiple cell types that are not solely inhibitory. ES210 is a bi-specific ADAPTIR molecule from Aptevo Therapeutics composed of an anti-CD86 fused with monomeric IL-10 that specifically induces IL-10R signaling in $CD86^+$ antigen-presenting cells. We tested whether ES210 could replace soluble-IL-10 during induction of tolerogenic DCs and alloantigen-specific Tr1 cells *in vitro*.

DC-10 and alloantigen-specific Tr1 cells (T-allo10) were differentiated in the presence of IL-10 as previously reported, or in the presence of ES210 (DC-ES210 and T-alloES210, respectively; n=12). DC-ES210 cytokine production was assessed by ELISA. Presence of $LAG3^+CD49b^+$ Tr1 cells was investigated in T-allo10/T-alloES210, together with alloantigen-specific proliferation, cytokine production and suppression of $CD4^+$ proliferation.

ES210 induced differentiation of tolerogenic DC that produced high levels of IL-10. DC-ES210 induced similar percentages of $LAG3^+CD49b^+$ Tr1 cells compared to DC-10. T-ES210 cell cultures presented alloantigen-specific anergy, high IL-10/IFN-γ production ratio and high suppressive properties comparable to those of T-allo10. Alloantigen-specific anergy of T-allo10 and T-alloES210 was maintained upon exposure to inflammatory cytokines.

In conclusion, ES210 induces differentiation of tolerogenic DC and functional alloantigen-specific Tr1 cells *in vitro*. As ES210 blocks T-cell expansion in a murine graft-versus-host model, our findings suggest this could be mediated by Tr1 cells. ES210 holds promise as a therapeutic agent *in vivo* in the context of immune-mediated diseases.

F.22. Rebooting Autoimmunity After Autologous Hematopoietic Stem Cell Transplantation in Systemic Sclerosis Patients by Newly-generated Regulatory T- and B-cells

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Autologous Hematopoietic Stem Cell Transplantation (AHSCT) is more effective to treat severe systemic sclerosis (SSc) than conventional immunosuppression (IS). However, its therapeutic mechanisms still need to be understood. Thirty-one transplanted and sixteen IS-treated SSc patients had PBMCs collected before and semiannually until 36 months after inclusion. Immune reconstitution analyses evidenced higher thymic function than baseline at 24 months as measured by β- and signal-joint (sj)-TCR excision circles (sjTREC) RT-qPCR, correlating with $CD3^+CD4^+CD31^+CD45RA^+$ recent thymic emigrants exportation. The TCR new generation sequencing (NGS) depicted higher TCR diversity from 1-2 years post-transplant. Additionally, at 6 months post-AHSCT, increased numbers of $CD8^+CD28^-CD57^+$ exhausted T-cells correlated with reduced telomere T/S ratio and reduced TCR diversity. $CD4^+CD25^{hi}FoxP3^+(GITR^+/CTLA\text{-}4^+)$ regulatory T-cells (Tregs) increased at 12 months post-transplantation, correlating with sjTREC values, having higher CD45RA expression and IL-10 production after anti-CD3/CD28 stimulation than baseline. Bone marrow output of naïve B-cells, as quantified by coding-joint (Cj) and sj-kappa-deleting recombination excision circles (sjKREC) RT-qPCR, increased from 12-36 months post-AHSCT, resulting in reduced B-cell division (N=LOG(Cj/sjKREC)/LOG2) and persistent increase of $CD19^+CD27^-IgD^+$ naïve and $CD19^+CD38^{low}IgD^+$ Bm2 B-cell counts. Finally, $CD19^+CD24^{hi}CD38^{hi}$ regulatory B-cell (Breg) counts increased

from 6-12 months post-AHSCT, correlating with sjKREC values and presenting higher IL-10 production after CpG±CD40L stimulation than baseline. Skin biopsies presented higher IL-10 expression at 6-12 months post-transplantation. Six transplanted patients relapsed, presenting lower FoxP3, GITR and CTLA-4 expressions, reduced Breg counts and lower TCR diversity. Our results suggest that increased counts of newly-generated regulatory B- and T-cells post-AHSCT are associated with clinical improvement in SSc patients.

F.23. Clonal Deletion and Anergy Play Dominant Role to Achieve Immune Tolerance After Reduced Intensity Unrelated Donor Cord Blood Transplantation (UCBT)

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The mechanism of immune tolerance after successful hematopoietic stem cell transplantation is not fully understood. Both central (clonal deletion) and peripheral (anergy,Treg,Tr1) mechanisms are conceivable and may even coexist. In this study, we set up serial experiments to test for the presence and relative contribution of these mechanisms after HLA-mismatched UCBT in the GvH direction.

Nine patients (age1m to9y) were transplanted and enrolled on a clinical trial (NCT01852370). Five immunocompetent patients were off immunosuppression therapy (IST) with no GVHD, while 4 had either limited or recently resolved GvHD and were still on IST when studied. Donor T cell chimerism was a median 97%. Purified T cells responses to host APCs were measured by MLR (3HTdR) and CTL reaction, along with cytokine secretion profiling (Bioplex). Host-reactive T cell clones were tracked with TCRVβ repertoire by ImmunoSEQ. In tolerant patients, there was no significant MLR or CTL response towards host APC. Similarly, cytokine profiles revealed non-responsiveness while T cells responded vigorously to 3rd party APC by all assays. Recipient-specific T cell clones that were identified from infused UCB stimulated by host APC pre-UCBT became undetectable. There was no indication of Treg or Tr1 cell involvement in sustaining tolerance as deletion by IL-2 immunotoxin or IL-10R blockade was unable to "break" tolerance. However, low dose IL-2 was able to restore some proliferation (S.I.=19;p=0.05) and IL-13,IFNγ,TNFα secretion, but in lesser degree in comparison to 3rd party responses (S.I.=112).

In summary, clonal deletion and anergy contribute to immune tolerance after UCBT with deletion being dominant.

Cytokines/chemokines

F.25. Expression and Regulation of Amphiregulin in Human Regulatory T Cells

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Regulatory T cells are essential for maintaining immune homeostasis and self-tolerance. Several lines of evidence suggest that adoptive transfer of Tregs can prevent, and in some cases cure, a variety of pathological conditions, from autoimmunity to transplant rejection. In addition to their effects on immune cells, there is also emerging evidence that Tregs have direct effects on tissue repair. Specifically, Tregs in mice promote tissue repair after infection or injury by producing the EGF family member amphiregulin (AREG) under the control of the alarmins IL-18 and IL-33. We investigated expression and regulation of AREG in human peripheral blood Tregs. TCR stimulation of Tregs ($CD4^+CD25^{hi}CD127^{lo}$) increased AREG mRNA and protein, particularly in the HLA-DR^- subset, but levels were lower than

in their Tconv counterparts. In contrast to reports from murine Tregs, IL-18 and IL-33 did not modulate human Treg production of AREG. *Ex vivo* Tregs expressed IL-18Rα, but expression of IL-33Rα (ST2) was not detectable. To more accurately measure human ST2 expression, we used phage display to generate a series of anti-ST2 mAbs. Experiments in ST2-transfected HEK-293T and transduced $CD4^+$ T cells revealed several candidate anti-ST2 mAbs that were superior to commercially available mAbs for flow cytometric detection of human ST2. Experiments to better define the localization and biology of human $ST2^+$ Tregs are in progress. Knowledge of whether human Tregs produce AREG is important to understand their potential to mediate tissue repair in addition to immunosuppression when used as a cell-based therapy.

F.26. Chemotaxis of Terminally Differentiated CD8+ cells (Temra) to the Sites of Sterile Infection: IL8-Receptors as an Answer?

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Terminally Differentiated Effector T cells (Temra) are defined as $CD3^+4/8^+45RA^+62L^-CCR7^-$ cells with poor proliferative capacity and contradictory functions, from exhausted cells to highly activated effector T cells (Teff). Recently, we revealed that enhanced levels of $CD8^+$TEMRA are associated with delayed healing of bone fractures. Interestingly, the Temra accumulate in the fracture hematoma and release inflammatory mediators, which inhibit mesenchymal stem/progenitor cell differentiation into osteoblasts (Reinke et al. STM 2013). Similar evidence on the impact of Temra have been observed in other diseases. As Temra seem to be a heterogenous population and to better understand which subsets are involved, we started deeper analyses of Temra. After performing screening of >250 different surface molecules of 9 donors (CD-Screen), we could find out, that a subgroup of Temra specifically express IL8-Receptors and assumed it to be a chemotactical mechanism of Temra wandering to the sites of the sterile infection (*e.g.* bone fracture). The analysis of Temra by using a multiparameter panel *via* Masscytometry (CyTOF) showed simultaneous expression of some other interesting markers on this IL8R+ subpopulation of Temra (activation molecules, NK-cell markers *etc.*). Based on the identified phenotypic Temra subset, we have implemented the subset analysis into the clinical biomarker studies, comparing corresponding blood and hematoma samples. Moreover, sorting of the subsets and analysis of their functional and molecular properties as well as TCR repertoire are ongoing.

F.27. Lupus Skin Is Characterized by a Robust Type I Interferon Signature Driven in Part by Epidermal Production of Interferon Kappa and Skewed Interferon Responses in Lupus Keratinocytes

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Cutaneous lupus erythematosus (CLE) rashes are disfiguring and often refractory to usual lupus therapies. A prominent and pathogenic feature of CLE is robust upregulation of type I interferon (IFN)-regulated genes, yet the drivers of this signature are poorly understood in the skin. Using microarray analysis of 90 CLE lesions, we confirmed a prominent type I IFN signature for both discoid and subacute cutaneous lupus characterized by a transcriptional network surrounding *STAT1* overexpression. To determine which type I IFNs were driving this IFN signature, we analyzed expression of all type I IFN genes in CLE lesional and control skin. Only two type I IFN transcripts were upregulated in CLE: IFNα10, presumably produced by plasmacytoid dendritic cells recruited to the dermis, and IFNκ, a keratinocyte-produced type I IFN. Immunofluorescence identified IFNκ as robustly produced in CLE epithelium compared to control skin. Both IFNα and IFNκ induced expression of IFN signature genes (*MX1*, *OAS1*, *IRF7*) in control and CLE keratinocytes. However, evaluation of genome-wide response to type I IFNs via RNA sequencing identified a distinct population of transcripts that were highly upregulated in CLE vs. control keratinocytes. Comparison of this unique mRNA population with CLE lesional microarray data identified the majority of CLE-IFN-skewed keratinocyte genes as also altered in CLE lesions. These data support a role for the epidermis in both production of and abnormal response to type I IFNs in CLE and provide a mechanism for the promising effects of type I IFN receptor blockade for CLE.

F.28. CD28 Co-Stimulation Negatively Regulates Differentiation of Human but Not Mouse Th17 Cells

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Th17 cells are important for protection from extracellular bacterial and fungal pathogens as well as homeostasis of commensal microbes. However, Th17 cells are best known for driving autoimmune diseases, and anti-IL-17 is now approved for therapy of psoriasis and ankylosing spondylitis. Generation of *in vitro* Th17 cells requires multiple inducing cytokines, and has been notoriously difficult for human T cells. CD28 is a critical costimulatory molecule for T-cell activation and is routinely added with anti-CD3 during Th17 differentiation. Our data found that CD28 co-stimulation suppressed Th17 cells in a dose-dependent manner, as detected by IL-17 and RORγt. Although activation of T cells with anti-CD3 alone resulted in very few $CD45RO^+$ activated T cells, addition of Th17-inducing cytokines overcame the deficit. Preliminary results indicate that Th17 cells generated in absence of CD28 are not anergic and maintain their Th17 phenotype. We are also investigating molecular mechanisms by which CD28 suppressed Th17 differentiation in human T cells. In contrast to human, addition of anti-CD28 to mouse Th17 cultures resulted in enhanced responses, fitting with known differences in CD28 costimulation effects between mouse and human. Together, these data provide new insight into mechanisms that regulate the generation of human Th17 cells, and have implications for approaches to target Th17 cells in autoimmune disease.

Diabetes and other autoimmune endocrine diseases

F.86. Using Antigen Specific Chimeric Antigen Receptor [CAR]-Redirected T Cells to Remodel Immune Balance in Type 1 Diabetes

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Previously we showed that an insulin B:9-23 register 3 specific monoclonal antibody (mAb287) delayed spontaneous type 1 diabetes (T1D) when administrated to NOD mice. Of note, mAb287 delayed progression to overt hyperglycemia even when administrated at a late pre-diabetic stage. Efficacy was associated with a decrease in islet infiltration of lymphocytes suggesting that mAb287 is acting to delete and/or reprogram antigen presenting cells expressing the cognate peptide:MHC complex. The recent successes of chimeric antigen receptor (CAR)-redirected CD8 T cells in cancer immunotherapy prompted us to investigate whether the efficacy of this monoclonal antibody therapy could be enhanced by delivery in a cellular format. Accordingly, we now describe our results using mAb287-CAR CD8 T cells and control CAR-T cells.

Using our optimized protocol, up to 35% of activated CD8 T cells can be transduced with mAb287-CAR retroviruses. *In vitro* stimulation experiments confirm that mAb287-CAR-CD8 cells maintain the antigen binding specificity of the parent antibody, secreting IFNg in a dose dependent manner in response to insulin B:9-23(R3)/I-A^{g7} complexes, but not controls. Similarly, mAb287-CAR-CD8 T cells delete antigen presenting cells with the target antigen on their surface *in vitro.* Following adoptive transfer to young NOD mice GFP expressing mAb287-CAR-CD8 T cells could be detected by flow cytometry at least 2 weeks post-transfer in the spleens and lymph nodes. Recipient animals showed no evidence of acute toxicity following transfer. We propose that the antigen specific mAb redirected CAR T cells may be a safe immune therapy to arrest the progression of autoimmune diabetes.

F.87. Type 1 Diabetes Is an Immunologically Heterogeneous Disease: Findings from a Longitudinal Study of CD4 and CD8 Cell Autoimmune Variation

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Brodin and Davis[1] recently reported that the variability of immune cell populations in healthy humans varies mostly between subjects, and levels within subjects remain relatively stable over time. We sought to investigate these results in a sample of 39 Type 1 Diabetes (T1D) patients. Variability of CD4 and CD8 T cell populations was assessed via random effects analysis to quantify the extent of between subject variation relative to within subject fluctuations of the cell population measurement. CD8 naïve, stem cell memory, central memory, transitional memory, effector memory, pre-terminal and terminal effector cell populations and CD4 naïve, stem cell memory, central memory, effector memory, and effector cell populations were included in analysis as fractions of the total CD4 and CD8 populations. Standard deviations measuring within-subject variability ranged from 0.21 to 5.77, and between-subject variability from 1.45 to 25.00. Percent variability from within-subject differences ranged from 2.83% to 35.08%, and between-subject from 64.92% to 97.17%. Thus, while the within-subject variability can be appreciable, the variation between subjects is typically much greater. We conclude that our findings in T1D patients are in general agreement with Brodin and Davis. However, the question remains whether this heterogeneity between subjects is linked to individual differences or reflective of different immunopathological processes (endotypes) as defined by Arif et. al[2]. We suggest that research should be conducted to both delineate endotypes and then identify subject-level correlates of this heterogeneous disease process in the hopes of improving our understanding and treatment approaches to this disease.

F.88. Altered IL-2 and IL-7 Networks in T1D are Revealed Through Systems Analyses

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Cellular phenotypes associated with T1D include multiple cell types which together, may offer clues to pathogenesis and guide therapeutic choices. Defining and ranking these phenotypes has been challenging due to patient heterogeneity, cellular variability, and limited global cellular analyses. Thus, we designed a large (n=100/group), age and gender matched, cross-sectional study of control and T1D subjects including approximately 300 reproducible (intra-assay CV; median 0.13) parameters of B and T cells using 3 steady-state and 4 cytokine stimulation flow cytometry panels. T cell activation (HLA-DR, CD25, CXCR3) and early B cell markers including IgD were most prominent in single parameter analyses between controls and T1D after applying multiple testing correction. To understand differing relationships between parameters, we correlated all pairs of parameters within cohorts and then compared these correlations between T1D and controls, revealing networks of significantly altered relationships. Prevalent altered relationships occurred between cell types, not within cell types, and were enriched for CCR4, cytokine responses, and early B-cell maturation markers. Using these same correlations to build networks between multiple parameters, control and T1D networks exhibited altered central parameters. IL-2 and IL-7 response and early B cell markers differed the most, with T1D networks being less connected indicating broad defects in these pathways. Together, our findings move beyond single parameter analyses of disease, offering a more comprehensive understanding of the phenotypes associated with T1D. Understanding these altered relationships between immune parameters has implications for selection and stratification of subjects for treatment of T1D with immune-mediated therapies.

F.89. T Cell Library Assay Identified Functional ZnT8-Specific Memory CD8+ T Cells in Patients with Type 1 Diabetes

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Recent studies identified pancreatic islet antigen-specific $CD8^+$ T cells in patients with type 1 diabetes (T1D) and healthy subjects with comparable frequencies, however the difference in the function was not fully understood. Other groups have used T cell library to assess $CD4^+$ T cell response to pathogens or myelin autoantigens, but $CD8^+$ T cell libraries have not been previously studied. We generated more than 8,000 $CD8^+$ T cell libraries from PBMCs of 17 patients and 9 healthy subjects and studied its reactivity against pooled islet antigen peptides including preproinsulin, IGRP, IA-2, and ZnT8. The frequency of positive libraries responding to the islet antigens with robust IFNg production was significantly higher in the memory $CD8^+$ fraction of T1D patients (3.83%) compared to healthy subjects (1.02%). The estimated ratio of these cells in the patients was 0.0019%. It declined over time after onset of disease (R^2=0.2365, p=0.0335). In addition to IFNg, these cells produced higher levels of proinflammatory cytokines including TNF-a and IL-6. Among 17 positive libraries from 3 patients were further analyzed for reactivity to antigenic peptides and all the libraries reacted with $ZnT8_{186\text{-}194}$ peptide suggesting that this is a major islet antigen recognized by $CD8^+$ T cells. TCR sequencing analysis on the libraries revealed that there is no or minimum shared clonality of ZnT8-speicific TCRs among the patients. These results imply that the library assay is useful for analyzing the islet antigen specific-$CD8^+$ T cells, which allows us to achieve immunological measures to evaluate the progression and intervention of disease.

T.70. Jurkats and Type 1 Diabetes: TCR and Receptor for Advanced Glycation Endproducts Signaling

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The receptor for advanced glycation endproducts (RAGE) may be found in adaptive immune cells but its role is not understood. Patients with type 1 diabetes (T1D) express increased levels of intracellular RAGE in T cells, compared to healthy controls, as do euglycemic at-risk relatives who progress to disease. We studied gene expression by nanostring in T cells from type 1 diabetics and found differences in pathways utilized by RAGE+ versus RAGE- T cells including those affecting apoptosis and survival via TNFs/NF-κB/Bcl-2 proteins, IL-12-induced IFN-γ production, and PDGF signaling through STATs and NF-κB proteins.

We studied RAGE signaling in Jurkat cells. We silenced intracellular RAGE with pooled siRNAs causing a 75% reduction in RAGE by flow cytometry FITC mean fluorescence index. Using knockdowns, we found T cell receptor (TCR) signaling interference. Western blots of RAGE siRNA transfected Jurkats showed decreased baseline levels of Zap70. There was decreased signal amplitude of phosphorylated Erk 1/2 with RAGE siRNA. The addition of high mobility group box 1 protein (HMGB1), a known RAGE ligand, increased the amplitude of phosphorylated Erk 1/2 signal in submaximally stimulated Jurkats transfected with negative control siRNA but not with RAGE silencing. RAGE siRNA did not affect CD3 surface expression.

RAGE stimulation may enhance TCR signal in T cells. Through the release of multiple ligands during inflammation this mechanisms may facilitate survival and activation of T cells.

T.71. Ultrasensitive Assays for Detection of Type 1 Diabetes (T1D) Autoimmune Reactivities

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In an ongoing study to assess the feasibility of developing multiplexed immunoassay panels for detection of organ-specific autoimmune biomarkers, MSD developed assays for the detection of T1D biomarkers. T1D affects approximately 0.3% of Americans and has a rapidly rising incidence. Autoimmune response markers of T1D can be detected prior to disease

onset, serving as important tools for T1D research. MSD® MULTI-ARRAY technology was used to develop a first-generation multiplexed assay to detect key T1D autoimmune response markers, namely, autoantibodies to glutamic acid decarboxylase (GADA), insulinoma 2 (IA2A), and insulin (IAA). Using assay proficiency evaluation samples from the Islet Autoantibody Standardization Program, these assays performed comparably to existing assays, with the advantages of low sample volume ((< 50 µL), high throughput, and no radioactivity. In the current study, next generation assays were developed using MSD's ultrasensitive assay format to detect the T1D-relevant reactivities IAA, GADA, and IA2A. Preliminary data show assay sensitivities/specificities improve from 60%/100% to 90%/89% (IAA detection), 65%/100% to 70%/100% (GADA detection), and 35%/100% to 50%/100% (IA2A detection). Furthermore, a new ultrasensitive assay was developed for detection of another key biomarker, ZnT8 autoantibody, that exhibits preliminary sensitivity and specificity of 35% and 89%, respectively. The ultrasensitive MSD assay format significantly improves the ability to detect serum samples with multiple T1D reactivities.

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T.72. The Effects of Oral Insulin in Individuals at Risk of Type 1 Diabetes; A TrialNet Randomized Clinical Trial

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We screened 138,385 relatives of individuals with type 1 diabetes for diabetes autoantibodies. 3,583 were identified as insulin autoantibody (mIAA) positive. Of these, 581 with insulin autoantibodies (miAA) and either ICA or GAD and IA-2 were placed into one of 4 stratum according to antibodies and first phase insulin release determined from intravenous glucose tolerance testing. All subjects had normal glucose tolerance at entry and therefore at stage 1 of diabetes. Subjects were randomized to treatment with 7.5 mg oral insulin daily or placebo and followed for development of stage 3 diabetes (clinical diabetes onset). A separate mechanistic study has randomized 92 individuals with mIAA and at least one other antibody to 6 months of either 67.5 mg daily oral insulin or 500 mg every other week to examine safety of higher doses and whether changes in antibodies or CD8 T cells can be detected. Results: This is the largest placebo control randomized trial ever conducted in those with Stage 1 of diabetes, with up to 10 years of follow-up (median 2.6 years). The median age at enrollment was 8.2 years. The first subject was randomized in 2007 and the last in December 2015. Results of the clinical trial will be presented at the ADA meeting, June 12, 2017 and are embargoed until then. At FOCIS, this and additional data will be available from the primary trial and the first 40 subjects of the companion mechanistic study.

T.73. Immunological and β Cell Changes in Response to Checkpoint Inhibition

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Autoimmune diabetes arises from destruction of pancreatic β cells by infiltrating autoreactive cytotoxic T cells. Cancer patients treated with checkpoint inhibitors can rapidly develop diabetes. While diabetes has been reported with monoclonal antibodies (mAbs) targeting the PD-1/PD-L1 axis, it has not been reported with anti-CTLA-4 mAbs alone. In addition, there is little understanding about why some individuals treated with these drugs develop diabetes. We hypothesized that the response of β cells to immune stressors may be responsible for the loss of tolerance with PD-1/PD-L1 but not CTLA-4 antagonists. Allogenic human islets were cultured with PBMCs from healthy donors in the presence of PD1 or CTLA4 mAbs or control immunoglobulin. In islet-PBMC cocultures treated with anti-PD-1, but not anti-CTLA-4 mAb, we observed increases in IL-2, IL-17, IL-13, and IFNγ. β cells from islets treated with IFNγ alone demonstrated increased expression of PD-L1, but not CD80 or CD86, the CTLA-4 ligands. We observed similar findings in β cells from NOD mice during progression of autoimmune diabetes. Although preliminary CyTOF analysis showed no difference in the frequency of β cell

antigen specific CD8 T cells by tetramer staining between patients treated with PD-1/PD-L1 antagonists who did and did not develop diabetes (N=3 each group), tetramer positive CD8 T cells in diabetics had higher levels of CD27 (p=0.006). We conclude that PD-L1 is induced on β cells with inflammatory mediators, possibly as a protective response. In those who develop diabetes, blockade of this inhibitory ligand may lead to activation of diabetogenic CD8+ T cells.

T.74. Transfer of Type 1 Diabetes by Bone Marrow Transplantation in Humans: Rare Clinical Cases of a Great Potential for Understanding Disease Pathogenesis

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In the early 1990s, a few cases of Type 1 Diabetes (T1D) transfer after allogeneic bone marrow transplantation (BMT) between HLA-identical siblings were reported as case reports but the mechanism responsible for this transfer has never been investigated. We believe that these "accidental experiments" provide the ideal clinical setting to gain insights into T1D pathogenesis.
Two cases of T1D transfer after BMT between HLA-identical adult and paediatric siblings occurred at our Hospital. The adult bone marrow recipient became diabetic 9 years post-transplant while the seven-year-old boy seroconverted one year after transplantation and he is currently T1D-free but 4 T1D-autoAb positive.
To dissect the mechanism of disease transfer we tested whether the same autoAg-specific T cells were present both in the donor and the recipient. Proliferative responses to T1D-associated autoantigens (GAD65 and pro-insulin) were measured by the CFSE dilution assay. The same frequencies of autoreactive T cells proliferating to GAD65 and pro-insulin were present both in donor and recipient while the frequencies of T cells to the recall Ag Tetanus Toxoid were different. Autoreactive T-cell clones were generated and current studies are testing the presence of shared TCR among the donors and the recipients. These rare clinical cases represent a unique opportunity to test the hypothesis that transfer of pathogenic T cells in humans can cause pancreas destruction in the absence of a diabetogenic-specific environmental trigger.

T.75. Interim analysis of UST1D: A pilot clinical trial of ustekinumab in recent-onset Type 1 Diabetes Mellitus.

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Preclinical studies suggest that blockade of pro-inflammatory T cells that secrete IL-17/IFN-γ may suppress the T1D auto-inflammatory response. We assessed the safety and optimal dosing of ustekinumab (a monoclonal antibody targeting the IL-17/IFN-γ pathway) for the treatment of adult recent-onset T1D in a phase I/II open-label clinical trial (NCT02117765).

We enrolled 10 patients within 100 days of T1D diagnosis, aged 18-35 years, and with a peak C-peptide of 0.2nmol/l or greater on MMTT. Subjects received either 45mg or 90mg ustekinumab every 3 months. The primary endpoint was safety (rate, frequency and severity of adverse events). We also measured the baseline-adjusted change in 2-h AUC C-peptide response to MMTT at 1 year and performed flow cytometric analyses before and after study drug administration to assess any change in T helper cell subsets.

Ustekinumab-treated patients had a total of 10 adverse events (1/10 was possibly attributed to study drug). At 1 year, the 90mg cohort had a mean reduction in C-peptide AUC of 0.1pmol/mL and after one dose of study drug demonstrated a mean 50% reduction in peripherally circulating Th17.1 cells. The 45mg cohort had a mean C-peptide AUC reduction of 0.26pmol/ml and no changes in Th17.1 cells were observed.

Our study confirms the safety of ustekinumab in the context T1D and indicates that a higher 90mg dosing regime is optimal. This pilot provides a rationale to proceed to a phase II/III study to definitively test the efficacy of ustekinumab in preserving endogenous insulin secretion in children with recent-onset T1D.

T.76. Comprehensive Immunophenotyping Identifies Novel Subset Associations with Age and Type 1 Diabetes Status

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OBJECTIVES: The goal of this project is to provide a detailed description of immune subsets from a cohort of T1D affected and unaffected donors and to assess the impact of age and disease status on circulating immunophenotypes. We hypothesize that establishing normal immunophenotypic baselines will facilitate biomarker identification.

METHODS: Blood donations were collected from a cohort of controls (n=164, age=26.7±11.2), relatives (n=342, age=31.8±16.0), and T1D patients (n=204, age=21.1±12.3). Whole blood was surface stained with six fluorescent antibody panels and complete blood counts (CBC) were measured, allowing for detailed adaptive and innate immune subset classification. In total, 351 percentage, mfi, CBC, and absolute count parameters were collected. Elastic net logistic regression analyses were used to predict the age and clinical statuses of donors using these immunophenotypes as covariates.

RESULTS: Circulating populations of immune cells were highly dynamic under age 30 and generally stable after age 40. In addition to the expansion of memory and contraction of naïve T cell populations, we also observed dramatic shifts in naïve $CD4^+CXCR3^+$, naïve $CD4^+CXCR5^{lo}$, $CD8^+CXCR5^{lo}$ Tem, and $CD4^+PD1^+$ Tcm subsets. Moreover, we were able to accurately predict donor age based on immunophenotypic parameters (predicted vs actual age Pearson r=0.997 for controls, r=0.978 for relatives, r=0.938 for T1D). Finally, several phenotypic differences were found in patients compared to age-matched controls, including increased naïve $CD8^+CXCR3^+$ ($p+CD185^+$ ($p< 0.01$), and decreased $CD8^+CD279^+$ Tcm ($p < 0.01$). Herein, we have constructed a comprehensive description of the immune repertoire and have identified several putative T1D biomarkers.

T.77. Selective Expansion of PD-1+TIGIT+ CD4 T Cells Following Depletion of CD2hi Cells with Alefacept in Recent Onset T1D

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In an ITN clinical trial, alefacept (an LFA3-Fc fusion protein targeting CD2) preserved endogenous insulin production in recent onset T1D a year following therapy cessation. Since alefacept may have deletional and agonistic effects on the immune system, we assessed lymphoid phenotypic and functional changes with therapy and developed an *in vitro* model mimicking alefacept mechanism of action. Immunological changes observed during treatment included 63-73 % depletion of CD2hi CD4 and CD8 memory T cells and CD56hi NK cells, preservation of Tregs, and 26-33% increases in PD-1+TIGIT+CD4 memory T cells in peripheral blood following treatment. PD1+TIGIT+ CD4 memory T cells failed to express CD57 and KLRG1, suggestive of an anergic/exhausted phenotype. Culture of alefacept with PBMCs from T1D subjects replicated depletion of CD2hi memory T cells and CD56hi NK cells including CD2hiPD1+ cells. In the same cultures, there was concomitant activation of CD56loCD16hi NK cells that correlated with a significant reduction of CD2hiCD4 effector memory cells and an increase in CD2loPD1+TIGIT+ memory CD4 T cells. To test the hypothesis that alefacept increases CD2loPD-1+TIGIT+ CD4 T cells in an agonistic manner, we performed mix-back experiments and found a 48% increase in PD-1+TIGIT+ cells in CD2loCD4 effector memory T cells. Together, these data suggest that alefacept may function through deletion of CD2hi cells and selective expansion of CD2loPD-1+TIGIT+CD4 T cells. Understanding the possible

role of therapy-induced PD-1+TIGIT+ CD4 T cells, and factors that maintain their functional properties associated with hyporesponsiveness may help explain the success of alefacept therapy.

T.78. A novel Population of **β** Cells That Resist Immunological Attack Develop During Progression of Autoimmune Diabetes in NOD Mice

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Type 1 diabetes (T1D) is a chronic autoimmune disease that involves immune mediated destruction of β cells. How β cells respond to immune attack is unknown. We identified a population of β cells during the progression of T1D in non-obese diabetic (NOD) mice that survives immune attack. This population develops from normal β cells confronted with islet infiltrates. Pathways involving cell movement, growth and proliferation, immune responses, and cell death and survival are activated in these cells. There is reduced expression of β cell identity genes and diabetes antigens and increased immune inhibitory markers and stemness genes. This new subpopulation is resistant to killing when diabetes is precipitated with cyclosphosphamide. Human β cells show similar changes when cultured with immune cells. These changes may account for the chronicity of the disease and the long-term survival of β cells in some patients.

T.79. Improving Efficacy of Tolerogenic DNA Vaccines for Type 1 diabetes

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Type 1 diabetes (T1D) is a beta cell-targeted autoimmune disease mediated by CD4 and CD8 T cells. Antigen-specific therapies (ASTs) aim to restore T cell tolerance by blocking or reprograming the response of diabetogenic T cells. However, various attempts at applying ASTs to treat T1D in humans have failed, likely due to lim ited antigen representation and inability to target neoantigen-specific T cells. DNA vaccines offer flexibility in expressing epitopes from multiple antigens as well as neoantigen-emulating mimotopes, but DNA-based endogenous antigen delivery primarily target CD8 T cells. Targeting of both CD4 and CD8 T cells across a broader range of antigen specificities will likely be required to reestablish tolerance fully and durably. Thus, we developed DNA constructs encoding major epitopes and mimotopes for several beta cell antigens, whereby groups of epitopes are differentially targeted within transfected cells for efficient and simultaneous engagement of CD4 and CD8 T cells, or alternatively secreted for acquisition and presentation by other antigen-presenting cells. Shortly after a single administration of these DNA vaccines *in vivo*, responses by multiple antigen-specific CD4 and CD8 T cells (adoptively transferred and/or identified by MHC tetramers) were evident in the draining lymph nodes (especially with the secreted antigens) and distinct from the response to endogenous antigens in pancreatic lymph nodes. This response included the induction of antigen-specific Foxp3+ CD4 T cells, suggestive of a tolerogenic outcome. Our studies suggest that pDNA tolerizing vaccination can efficiently target diabetogenic CD4 and CD8 T cells and enhance protection against T1D

T.80. Helios and CD127 Expression in CD8 Central Memory Cells are Associated with Disease Progression in T1D

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Residual C-peptide in Type 1 diabetes (T1D) indicates the persistence of beta cell function and insulin production and is a key therapeutic goal in the treatment of T1D. T cells are known to be involved in T1D pathogenesis. Thus, we designed a study to test T cell immune correlates of residual C-peptide using a cross-sectional cohort of T1D patients with varying levels of disease progression; slow progressors (n=33) maintained at least 0.1ng/mL of C-peptide, whereas rapid progressors (n=32) had C-peptide levels less than 0.1ng/mL within 5 years of diagnosis. We characterized PBMCs using multicolor flow cytometry and then compared T cell populations with clinical data. Compared to rapid progressors, slow progressors had significantly increased CD127 expression on CD8 central memory (CM) cells and decreased Helios expression on CD8 effector memory RA cells. As a continuous variable, higher C-peptide levels correlated with diminished total CD8 CM cells, and decreased Helios and CD28 expression within CD8 CM. Increased CD127 expression on CD8 CM cells was also observed in patients diagnosed at a later age. In an independent set of T1D samples analyzed by mass cytometry, islet-specific CD8 T cells were marked by increased CD127 expression compared to virus-reactive cells. Together, these observations suggest that CD127 and Helios on CD8 memory subsets may be an immune correlate of disease progression, and suggest involvement of a chronic activation phenotype in CD8 CM cells with more aggressive disease. Future studies will validate these findings and address the associations of this phenotype with genetic risk profile.

T.81. T cell Recognition of Hybrid Insulin Peptides in Human Subjects with Type 1 Diabetes

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T cell clones isolated from the pancreatic infiltrates of nonobese diabetic (NOD) mice have been shown to recognize epitopes formed by covalent cross-linking of proinsulin peptides to other peptides present in secretory granules. Formation of such hybrid insulin peptides (HIPs) has been confirmed through mass spectrometry. In addition, responses to HIPs were recently observed among CD4+ T cells isolated from the pancreatic islets of two organ donors who had T1D. However, unanswered questions remain about the prevalence of HIP-specific T cells in human subjects, the diversity of hybrid sequences they recognize, and their role in human disease. Here we demonstrate that HIP-specific CD4+ T cells can be readily detected in the peripheral blood of patients with T1D through direct *ex vivo* analysis with HLA class II tetramers. T cell clones isolated from these subjects typically recognized HIP peptide with high affinity. Relevant HIPs discovered in the NOD are restricted by I-Ag7, implying that in human subjects HIPs might be recognized primarily in the context of DQ8. However, we identified six novel HIP sequences that are recognized in the context of DRB1*04:01. Interestingly, these diverse HIPs are derived from the insulin A chain, B chain, and C peptide covalently linked to one another (intra-antigen HIPs) or to other secretory granule proteins (inter-antigen HIPs). These results support the relevance of HIPs in human disease and may help to establish a new mechanism that contributes to the loss of peripheral tolerance in T1D.

T.82. Broad Repertoire of Autoreactive T Cells from the Islets of Donors with Type 1 Diabetes

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By necessity, most of our knowledge concerning islet-autoreactive T cells comes from the peripheral blood of individuals with type 1 diabetes (T1D). A significant knowledge gap exists in our understanding of the repertoire of islet-infiltrating,

islet-autoreactive T cells. From isolated, handpicked islets from pancreata of 12 donors (5 month-20 year disease duration) with T1D,we isolated, by direct growth from islets or by FACS-sorting of T cells from enzyme-dispersed islets, 300 T cell clones/lines, 5 T cell lines from 1/7 donors without T1D, and no lines from 2 donors with type 2 diabetes. To date, in this ongoing project, from 50 islet-derived T cell lines or clones have been examined and nineteen CD4+ T cell lines/clones were found to be reactive with a broad range of islet-derived peptides. Thirteen lines/clones reactive were reactive with known peptide targets and 6 lines/clones were reactive with post-translationally modified islet peptides. All autoreactive T cell lines secreted pro-inflammatory cytokines. The remaining T cell lines/clones await determination of reactivity: this includes 30 CD8+ T cell lines and the separation and purification of 102 T cell lines which grew from islets of as mixtures of CD4+ and CD8+ T cells. These studies are a major step forward in defining the repertoire of islet infiltrating, autoreactive T cells. These reactivities should impact the design of the induction of autoantigen-specific therapeutic efforts attempting to benefit subjects at-risk for, with recently diagnosed, and with established T1D in combination with islet replacement or regeneration.

T.83. The Role of Id2 and Id3 in Regulating NKT Cells of the Adipose Tissue

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Disorders involving obesity, such as diabetes, heart disease, and metabolic syndrome have become a predominant health issues in the United States and around the world. According to the CDC 36% of adults in the US qualify as obese, leading to a variety of negative health outcomes and billions of dollars in medical costs.

One of the hallmarks of obesity is inflammation of the adipose tissue mediated by a combination of hormones and cytokines produced by immune cells. Natural killer T (NKT) cells are a heterogeneous population of innate immune cells defined, in part, by their ability to quickly release cytokines after activation. NKT cells have been implicated in the maintenance of healthy adipose tissue and loss of NKT cells in obesity has been correlated with increased inflammation and both glucose and insulin intolerance.

We hope to further elucidate NKT cells' role in adipose tissue, after activation. We also hope to define the molecules responsible for mediating NKT cells' activity in a healthy adipose environment, with the goal of modulating these cells to improve health outcomes in the disease state.

The inhibitor of DNA binding proteins, Id2 and Id3, have been implicated in the proper maturation of NKT cells in the thymus. Though their roles in NKT cell maintenance and activation in the periphery have been relatively unexplored. Here we use both a GFP reporter and conditionally deficient constructs to determine the roles that Id2 and Id3 play in NKT cell activation in adipose tissue.

T.84. Natural Killer Cell Frequency and Phenotype in Patients with Type 1 Diabetes

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Multiple lines of evidence suggest viral infections may play a key role in the initiation of type 1 diabetes (T1D), including a type 1 interferon signature, MHC class I hyperexpression in pancreatic islets, and the presence of autoreactive $CD8^+$ T cells thought to be directly cytotoxic to b-cells. However, there remains a paucity of data regarding the frequency, subsets, and activation state of natural killer (NK) cells in T1D. Here, we report on NK cells in a cross-sectional cohort of patients with established (> 3months duration) T1D (n=39; 25.7±16.1 y) first-degree relatives (n=26; 35.7±16.3 y) and healthy controls (n=36; 21.7±10.9 y). Whole peripheral blood was collected and analyzed by flow cytometry for the markers CD3, CD56, CD16, CD57, CD25, NKG2A, CD226 and TIGIT. In contrast to prior reports suggesting deficiencies, our results showed no significant differences when comparing patients to controls in the frequency of total NK cells or in the

associated immature CD56^{bright} and terminally differentiated CD57^{+} subsets. However, we did observe increased expression of the costimulatory receptor CD226 on NK cells in patients with T1D (P=0.0394), while expression of the negative regulatory TIGIT was comparable with that of controls. Moreover, we noted a decrease in the expression of the activation-dependent *IL2RA* (CD25), a T1D candidate gene, on NK cells in subjects with T1D (P=0.0084). Altogether, this study shows differences in NK cell receptor expression and activity in T1D, which may be useful for the development of biomarkers or rational therapies in the context of precision genomic medicine.

T.85. Relation of Islets Cell Antibody with Beta Cell Function in Type II Diabetic Patients of Bangladesh

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Background: Presence of Islet cell autoantibody (ICA) at the time of diagnosis predicts deteriorating β-cell function of pancreas measured by C-peptide in type II diabetic patients. ICA positivity and measurement of C-peptide may predict early insulin requirement.

Objective: To assess pancreatic β cell function (measured by C-peptide) in patients with ICA positive and negative type II diabetic patients.

Methods: 173 type II diabetic adult patients of both sexes was selected and tested for presence of ICA autoantibody and C-peptide level using ELISA kits (both from DRG, USA) at the time of diagnosis, after 6 month and one year interval.

Results: Statistically significant difference was found in respect of ICA value in 173 type II DM patients ($p < 0.001$). ICA positive (12.7%) type II patients' mean value was 1.72±0.64 and was 0.55±0.16 in ICA (83.3%) negative cases. No statistically significant difference was found in age, gender and BMI in ICA positive and negative patients. The mean C-peptide level was 8.20±4.78 ng/ml in patients' positive for ICA antibody. After six months and one year follow up the mean C-peptide was 3.93±3.38 ng/ml and 2.50±2.05 ng/ml respectively. The changes of mean C-peptide was statistically significant ($p < 0.001$) in ICA positive patients after six months follow up in comparison to non-ICA negative patients.

Conclusion: We found deteriorating pancreatic β-cell function among ICA positive type II diabetic patients much earlier than found elsewhere in other studies (2-3 years). They may need insulin (instead of oral) much earlier for effective control of diabetes.

General autoimmunity

F.47. Literature Mining and Bioinformatics Analysis of Gene Expression Identifies Drug Targets for Treatment of Systemic Lupus Erythematosus (SLE)

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Since new therapies for lupus have been extremely slow to develop and lupus patients have a great unmet medical need, an independent pharma-external effort has been undertaken to evaluate all FDA approved drugs for potential use in this autoimmune disease. In 2013, literature mining evaluated the >1000 compounds approved by the FDA and identified 157 possible lupus therapies. These were ranked using an evidence-based Composite Lupus Treatment Scoring (CoLTs) approach (Lupus 25:1150, 2016). Between 2014-2017, the FDA has approved 125 new drugs, seven of

which targeted an autoimmune/inflammatory disease and none of which was approved for use in SLE. These drugs were evaluated for possible utility in autoimmune diseases and 43 were identified as possible therapies and ranked by the CoLT-scoring system. Nine were identified as high-priority candidates for repositioning into lupus including drugs targeting cellular metabolism, kinases and the immune system. In addition, potential utility of drug candidates in SLE was gauged by bioinformatic analysis of gene expression profiles from lupus tissues (kidney, skin, synovium) and the periphery (Whole Blood, B cells, T cells, myeloid cells). Connectivity scoring with gene expression profiles available in the Library of Integrated Network Cellular Signatures (LINCS) confirmed many of the drug candidates and also suggested some unique potential drug therapies. The combination of literature mining and bioinformatic analysis of gene expression profiles has yielded a group of high priority candidates that have promise as therapies for SLE.

F.48. Genetic vs. Selective Forces in Formation of the Human Thymic TCR Repertoire

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Our knowledge about the formation of a TCR repertoire in the human thymus is limited. We used high throughput TCR sequencing to assess the degree of similarity of TCR repertoires of human thymocytes generated in human thymi of humanized mice generated with the same HSCs and autologous and allogeneic thymi. We observed that human thymus in humanized mice selects a very diverse TCR repertoire. Formation of the human TCR repertoire is largely stochastic and can be almost totally divergent in identical thymi generating thymocytes derived from the same pool of HSCs. We demonstrated the impact of thymic selection in narrowing the TCR repertoire. The proportion of overlapping CDR3βs was similar between identical and allogeneic thymi, showing that they may be cross-reactive and selected by different thymi or that there are shared epitopes that participate in selection in the context of different HLAs. We observed an increased overlap in β chains with higher frequencies. Length of more abundant CDR3βs decreased during thymic selection. CDR3β overlap was also detected between different SP cell populations, often with different Vβ gene usage. As the HLAs that SP CD4 and SP CD8 cells are selected on are different, the most likely explanation is that the shared CDR3βs are highly cross-reactive. We also showed that genetic background and thymic selection does not dramatically change the V and J gene usage in forming the TCR repertoire. Pairing of V and J genes is random and is only determined by the amount of expression of each gene.

F.49. Long Non-Coding RNA and Methylome Profiles of Human Regulatory T Cells in Health and Disease

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Regulatory T cells (Tregs) play a pivotal role in regulating immune responses to self and foreign antigens. Recent findings highlighted that long-non-coding (lnc) RNAs control biological processes, acting as regulators of gene expression. We hypothesize that defects in lncRNA-dependent post-transcriptional or chromatin regulation in Tregs may affect gene expression resulting in altered Treg development, function or fitness in autoimmune and inflammatory disorders. Tregs ($CD4^+CD25^+CD127^{low}$) and effector T (Teff) ($CD4^+CD25^-CD127^{-/+}$) were purified by flow cytometry from the peripheral blood of healthy subjects (n=8) and the transcriptome and DNA-methylation profile were assessed. By RNA-Seq we identified 82 differentially expressed annotated lncRNAs in Tregs vs Teff (fdr2). A good proportion of the transcripts overlapped with $CD4^+$ T-cell regulatory elements, Treg-associated coding genes, known FOXP3-binding sites, or are proximal to Treg-specific differentially methylated regions (DMRs), thus suggesting a function in the regulation of Treg biology. One of them hosts known autoimmune-disease associated single nucleotide polymorphisms. Preliminary data

indicate that expression of Treg-specific lncRNAs is FOXP3-independent, since it is maintained in FOXP3-mutated Tregs from IPEX patients and is not induced upon ectopic over-expression of FOXP3 in $CD4^+$ T cells. Together with known hypomethylated regions in Treg-associated genes, Medip-Seq analysis of the same samples identified a DMR in a previously not reported Treg-specific regulatory element proximal to the *CTLA4*-gene. We are currently investigating the functional relevance of Treg-specific lncRNAs and DMRs in Tregs from autoimmune patients. Our results will potentially enable the identification of novel targets to modulate Treg function in health and disease.

F.50. Immunoediting Through Somatic Loss of HLA Class I Alleles Uncovers the Role of HLA Class I-Mediated Autoimmunity in Acquired Aplastic Anemia

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Acquired aplastic anemia (aAA) is an acquired bone marrow failure syndrome associated with predisposition to myelodysplastic syndrome and leukemia. Although its exact pathogenesis is unknown, aAA is believed to be immune-mediated. Recently, copy number-neutral loss of heterozygosity of chromosome arm 6p (6p CN-LOH), the site of the Major Histocompatibility Complex and Human Leukocyte Antigen (HLA) genes—was identified as a recurrent somatic change in aAA, thought to emerge due to immune escape of cells lacking certain HLA alleles. However, the linkage disequilibrium between HLA loci as well as other genes in the region made it difficult to pinpoint the etiologic driver of acquired 6p CN-LOH. Recently, we identified two aAA patients with somatic loss-of-function mutations in HLA class I genes. This led us to hypothesize that inactivating HLA gene mutations represent another mechanism of immune escape in aAA and may enable a more precise characterization of aAA immune specificity. To screen for HLA mutations, we performed targeted next-generation sequencing of HLA class I genes in 66 aAA patients. Eleven patients (17%) had somatic HLA loss, with recurrent loss-of-function mutations in *HLA-A**33:03, *HLA-A**68:01, *HLA-B**14:02 and *HLA-B**40:02 alleles. Patients who inherited these four HLA alleles had a more severe disease course with more clonal complications as assessed by whole exome sequencing (WES), SNP-array genotyping, and metaphase cytogenetics, and were also more likely to develop myelodysplastic syndrome. Our results demonstrate the role of HLA class I-driven autoimmunity in aAA, and establish a novel link between aAA patients' immunogenetics and risk of hematologic malignancies.

F.52. Clinical Data Specification and Coding for Cross-Ana**lyses with Omics Data in Autoimmune Diseases' Trials**

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Autoimmune and inflammatory diseases (AIDs) form a continuum of diseases with variable contribution of autoimmunity and inflammation. Yet, the nosography of AIDs remains mostly based on syndromic classification. The TRANSIMMUNOM observational clinical trial (NCT02466217) aims at re-evaluating AIDs nosography through multi-omics investigations of 1000 patients with one of nineteen selected AIDs. During patients' visits, clinical records and biological samples are gathered. To allow cross analyses of clinical data together with omics results, there is a need for specifying and then coding each type of data. To this end: i) a clinical expert consortium selected hundreds of relevant clinical features and clinical laboratory values to be collected for all patients, such as to homogeneously describe clinico-biological status of

every patients irrespective of their disease; ii) a cohort management team, involving biologists, clinicians and computer scientists designed an electronic Case Report Form (eCRF) using an open-source CFR-part 11 compliant electronic data capture system and iii) used CDISC standards for the coding of more than 700 heterogeneous parameters. For each parameter, the values to be recorded were specified and coded. Example of such coding are Yes/No check boxes for clinical investigation, numerical values with a list of relevant units according to the parameter, disease scores as a result of the automatized sum of several scores, treatment description including the coding of possible formula, dosage and posology. Altogether, a truly multidisciplinary endeavor led to design and implement a collection of 24 CRF capturing more than 8000 coded values that are now used in TRANSIMMUNOM.

F.53. Retained Inflammatory Signaling in Peripheral Blood Mononuclear Cells from Children with Quiescent Juvenile Dermatomyositis and Active Psoriasis

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Background: The Cure JM Registry/Repository follows 492 JDM and sequentially collects clinical information and biological samples, including peripheral blood mononuclear cells (PBMCs). Approximately 8% of patients later develop a secondary autoimmune disease, most commonly psoriasis (~2%). This study compared the immunological pattern for PBMC RNASeq during active JDM with the same child's PBMC pattern during Psoriasis and to matched controls.

Methods: We identified JDM patients (n=5) with stored PBMCs from the time of active JDM and PBMCs obtained after the onset of Psoriasis, approximately 10 years later, as well as 2 sets of controls, age/sex matched to the PBMC samples (n=5). The PBMC transcriptome was assessed using stranded RNA sequencing.

Results: Using a paired-sample analysis, the JDM-only PBMCs were essentially the same as the PBMCs from active psoriasis, with only a single differentially expressed gene. In contrast, the JDM samples, compared to controls, exhibited numerous genes significantly increased fold-change (FC) greater than 2 (n=115) and decreased less than -2 (n=9), encompassing inflammatory genes, such as *JUN* (4.3 FC; $p < 0.0001$), *MAP3K8* (FC 3.0, $p < 0.01$), and lipid processing, *OLR1* (FC 33.1, $p < 0.01$). The genes significantly increased at least 2-fold were enriched for the NK-kappa B and TNF signaling pathways.

Conclusions: As expected, PBMCs from symptomatic JDM displayed increased immunological activity compared to controls, with enrichment of several key pathways. However, this signature had not significantly altered a decade later, when they developed psoriasis, suggesting a shared genetic liability for both JDM and Psoriasis, and, perhaps, other autoimmune diseases.

F.54. Generation of Powerful Human Tolerogenic Dendritic Cells By LV-mediated Human IL-10 Gene Transfer

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Tolerogenic dendritic cells (tolDC) are a promising tool for cell-based approaches to induce tolerance in transplantation and autoimmunity. Among others, IL-10-modulated DC have been recently identified as the best-suited cells for tolDC-based therapies. An example of tolDC are DC-10, an inducible subset of human DC endowed with the ability of spontaneously and upon activation releasing high levels of IL-10, and of inducing T regulatory Type 1 (Tr1) cells. In this study, we assessed the use of a lentiviral vector encoding for IL-10 and Ã¢Ë†â€ NGFR, as marker gene (LV-IL-10/Ã¢Ë†â€ NGFR), to generate DC-10-like cells. During human DC differentiation, monocytes were treated with viral-like particles containing the simian immunodeficiency virus (SIV)-derived accessory protein Vpx and then transduced with LV-IL-

10/Ã¢Ë†â€ NGFR. As control, classical DC-10 generated from monocytes in the presence of IL-10 were used. Compared to classical DC-10, IL-10-transduced DC secreted spontaneously, and upon LPS stimulation, higher levels of IL-10, in the absence of IL-12. Moreover, IL-10-transduced DC displayed DC-10 features: expression of the tolerogenic molecules HLA-G and ILT4, induction of hypo-responsiveness in allogeneic $CD4^+$ and $CD8^+$ T cells, and of anergic allo-specific T cells. Interestingly, IL-10-transduced DC are more powerful than classical DC-10 in promoting *in vitro* differentiation of allo-specific Tr1 cells. These results showed that constitutive over-expression of IL-10 in human monocyte-derived DC leads to a cell population that recapitulates phenotype and functions of DC-10 and open new perspectives in their clinical use in T-cell mediated diseases.

T.33. Tolerance Induction by Teplizumab Is Modulated by Gut Microbiota

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We have shown that teplizumab, a non-FcR binding anti-CD3 mAb, induces tolerance by activating tolerogenic, IL-10 producing T cells in the gut of normal and humanized mice. Clinical studies revealed that not all patients respond to teplizumab treatment. Due to the intimate relationship between the immune system and microbiota in the gut, we hypothesized that gut microbiota can affect the efficacy of teplizumab treatment. We studied the effects of modulating the gut microbiome on the prevention of xenograft rejection in humanized mice. Teplizumab treatment delayed the rejection of B6 skin grafts on humanized NSG mice ($p=0.001$) and induced IL-10 secretion in the serum. Treatment with a cocktail of 4 antibiotics (Ampicillin, Neomycin, Metronidazole, and Vancomycin) but not the individual antibiotics prevented the activity of teplizumab and the induction of IL-10 ($p=0.034$). Teplizumab induced immune cell activation in gut infiltrating immune cells that was reduced when antibiotics were given. To directly study the activation properties of the microbiota of antibiotic-treated or untreated mice, we cultured splenocytes with microbiota and determined the cytokine production. Gut microbiota from antibiotic-treated mice induced less IL-10 production by splenocytes from humanized mice($p=0.0014$). Moreover, human stool samples from antibiotic treated children also showed reduced induction of IL-10 by peripheral blood mononuclear cells. In conclusion, our results suggest that the microbiota are critical modulators of tolerance induced by biologics. The microbiome from antibiotic humans induce less activation of human immune cells that is needed for acquisition of tolerance.

T.34. Altered Treg Function and Selection Following Aire-deficiency, New Insights on the Role of Aire

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Auto-immune diseases can be caused by a break in self-tolerance. The Auto-immune regulator (Aire) is an important transcription regulator that plays a critical role in central tolerance via promoting the expression of tissue restricted antigens in the thymus. The role of Aire on the selection and function of Tregs is still unresolved and controversial and its role in transplantation has never been addressed.

We demonstrated that there is a similar pattern of expression of Aire between rat and human in the thymus and lymph nodes. We then generated a model of Aire-deficient rats by disrupting 7-base pairs in exon 3 emulating a frequent human mutation. We observed that Aire-deficient rats displayed an APECED-like disease with strong auto-immune symptoms such as alopecia and vitiligo occurring at 6-month-old, auto-immune histological injuries, numerous circulating auto-antibodies and a strong increase of circulating IgM and IgA. We demonstrated an altered suppressive capacity of both $CD4^+CD25^+$ and $CD8^+CD45RC^{low}$ Tregs in $Aire^{-/-}$rats *in vitro* in a MLR assay. Preliminary results from *in vivo* model of

wasting disease in IL2Rg$^{-/-}$ rats suggested that Tregs from Aire$^{-/-}$ rats failed to control the disease in contrast to Tregs from control rats. We also showed a significant bias in the TCR repertoire of CD8^{+}CD45RC^{low} Tregs with selection of a restricted private Vβ20 repertoire, but not in CD4^{+}CD25^{hi} Tregs. Altogether, our results demonstrate that Aire-deficient rats expressed APECED-type auto-immune features and suggest a role for Aire in Treg function and selection.

T.35. Regulation of B Lymphocyte Metabolism by the PI3K/Akt Pathway

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The phosphatidylinositol-3-kinase (PI3K) pathway has been shown to regulate cellular metabolism. Disturbance of the PI3K pathway causes abnormal activation of immune cells that may result in autoimmunity or leukemia/lymphoma development. Tandem pleckstrin homology (PH) domain proteins (TAPPs) bind to phosphatidylinositol-3,4-bisphosphate (PI(3,4)P_2), a product of PI3K. The serine/threonine kinase known as Akt is also recruited to PI(3,4)P_2 where it becomes activated. This results in the activation of signalling pathways that regulate many biological processes including cellular metabolism. Here we investigate how the PI3K pathway and specifically how TAPP proteins control B lymphocyte activation. TAPP knock-in (KI) mice contain a knock-in mutation that results in TAPPs with a defective C-terminal PH domain, preventing its binding to PI(3,4)P_2. Using wild-type (Wt) and TAPP KI mice, we studied the effect of this mutation and how it impacted B cell metabolism. TAPP KI B cells exhibit increased proliferation, increased cell size and increased Akt phosphorylation upon activation *in vitro*. These cells show elevated metabolic activity (measured by oxygen consumption rate and extracellular acidification rate), increased expression of glucose transporter Glut1 and increased glucose uptake. Treatment of TAPP KI B cells with pharmaceutical inhibitors of PI3K decreased Glut1 expression and glucose uptake, and reduced proliferation. TAPP KI mice develop B cell-mediated autoimmunity that could be partially reversed by treatment with the inhibitor of glycolysis 2-deoxyglucose. Our data demonstrate that PI3K controls B cell metabolism, suggesting that targeting abnormal B cell metabolism may have therapeutic benefit.

T.36. Coxsackievirus B3 Viral Protein 1 Possesses Multiple Immunodominant Epitopes That Induce Differential Antigen-Specific, CD4 and CD8 T Cell Responses in A/J Mice

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Coxsackievirus B3 (CVB) is commonly implicated in myocarditis that can lead to dilated cardiomyopathy possibly by triggering an autoimmune response, and there are no commercially available vaccines to prevent this infection in humans. In fact, by using major histocompatibility complex (MHC) class II dextramers for cardiac myosin, we recently demonstrated that A/J mice infected with CVB can show the generation of myosin-specific CD4 T cells that can transfer disease to naïve recipients. Thus, it is critical to design vaccines that are free of side effects like autoimmunity. We report here identification of viral protein$_1$ (VP$_1$) epitopes that permit us to track the generation of virus-specific T cell responses in infected animals. These include VP$_1$ 681-700, VP$_1$ 721-740 and VP$_1$ 771-790. While, all epitopes induce CD4 T cell responses as evaluated by proliferation assay and MHC dextramer staining, VP$_1$ 721-740 and VP$_1$ 771-790 could also induce CD8 T cell responses. Furthermore, by creating T cell hybridomas for VP$_1$ 771-790, we demonstrate that a proportion of splenocytes containing mononuclear cells express viral proteins in CVB infected mice. Together, the use of MHC dextramers in conjunction with T cell hybridomas may permit evaluation of antigen-specific T cell responses in infection and vaccine studies with CVB.

T.37. CD25lowFOXP3+ T Cells Are Derived from a Regulatory T Cell Lineage and Are Circulating Markers of Active Autoimmunity

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Identification of alterations in the cellular composition of the human immune system is key to understanding the autoimmune process. Although the disease mechanisms leading to the break of tolerance are largely unknown, alterations in FOXP3$^+$ regulatory CD4$^+$ T cells (Tregs) have been implicated in the pathogenesis of several autoimmune diseases, including type 1 diabetes (T1D) and systemic lupus erythematosus (SLE). Here we found that the frequency of FOXP3$^+$ cells within CD127^{low}CD25^{low} CD4$^+$ T cells (here defined as CD25^{low}FOXP3$^+$ T cells) is increased compared to healthy donors in several autoimmune diseases of varying disease severity, including SLE, severe immunodeficiency patients with active autoimmunity and T1D patients. In this study, we show that CD25^{low}FOXP3$^+$ T cells share phenotypic features resembling conventional CD127^{low}CD25^{high}FOXP3$^+$ Tregs, including demethylation of the Treg-specific epigenetic control region in *FOXP3* and lack of IL-2 production. As compared to conventional Tregs, more CD25^{low}FOXP3$^+$ T cells are in cell cycle (29.9% vs 22.0% Ki-67$^+$; $P = 2.7 \times 10^{-7}$) and express the late-stage inhibitory receptor PD-1 (65.1% vs 38.3%; $P = 1.7 \times 10^{-17}$), while downregulating the expression of the early-stage inhibitory receptor CTLA-4, as well as other classical Treg markers, such as FOXP3, HELIOS and TIGIT. These findings suggest that CD25^{low}FOXP3$^+$ T cells represent a late stage of Treg differentiation *in vivo*, and are a peripheral biomarker of recent Treg expansion in response to an autoimmune reaction in tissues.

T.38. Unaffected Relatives of Systemic Lupus Erythematosus (SLE) Patients Are Discerned by Soluble Mediators, Autoantibodies, and Connective Tissue Disease Questionnaire Scores from SLE Patients and Controls in a Confirmatory Cohort

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Identifying populations at risk of SLE is essential to curtail inflammatory damage and implement prevention strategies. Using unique participant resources, this study examined an initial cohort of previously unaffected blood relatives who transitioned to classified SLE (n=45), plus a confirmatory cohort of European-American (EA) (n=50) and African-American (AA) (n=50) SLE patients, matched by race and gender to unaffected relatives (n=190) and unrelated healthy controls (Ctls, n=145). Participants provided clinical and demographic information, and completed the SLE-specific portion of the CTD Screening Questionnaire (SLE-CSQ). Plasma samples were assessed for autoantibody production and for 52 soluble mediators by multiplexed bead-based assays or ELISA. In both cohorts, compared to unaffected relatives, Ctls had significantly lower ($p=0.001$), while SLE patients had significantly higher ($p\leq0.001$), SLE-CSQ scores. Irrespective of race, a strong correlation existed between SLE-CSQ scores, number of ACR criteria, and number of autoantibody specificities ($p\leq0.001$). A strong correlation was noted between these three aforementioned variables and select soluble mediators, including TNF superfamily member BLyS, pro-inflammatory mediator stem cell factor (SCF), and the regulatory mediator IL-10 ($p\leq0.004$ for each mediator), among others. Levels of BLyS in unaffected relatives and Ctls were significantly lower than SLE patients ($p\leq0.001$). Unaffected relatives had intermediate levels of SCF between SLE patients ($p\leq0.01$) and Ctls ($p\leq0.03$). In contrast, unaffected relatives had significantly higher levels of the regulatory mediator IL-10 than SLE patients

($p \leq 0.001$). Identification of factors which discern unaffected lupus relatives from SLE patients may be beneficial to identify potential treatments to curtail inflammatory damage for prevention trials.

T.39. Anti-reticulin Autoantibodies Revisited: New Clinical Associations

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Introduction: Reticulin is a type III collagen and specific anti-reticulin antibodies (ARA) had been used as diagnostic markers of celiac disease, although currently obsolete. Five types of indirect immunofluorescence (IIF) patterns associated with these autoantibodies have been observed, whose clinical implications are unknown.

Methods: Fifty-eight consecutive patients with positive ARA from a total of 490 serum samples routinely sent to perform triple tissue IIF to our laboratory were studied (*NIKON Y-THS* microscope and HELIOS® AESKU Systems). ARA patterns' distribution were as follows: R1: peritubular and periglomerular in kidney; perivascular and parenchymatic in liver; and intergastric fibers and subgladular muscle in the stomach. R2: Blood vessels in kidney and periportal in liver; blood vessels and intergastric fibers in the stomach. Rs: liver sinusoids and renal brush border. R3: Kuppfer's cells in liver.

Results: We observed 28 R2 out of 58 (48%); 19 Rs (33%); 7 R1 patients (12%); 4 R3 (7%). Of these, 34.5% of patients with positive ARA show chronic hepatopathy. A total of 41.4% reported chronic digestive disorders, the majority with R2 pattern. A 27.6% had signs of malignancy or familial cancer. Interestingly, 8 of R2 positive (28.6%) showed type 2 Diabetes Mellitus (T2DM).

Conclusions: Type 2 ARA was the commonest pattern followed by Rs. Most of ARA positive patients had history of chronic hepatopathy and other digestive disorders, as well as underlying cancer. An interesting preliminary result was a third part of R2 patients associated T2DM. Further studies may shed light of the clinical significance of these autoantibodies.

T.40. Alterations on the Interleukin-13 Pathway Impair iNKT Cell Mediated Regulation in Type 1 Diabetes

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Tolerance breakdown in Type 1 Diabetes (T1D) has been associated in part to frequency and functional defects of iNKT cells favoring the development of T1D in NOD mice. Thus, to determine if human NKT cells could play a role in the control of T1D development, we have studied if iNKT cells derived from human T1D patients and healthy donors could exert a regulatory function.

We demonstrated that iNKT cells have the capacity to suppress the proliferation of T effector cells (Teff). Interestingly, suppression was dependent on the secretion of the cytokine IL-13 since addition of a blocking antibody to IL-13 resulted on Teff cell proliferation and IL2 secretion recovery. However, this regulatory mechanism was clearly impaired in iNKT cells isolated from blood or pancreas of T1D patients at disease onset. Further, their functional defect could be related both to a decreased secretion of IL-13 by iNKT cells and to a different expression pattern of the IL13 receptor in Teff cells from these patients compared to controls. These results indicated that the alteration of this regulatory pathway in T1D patients can favor progression of the autoimmune response to T1D. In order to elucidate the reason behind this functional defect a gene expression analysis has been performed to identify molecules and/or pathways that could be related to the regulation impairment in T1D patients. Confirmation of these results should provide a new immunotherapeutic approach for T1D using iNKT cells.

T.41. Immunophenotypic Heterogeneity of Regulatory and Helper T Lymphocytes in Patients with Genetic Autoimmunities

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Immunological studies of patients with genetic immune mediated disease with autoimmunity offer a unique opportunity to understand the role of regulatory T (Treg) cells in immune homeostasis. IPEX and CTLA-4 deficiency are examples of mono-genetic diseases presenting with severe autoimmunity. Treg cells maintain self-tolerance, and their impaired function in CTLA-4 deficient and IPEX patients leads to the characteristic clinical phenotype. Human CD4+ CD25hi CD127lo/- FOXP3+ regulatory T (Treg) cells express both CTLA-4 and FOXP3 constitutively, which are dysfunctional in CTLA-4 deficient and IPEX patients, respectively. Furthermore, low frequency of Treg cells can also result in autoimmunity in other primary immunodeficiencies, such as DiGeorge syndrome (DGS).

To study patients with immune dysregulation we have combined the use of mass cytometry (CyTOF), with functional assays. Using CyTOF, we can perform a comprehensive analysis of CD4+ Treg and T helper (Th) type 1 (Th1), Th2, Th17 and follicular T helper (Tfh) cells on a single patient sample. This allows us to evaluate how the detected alterations in CD4+ T cell subpopulations correlate with autoimmunity due to different single mutations with overlapping clinical phenotype.

The IPEX patients analyzed showed increased Treg cell frequency with low FOXP3 expression compared to age-matched healthy controls. In contrast, DGS and CTLA-4 deficient patients had normal FOXP3 expression, but a low frequency of Treg cells. Each disease, however, had a unique signature of abnormal Th1, Th2, Th17, and Tfh cell frequency and function, providing new insights into each disease mechanism.

T.42. Role and Regulation of CD11chi T-bet+ B cells in SLE

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We examined a large cohort of systemic lupus erythematosus (SLE) patients and found an unusual B cell subset highly expanded. These B cells displayed a unique phenotype and expressed antigens not present on other B cell populations, including high densities of CD11c, FcRL5, and the T-box *transcription factor,* T-bet, but unexpectedly, were $CD40^{lo}$ and $CD24^{-}$. Although these $CD11c^{hi}$ cells were largely negative for the memory B cell antigen CD27, their telomere length was consistent with memory, rather than naïve B cells. Notably, the increase of $CD11c^{hi}$ B cells in SLE significantly correlated with disease severity scores as well as with other markers of disease activity including serum IL-21. While memory B cells characteristically express low densities of IL-21R, these memory $CD11c^{hi}$ B cells were IL-21R bright. In culture, IL-21 was a potent driver of CD11c expression when B cells were co-stimulated through the B cell receptor and CD40. Lastly, $CD11c^{hi}$ B cells in SLE significantly correlated with blood plasma cells as well as a distinct set of autoantibodies, and importantly, were efficiently driven to differentiate into plasma cells in response to activated T cells. These data indicate that among the many roles of IL-21 in regard to B cell activation and differentiation, this pleiotropic cytokine also regulates $CD11c^{hi}$ B cells, and presumably contributes to autoimmunity through the differentiation of autoreactive $CD11c^{hi}$ B cells in SLE.

T.43. CD19+CD3+ cells: heterogeneous population that may potentially be involved in autoimmunity.

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In this study, we are interested in demonstrating the existence of $CD19^+CD3^+$ cells and whether they have a role in autoimmune diseases. We sought to evaluate whether $CD3^+CD19^+$ cells were differentially present in the peripheral blood of patients with autoimmune disease compared with healthy donors.

Peripheral blood was obtained from patients with confirmed diagnosis of autoimmune disease (Psoriasis; N=121, Multiple Sclerosis (MS); N=52, Sarcodiosis; N=66, Lupus (SLE); N=55, Inflammatory Bowel disease-IBD; N=33) who were untreated prior to sample acquisition or from healthy donors (N=53). Samples used for these studies were from NIH IRB-approved protocols. Phenotypic analysis of B and T cells was performed using multicolor FACS. Image-flow cytometry using AMNIS-image stream was used to analyze expression of markers at single cell level.

In comparison to the healthy controls, we observed a significantly higher frequency of CD3+CD19+ cells in MS, Sarcodiosis and Psoriasis but no differences in SLE or IBD. The CD3+CD19+ cells were found to be expressing CD20, HLA-DR, CD23, and CD27. CD86, Ig, CD38, CCR7.

In summary, we show that in autoimmune diseases the presence of a previously neglected T cell subset, $CD3^+CD19^+$ T cells. Further studies will be required to understand the developmental origin and evolutionary relevance of $CD3^+$ CD19+ cells and whether they differ functionally from CD19- or recently discussed $CD20^+$ T cells. Understanding the pathological relevance of this T cell subset in autoimmune disorders will likely broaden our understanding of human autoimmunity and may reveal novel therapeutic avenues.

T.44. Analysis of the Cytoplasm-Nuclear Translocation of Signal Transducers and Activator of Transcription 3 (STAT3) Gain-of-Function and Loss-of Functions Mutants. A New Technological Approach

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Patients with germline STAT3 gain-of-function (GOF) and loss-of-function (LOF) mutations were identified by whole-exome sequencing. GOF STAT3 mutations increased the expression of downstream target genes like Suppressor of Cytokine Signaling 3 (SOCS3). Although transcriptional activity was increased, phosphorylation of STAT3 both at baseline and upon stimulation was normal. To elucidate the mechanism by which normal phosphorylation of GOF STAT3 leads to altered target gene expression and immune dysfunctions, we studies STAT3 dynamic at single cell levels with the Amnis ImageStream flow cytometer. We analyzed the ratio of similarity scores between IL-6 stimulated vs. nonstimulated nuclear and cytoplasmic localization of STAT3 at different time points. In comparison with cells carrying wild type STAT3, we found that GOF STAT3 and LOF STAT3 cells have different behaviors during prolonged IL-6 stimulation. The ratio of the wild type STAT3 cells changed very little when the cells were stimulated with IL-6 from 15 to 120 min. Interestingly, the ratio of similarity score for GOF STAT3 cells were higher at 15 min and 30 min after IL-6 stimulation, and decreased for an extended stimulation of up to 120 min. Unexpectedly, the ratio of LOF STAT3 cells were lower than the wild type and the GOF cells for the shorter stimulation times of up to 30 minutes, but it increased with the time of IL-6 stimulation. These preliminary results provide more information about STAT3 behavior in a complex biological system, which may help us understand the mechanism linked to mutated STAT3 dysfunctions.

T.45. Mechanisms Driving an Autoimmune-Like Syndrome in Humanized Mice

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Spontaneous development of a graft-versus-host disease (GVHD)/autoimmunity syndrome after around 25-40 weeks post-transplantation limits the experimental window for humanized mice generated by transplantation of human hematopoietic stem cells (HSCs) with/without a human thymus to immunodeficient NOD/SCID/gamma (NSG) mice. The cause of this disease is unknown. Skin, lungs, spleen, liver and intestine are infiltrated with mononuclear cells, neutrophils and histiocytes. Target organs and the lymphoid tissues of affected mice contained very high levels of CD4 and CD8 effector/memory cells, suggesting that the disease targets antigens presented on both MHC classes I and II. Measures were taken to exhaustively deplete pre-existing thymocytes and analysis of infiltrates in animals with different thymus vs HSC donors indicated that the T cell infiltrates were largely HSC-derived. Thymectomized NSG recipients, which fail to develop human T cells, did not develop autoimmunity/GVHD. Humanized mice constructed with an intact native mouse thymus, which supports a low thymic output, demonstrated earlier disease onset (20-45 weeks) compared to mice with thymocyte-depleted human thymus grafts (35-60 weeks) that support higher thymic output. Adoptive transfer of T cells to T cell-deficient mice with autologous human APCs, revealed that human APC-dependent lymphopenia-induced proliferation (LIP) of T cells significantly accelerated disease onset. In conclusion, while both mouse and human thymi produce T cells causing this syndrome, more prominent LIP of T cells developing in mouse thymus accelerated the disease, whose etiology may be multifactorial.

T.45. PD1 and PD-**L1 expression in Graves' disease's** Thyroid Glands. A Clue to Better Understand The Pathogenesis And Immune Checkpoint Immunotherapy-Associated Thyroid Autoimmunity

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It has recently been reported that thyroid autoimmunity -including cases of Graves' disease (GD)- may arise as a consequence of the new cancer immunotherapies focused on blocking the immune checkpoints PD-1 and CTLA-4. On the other side, thyroid autoinmune tissue show a clear IFN signature, and molecules like PD-L1 are inducible by INF-gamma. The aim of the study was to investigate imnunocheckpoint molecules expresion induced by INF-gamma in thyroid samples

Cryostat sections of GD, Hashimoto thyroiditis, multinodular goiter with focal thyroiditis and lymphoid control organs were stained by single and double immunofluorescence for PD-1, PD-L1, as well as for HLA-I/II, Cytokeratin-18, TPO and lymphocyte phenotypic markers. Primary thyroid and SV-40 thyroid- derived cell line HT93 cultures were supplemented with increasing doses of IFN-gamma and stained at 48h-72h to assess HLA and PD-L1 induction.

In 11 out of 12 GD glands PD-L1 was detected clearly in thyroid follicular cells (TFCs). PD-L1 staining was confined to follicles close to lymphoid infiltrates and was much less extensive than HLA class II. In multinodular goiter glands traces of PD-L1 staining was visible in TFCs close to infiltrates. Importantly, PD1 was highly expressed by the infiltrating T cells in GD. PD-L1 expression could be readily induced by IFN-gamma in TFC cultures in a time-and dose- dependent manner, as assessed by FACS and RT-PCR.

These results suggest that silent thyroid focal thyroiditis could evolve into thyroid autoimmunity due to the release of infiltrating lymphocytes from PD1/PD1-L inhibitory interaction.

Genetics

F.29. Enhancer Connectome in Primary Human T Cells Reveals Target Genes of Autoimmune Disease-associated DNA Elements

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The imprecision of linking intergenic mutations to target genes has limited molecular understanding of diverse human diseases. Here, we show H3K27ac HiChIP generates high-resolution contact maps of active enhancers and target genes in rare primary human T cell subtypes. Differentiation of naïve T cells to either T helper 17 cells or regulatory T cells create subtype-specific enhancer-promoter interactions, specifically at regions of shared DNA accessibility. We provide an atlas for assigning molecular functions to autoimmune disease risk variants, linking hundreds of noncoding variants to putative gene targets. HiChIP target genes are supported by high-resolution CRISPR screens, expression quantitative trait loci, and allele-specific enhancer loops in patient-derived primary cells. The majority of disease-associated enhancers contact genes beyond the nearest gene in the linear genome, leading to a four-fold expansion of target genes for autoimmune diseases.

F.30. TTC7A Mutation in a Patient with Crohn's Disease and Recurrent Sinus Infections

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Biallelic TTC7A mutation has been implicated in Multiple Intestinal Atresia- Severe Combined Immunodeficiency (MIA-SCID), as well as Very Early Onset Inflammatory Bowel Disease (VEO-IBD). We present a patient with a TTC7A (R163W) mutation presenting with Crohn's disease at 9 years of age. Whole blood was obtained from the patient, PBMCs were then isolated and stimulated with pokeweed, PBS, CD79b, or IL-4/CD40. The samples were then stained and for CXCR4 and CCR7. In the second Stimulation Assay, CD3/28 was used in addition to the previous stimulants, and the cells were stimulated overnight. For the Proliferation Assay, PBMCs were labeled with CFSE, stimulated with CD3/28, and incubated for 3 days at 37ºC. The Stimulation Assay indicated that the patient exhibits lower levels of baseline CXCR4 expression compared to controls. In the healthy control, stimulation resulted in down-regulation of CXCR4, while in the TTC7A patient, CXCR4 was up-regulated. CCR7 expression does not change much in response to stimulation in a healthy control, but down-regulates in the TTC7A patient and up-regulates in the TTC7A carrier mother. The proliferation assay revealed that the TTC7A patient had increased proliferation of T cells compared to the healthy control and mother. TTC7A plays a role in apicobasal polarity of enterocytes, as well as the migration and adhesion capabilities of lymphocytes. This case study shows that carrying one mutant TTC7A allele results in immunologic abnormalities, especially in the molecules that play a role in lymphocyte migration.

F.31. Using Machine Learning Neural Networks Improves Assessment of T1D Genetic Risk

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BACKGROUND: Over the years, models that assess the combined genetic burden have been refined as more putative risk SNPs have been identified and with the utilization of logistic regression genetic risk score (logitGRS) models. However, these models do not account for potential interaction effects between loci. Our OBJECTIVE was to apply a logitGRS model to a novel cohort from the southeast U.S., and to compare its performance to a machine learning neural network genetic risk (nnGR) model. We hypothesized that the nnGR would be capable of training itself to account for locus interaction effects and could therefore more accurately classify subjects as patients and controls. METHODS: A cohort consisting of 325 controls, 543 first-degree relatives, and 519 T1D subjects were genotyped on a custom SNP array for 3 SNPs to impute HLA DR3 and DR4 and 30 additional T1D-risk loci. The logitGRS calculation incorporated the odds ratios and number of risk alleles carried for each locus. The nnGR model input was the number of minor alleles carried at each SNP. RESULTS: The logitGRS yielded a T1D vs control ROC-AUC of 0.844 (P50th T1D-centile was indicative of T1D with

92.9% specificity. Interestingly, the logitGRS negatively correlated with age at diagnosis (Pearson P=0.0002). The logitGRS had a peak balanced accuracy of 63.5% at classifying T1D and control subjects, while the nnGR model peaked at 65.8%. This modest improvement may aid in cohort stratification, which will improve functional studies, biomarker identification, and subject selection for interventional and natural history trials.

F.32. Single cell Expression Quantitative Trait loci (eQTL) Analysis of Established Systemic Lupus Erythematosus (SLE)-risk loci in Lupus Patient Monocytes

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Most confirmed SLE-risk loci are found near or in genes with immune function, yet how these loci influence diverse immune cell subsets remains unknown. We performed single cell eQTL analysis in SLE monocytes to determine the impact of SLE-risk loci in single human monocytes. Purified classical (CL) and non-classical (NCL) monocytes from SLE patientsÃ‚Â were analyzed for expression of 90 genes, and patients were genotyped for 7 SLE-risk SNPs. Each monocyte subset was analyzed separately using non-parametric analyses. We observed a large number of significant eQTL associations that surpassed the 5% FDR. The SLE-associated SNPs demonstrated more eQTLs in NCLs as compared to CLs ($p=2.5x10^{-8}$). For a given SNP, the eQTL associated transcripts differed between monocyte subsets ($p < 0.001$ for all 7 SNPs for discordance). For NCLs, TNFAIP3, IRF5, IRF7, PTPN22, and SPP1 shared a significant proportion of eQTL associations. For CLs, TNFAIP3 shared many eQTLs with SPP1 and ITGAM, while SPP1 and ITGAM showed limited overlap. Thus, SLE-associated risk loci exert coordinated effects on gene expression within individual human monocytes, and the risk loci interact in different ways in different cell types. Our study revealed striking differences, using single cell gene expression, in the occurrence and interaction of SLE risk associated eQTLs within different but closely related cell types. This suggests pleiotropic effects from each locus across various immune cell types, and a high degree of complexity when considering how these loci impact the immune system.

F.33. The 10,000 Immunomes Project: A Data Resource of Human Immunology

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Despite increasing appreciation of the promise of clinical immunology in many areas of medicine, including cancer therapy, metabolism, and neurobiology, to date there is no large, representative data resource for the multitude of assays in human immunology. The ability to translate observations from model organisms to humans, the generation of new basic science hypotheses, and the interpretation of seldom-measured analytes are currently hampered by the lack of a reference human 'immunome.' The 10,000 Immunomes Project aims to generate such a reference by compiling and harmonizing the measurements available on nearly 10,000 control subjects from the Immunology and Data Analysis Portal (ImmPort, www.immport.org), an archival repository for immunological research and clinical trials funded by NIAID. Data in the resource include ELISA, Luminex, flow cytometry, CyTOF, gene expression, HAI titers, clinical lab tests, and others. These data will be available through a web interface, allowing researchers to view, analyze, and download data across the 84 studies that contribute to the dataset. Our presentation will describe the demographics of the 10,000 Immunomes cohort, our data curation and standardization process, and the datasets we have successfully harmonized, including gene expression, ELISA, Luminex, and clinical lab tests. We will also present preliminary meta-analyses of these analytes. For example, we find that many soluble protein measurements vary significantly with age, gender, or ethnicity, implying that taking these variables into consideration will be important for the practice of clinical immunology and the advancement of precision medicine.

F.34. Next Generation Sequencing-Based Panel Screening in Patients with Undifferentiated Autoinflammatory Diseases: Friend or Foe?

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A relevant number of patients with clinical phenotype clearly consistent with an autoinflammatory diseases cannot be identified on the molecular basis. Aim of this study is to test a next generation sequencing (NGS)-based clinically-oriented protocol in these patients. We designed a NGS panel of 41 genes clustered in seven subsets according to the clinical phenotypes usually associated with their mutation (i. periodic fever, ii. chronic urticaria, iii. skin/bone/joints involvement, iv. intestinal involvement, v. type 1 interferonopathies and familial lupus, vi. Aicardi-Goutières syndrome, vii. miscellaneous). Patients were classified into one or more clinical subsets according to their clinical features and then massively sequenced for the coding portions of 41 genes. Each variant of the genes included in the selected clinical subsets was confirmed by Sanger method. Fifty patients (27 M, 23 F) were enrolled. All patients were already screened for at least one gene (mean 3; range 1-7). The mean age was 12.5 years (range 5-38) with mean duration of disease of eight years. The vast majority (43 patients; 86%) displayed periodic fever episodes; five (10%) a prevalent skin/bone/joints involvement without fever; one a prevalent intestinal involvement. Seven patients (14%) were suspected of type 1 interferonopathies. In total 128 variants were found (mean 3 for patient; range 0-7), of which only 29 (23%) in genes belonging to the suspected clinical subsets. Variants with suspected pathogenic relevance were found in 8 patients (16%). In this sample of highly selected patients, our NGS-based clinically-oriented protocol was associated with a good molecular diagnosis rate.

F.35. Rapid Functional Characterization of Rare and Common Immune Variants

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Immunologic research has revealed extensive mechanistic information about the cellular and macromolecular components of the immune system. Mono- and polygenic disorders causing autoimmune and immunodeficiency disease affect these very components. However, the functional consequences of causative genetic variants, both rare mutations (monogenic disorders) and common SNPs (polygenetic diseases) are regularly not known. An asymmetry between our ability to identify variants versus our ability to functionally characterize them produces these mysterious variants of "unknown significance", hampering investigation into monogenetic clinical decision making and polygenetic disease etiology. We present a pipeline combining CRISPR/Cas9 primary human immune cell editing with classical immunologic phenotypic assays to functionally characterize putative causative variants in primary immune cells on an accelerated time scale. As a demonstration in monogenic disorders, we introduce patient mutations in IL2RA and CTLA4 into healthy donor cells and recapitulate the patient's *ex vivo* functional deficients. Expanding to common SNPs, we discover a novel functional deficit in cells carrying a SNP associated with Crohn's disease, but protective for Type 1 Diabetes. Overall we describe a rapid method for removing "unknown significance" labels from variants in clinical diagnostics and basic research that represents a powerful personalized diagnostic.

F.36. Predominant Male Prevalence of HLA B27 Alleles in Referred Patients to a Tertiary Medical Centre of Bangladesh

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Background: Human Leukocyte Antigen B27 (HLA-B27) (subtypes B*2701-*2759) is a class I surface antigen encoded by the B locus in the major histocompatibility complex (MHC) is strongly associated with ankylosing spondylitis (AS) and other associated inflammatory diseases, referred to collectively as 'spondyloarthritis'. The Prevalence of HLA-B27 varies regionally. This is the first report on the prevalence of HLA-B27 in referred patients in a tertiary medical centre of Bangladesh.

Methods: 2651 referred patients to Popular Diagnostic Centre Ltd., Dhaka from October, 2015 to December, 2016 with the complaints of back pain, joint pain, eye pain etc. has been included. The test was performed in LightCycler 480 (Roche Applied Sciences, Switzerland) platform using LightMix HLA-B27 kits from TIB Molbiol Gmbh (Germany).

Results: Among 2651 referred adult patients (21 to 68 years), 1551 were male (58.5%) and 1100 were female (41.5%). Overall, 715 (27%) has been detected positive and 1936 (73%) were negative for HLA-B27. 29% (451) of male referred patients and 14% (154) of female patients were found positive. Significant association (p

Conclusion: Female referred patients were found more protective than compared with male. Epidemiological studies with large cohort may be undertaken to strengthen this finding in future.

Immune monitoring

F.37. High-Dimensional Analysis of Human Regulatory T Cells Using Mass Cytometry

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Regulatory T cells (Tregs) are critical for maintenance of peripheral tolerance in a variety of tissues. Evidence that Tregs seem to be a phenotypically diverse population that varies between individuals and tissues suggests that there may be functionally-specialized subsets of Tregs which vary depending on location and/or disease state. We developed a new mass cytometry-based method to directly measure FOXP3 in combination with 20 other parameters to characterize the phenotype of human Tregs in different tissues, and to enable analysis of antigen-specific cells from whole blood. We first developed and characterized a protocol to directly detect FOXP3, the Treg lineage-defining transcription factor, via mass cytometry. We found this optimized protocol is more sensitive than commercially-available methods to detect FOXP3 by mass cytometry and is compatible with staining of whole blood, previously-frozen samples and other intracellular targets such as cytokines and other transcription factors. To ask how Treg populations differ depending on location and disease state, we stained mononuclear cells from adult peripheral blood, pediatric thymus and cord blood, as well as synovial fluid from pediatric subjects with juvenile idiopathic arthritis with our Treg-specific mass cytometry panel. The resulting data were analyzed using several bioinformatics approaches, including viSNE, which revealed diversity in the heterogeneity and phenotype of Tregs depending on their origin. The ability to stain FOXP3 effectively using mass cytometry will facilitate the further characterization of Tregs in health versus disease to understand how Tregs are functionally specialized in the context of different tissues and disease.

F.38. Understanding Immune System Sex Differences in the Healthy Human Transcriptome

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Sex and gender biases in the incidence of autoimmunity and infection imply that women have stronger immune responses. Women are at higher risk of autoimmune diseases, while men are more likely to die of infectious disease. Molecular

factors driving this phenomenon may be detectable in the transcriptome, as it reflects immune activation, hormonal regulation, and chromosome status. We performed an immunologically focused investigation of transcriptional sex differences across global populations. First, we performed an integrated multi-cohort analysis of 6 cohorts consisting of 458 individuals to identify a 178-gene signature, called the Immune Sex Expression Signature (iSEXS), which is differentially expressed between healthy men and women in the blood across populations. We validated iSEXS in 3 additional cohorts of 128 samples. Second, we examined whether iSEXS was driven by cell frequencies. Using deconvolution, a method of predicting cell frequencies from bulk gene expression, we performed a meta-analysis of sex differences in cell frequencies. We validated our results in an independent mass cytometry dataset and found that males had higher frequencies of monocytes. Third, we examined the role of sex hormones and chromosomes in iSEXS. We observed that 25% of iSEXS is located on sex chromosomes. Importantly, in a cohort of disorders of sexual development, XY-individuals with normal female genitalia expressed iSEXS at similar levels as XY-males, indicating that iSEXS is primarily driven by chromosomal differences. As a robust gene signature across populations, iSEXS has applications in understanding why women and men have differential risks of autoimmunity and infection.

F.39. Cloud-based Highly Parallel Execution of t-SNE and SPADE with Metaclustering for Analysis and Visualization of Large Single-cell Datasets

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The use of machine learning techniques, in particular unsupervised clustering and dimensionality reduction algorithms, is quickly becoming a standard workflow for identifying and visualizing biological populations from within high-dimensional data. These methods allow researchers to approach data analysis without the bias and subjectivity that has traditionally been standard in the field.

Algorithms have context-dependent strengths and weaknesses. Across algorithms, an inability to scale computation to large datasets is a common theme. Most algorithms are designed and distributed to run on individual computers where memory and CPU are quickly exhausted by large datasets. Even when high-performance compute resources are available, algorithms often don't scale to large datasets as a fundamental property of their design. If they do, it might result in an untenable increase in runtime or diminished quality of results.

t-SNE and SPADE are two well-published algorithms that suffer problems as discussed above after datasets exceed a number of observations on the order of 1 million. This study introduces an alternative approach to the use of SPADE and t-SNE whereby a dataset is divided and distributed across numerous compute nodes in the cloud to process independently in parallel. The results of each computation are then combined in a metaclustering step for final visualization and analysis. The improvement in execution speed as a function of degree of parallelization is established. The method is validated against a non-parallel analysis of the same dataset to establish concordance of identified populations. The workflow is executed on Cytobank for portability to other researchers.

F.40. Production and Use of External Standards for Antigen-specific T-cell Assays

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Immunological assays for monitoring antigen-specific T-cell responses like Elispot, ICS, or MHC-multimer staining, are complex and prone to significant variation at all assay stages (e.g. sample acquisition, storage, shipment, processing, and analysis). However, these assays are generally poorly standardized. In particular, the lack of external assay standards reduces the comparability of results between runs and across institutions. A recently established technology provides a new type of reference T-cell sample based on T-cell-receptor-engineering. These T-cell engineered reference samples (TERS) can be adjusted with respect to the frequency of specific TCR-bearing T-cells in the sample, even permitting the use of high and low controls from the same source in parallel. They deliver stable signals over time and can be used in connection with stimulation based assays (Elispot, ICS) or MHC-multimer staining. The recent commercial availability of TERS kits with different TCR-specificities allows users to produce their own tailor-made batches of TERS and is likely to become a game changer in regards to the use external standards for the mentioned assay platforms. Here, we show our initial results produced with commercially available TERS kits, highlighting their usefulness for example in assay calibration and assay monitoring over time. The use of TERS facilitates the detection of even small variations in assay performance and may ultimately allow users to accept or reject assay runs based on an objective control. Our data showcases both the ease of use and the robustness of this approach on different platforms and highlights the advantages associated with proper assay standards.

F.41. Dose-related Immunomodulation by Placenta-Expanded, Mesenchymal Cells Improves Muscle Function Following Hip Arthroplasty: A Randomized Controlled Pilot Phase I/IIa trial

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No regenerative approach has thus far been shown to be effective in skeletal muscle injuries, despite high frequency and associated functional deficits. We sought to address surgical trauma related muscle injuries using local intraoperative application of allogeneic placental-expanded, mesenchymal-like adherent cells (PLX-PAD), using hip arthroplasty as a standardized injury model, because of their high regenerative and immunomodulatory potency. Our pilot phase I/IIa study was prospective, randomized, double blind and placebo-controlled. Twenty patients undergoing hip arthroplasty via a direct lateral approach received 3.0Ã—108, 1.5Ã—108 PLX-PAD, or a placebo into their gluteus medius muscle. We did not observe any relevant PLX-PAD-related adverse events at the 2-year follow-up. Improved gluteus medius strength was noted as early as week 6 in the treatment-groups. Surprisingly, until week 26 the low-dose outperformed the high-dose group and reached significantly improved strength compared to placebo, mirrored by an increase in muscle volume. Histology indicated accelerated healing after cell therapy. Biomarker studies revealed that low-dose treatment reduced the surgery-related immunological stress reaction more than high-dose. Signs of late-onset immune reactivity after high-dose treatment corresponded to reduced functional improvement. In conclusion allogeneic PLX-PAD therapy improved strength and volume of injured skeletal muscle with a reasonable safety profile. Outcomes could be positively correlated with the modulation of early postoperative stress-related immunological reactions.

F.42. Impact of Age, Sex and Genetics on Transcriptional Immune Responses to Bacterial, Viral and Fungal Challenges

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Immune responses are highly variable between individuals and populations, with this variance mediated by many factors including age, sex, and genetics. To dissect how each of these factors contributes to differential immune responses we

recruited 1,000 healthy donors as part of the *Milieu Intereiur* cohort that were equally stratified by age (20-70 years old) and sex (50:50). Whole blood from each donor was stimulated in a standardized approach with *Escherichia coli*, BCG, *Stapholocus aureus*, SEB, *Candida albicans* and Influenza virus and the transcriptomic response was analyzed by Nanostring gene expression arrays. We show that age affects gene expression in a stimuli specific manner, while the effect of sex is more common across conditions. Both age and sex contribute to the expression of many immune-related genes but at a low level, while in contrast, the genetic effects are much stronger but relevant to fewer genes. Genetic analysis identified hundreds of eQTLs, regulated in both cis and trans, for all stimuli induced responses. We confirm the presence of a TLR1 master regulator recently shown to be an important factor controlling variable immunity in European populations. Finally, we integrated transcriptomic and cellular data sets in an integrative model to describe how age and sex mediated their effects through different immune cell populations. These results lay the foundations for new patient stratification strategies that consider age, sex, and genetics as impacting variable immune response outcomes.

F.43. T lymphocytes Infiltrating Liver Metastases of Uveal Melanoma Patients

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Liver metastases develop in 20% uveal melanoma (UM) patients. Only 10% of these patients are eligible for hepatic resection, which can improve patient outcome. In the absence of proven standard of care, anti-checkpoint therapies have been tried with only anecdotal tumor regression. The characterization of the immune response in UM metastases may provide information to optimize immunotherapies in this disease.

Herein, we studied the lymphocytes present in the metastases as well as in the juxta-tumoral liver. We also characterized the circulating lymphocytes in peripheral blood (PBL) before and after hepatic metastasectomy. We studied 30 UM patients whose primary tumor had been treated by enucleation (14) or radiotherapy (16). Infiltrating lymphocytes were isolated from liver samples by enzymatic digestion. Both liver lymphocytes and PBL were analyzed by flow cytometry using a panel of 13 antibodies.

CD8+ T-cells were in similar proportion in juxta-tumoral liver and metastases. In contrast, the proportion of CD4+ T-cells, notably the Tregs and the chronically activated effector CD4 T-cell subsets were increased in the metastases. MAIT cells were abundant in the healthy liver but were underrepresented in the metastases. NKT cells, which are rare in the blood, represented up to 4% of the lymphocytes in juxta-tumoral and metastatic liver.

Thus, liver metastases are infiltrated with effector CD4 T-cells that dilute out the resident MAIT cells. No obvious change in the phenotype and proportion of CD8 T-cells was observed. The increased proportion of Treg may be implicated in the absence of productive anti-tumor immune response in UM patients.

F.44. Inter- and Intra-Laboratory Variability of Polyfunctional Responses Assessed by Intracellular Staining Assay as Part of the External Quality Assurance Program Oversight Laboratory Flow Cytometry Proficiency Testing Program

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Polyfunctional ICS responses play a significant role in the context of immune monitoring to evaluate vaccines and immunotherapies. Studies suggest that polyfunctional ICS responses are associated with immunologic control of HIV and represent correlates of protection post vaccination. Additionally, many laboratories are currently utilizing ICS assays to profile anti-tumor responses in the context of cancer vaccine trials and novel immunotherapies.

While polyfunctional cells are routinely measured in the context of clinical trials, there is a paucity of data describing the variability of these measures between and within laboratories. A limited number of proficiency programs assess the performance of ICS assays across laboratories, albeit these programs have focused primarily on assessing total functional ICS responses, rather than polyfunctional responses.

The EQAPOL Flow Cytometry Program assesses the proficiency of NIH/NIAID/DAIDS-supported laboratories in performing ICS assays. The EQAPOL Program currently assesses site performance of ICS assays based on total functional responses. Introducing an evaluation of polyfunctional ICS responses represents the next logical step for advancing the EQAPOL Program. Therefore, we devised a pilot study to determine the feasibility of assessing polyfunctional ICS responses as part of the EQAPOL Flow Cytometry Proficiency Testing Program. EQAPOL compared total functional and polyfunctional ICS data, collected across three proficiency testing surveys with an average of 16 participating laboratories. Respectively, inter- and intra-laboratory variance estimate ranges were 0.000-0.032 and 0.000-0.005 for polyfunctional and 0.008-0.035 and 0.000-0.009 for total cytokine ICS responses. Overall, the inter- and intra-laboratory variability of polyfunctional ICS responses were similar to total functional ICS responses.

F.45. Comparative Study of Human and Cynomolgus T-cell Depletion with Rabbit Anti-Thymocyte Globulin (rATG)

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Rabbit-antithymocyte globulin (rATG) is commonly used in solid organ transplantation to prevent and treat allograft rejection. Also, immunosuppressive drug is used for graft to permit long-term survival in animal transplantation model. Although rATG is also commonly used in non-human primates, the optimal dose has not been reported. In this study, we evaluated which cumulative dose of rATG was most appropriate for transplantation in non-human primate, compared to clinical cases.

In this study, cynomolgus monkeys were treated with 5 mg/kg of rATG (Thymoglobulin®, Genzyme Ltd., UK) intravenously twice (totally 10 mg/kg, n=2) or 4 times (totally 20mg/kg, n=4). Their peripheral blood were collected and analyzed by flow cytometry. Also their subpopulation of T cells like effector memory cells, central memory cell and naïve T cells were analyzed serially. And, to assess the effect of T-cell depletion in lymphoid organ, their lymph node and spleen were also collected and analyzed by immunohistochemistry at the indicated time points.

The absolute number of lymphocytes were decreased rapidly at day 1 and then maintained for 2 weeks in 20mg/kg rATG group but not maintained in 10 mg/kg rATG group. Experimental data of lymphoid tissues demonstrate that rATG monotherapy with 20mg/kg affects T cell-depletion, especially in spleen, although Lymph node was weakly affected by rATG induction. During the early period of rATG treatment, the pattern of changes in T cell subpopulation in 20mg/kg group showed similar with that in kidney transplant patients treated with ATG (totally 4.5 mg/kg). Therefore, we performed kidney transplantation in non-human primate with 20 mg/kg rATG induction and compared these to the results of a human KT.

These data show that lymphocyte-depletion induced by rATG was influenced by the cumulative dose, and show that an rATG dose of 20 mg/kg is suitable for induction therapy for renal transplantation in the cynomolgus monkey when compared to human kidney transplantation.

F.46. DAFi: A Novel and Robust Auto-Gating Approach to Computational Identification of Cell Populations from High-Dimensional Flow Cytometry Data

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Background: Manual gating analysis of cytometry data is subjective, irreproducible, and inaccurate. The dimensionality of cytometry data keeps increasing, making auto-gating methods both desirable and essential. However, the assessment and validation of the results from auto-gating methods is highly challenging, making the adoption of auto-gating approaches nontrivial.

Methods: This work presents a new computational approach – DAFi (Directed Automated Filtering and Identification of Cell Populations) for robust auto-gating analysis of high-dimensional flow cytometry data. DAFi is designed and implemented with multiple auto-gating options, including clustering, bisecting, slope-based, reversed-gating, and their combinations to identify different types of cell populations defined in user manual gating strategy. Through mapping high-dimensional data clusters into user-defined gating hierarchy, DAFi not only preserves the data clusters generated in unsupervised learning, but also makes the results easily interpretable as in supervised methods.

Results and Conclusions: Both quantitative assessment and visual examination of dot plots are used to evaluate the performance of DAFi against manual gating analysis across a variety of translational research projects as well as the diagnostics of blood cancers (AML and CLL). The results show that DAFi not only preserves natural shapes of both major and small lymphocyte subsets in an interpretable and reproducible way but also identifies novel cell subsets that differ between subject groups, which manual gating cannot find. Based on the results, we conclude that DAFi is highly promising to substitute manual gating for reproducible and exploratory analysis of high-dimensional flow cytometry data for biomarker identification in translational and clinical immunology research.

Immunity & infection

F.51. Acute Influenza a Virus (IAV) Infection in Humans Leads to Expansion of Highly Diverse CD8 T Cell Repertoires Crossreactive with Persistent Epstein Barr Virus (EBV)

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The competence of T cell responses predominantly depends on how efficient T cell receptors (TCRs) are at recognizing antigenic epitopes. We show here that during acute severely symptomatic IAV infection there was an expansion of IAV-M1/EBV-BRLF1 and IAV-M1/EBV-BMLF1 double-tetramer+ cells directly *ex vivo* in 5 HLA-A2+ patients. We questioned whether this expansion specific to these two different crossreactive responses would lead to alterations in the TCR repertoire of the IAV-M158, EBV-BRLF1109 and -BMLF1280 from before, during and following acute IAV infection. Using staining with VB mAb we found that T cell responses generated to these epitopes became surprisingly more polyclonal, with the sharing of Vb between M1, BMLF1 and BRLF1 populations which is not seen in healthy donors and which decreased 2 months later consistent with crossreactive expansion. Furthermore, by using single-cell analysis of TCRα and TCRβ repertoire of tetramer sorted IAV-M1 cells we showed dramatic changes in specific clonotype usage and in JA and JB family usage during acute IAV infection compared to before infection. In summary, these changes in TCR repertoire during acute symptomatic IAV infection suggest that during severe infection there is a preferential expansion of highly diverse crossreactive responses between IAV and the persistent virus, EBV, which leads to permanent changes in TCR repertoires to both of these two viruses (NIHAI49320).

F.56. Analysis of Genetic Determinants of Healthy Immune Responses to Common Pathogens reveals Associations with HLA Class II Molecules

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Healthy humans are regularly infected with pathogens that have the ability to persist through life. Our hypothesis is that host factors influence (i) the rate of seroconversion upon exposure, (ii) the intensity and characteristics of immune responses. To test this, we used a systems biology approach, integrating serological testing, genome-wide association studies (GWAS) and extensive immunophenotyping in 1000 healthy individuals recruited by the *Milieu Interieur* Consortium. Blood composition was assessed by flow cytometry. Serums were used for measurement of IgGs against (i) persistent or recurrent viruses - Cytomegalovirus (CMV), Epstein-Barr virus (EBV), Herpes simplex virus 1 & 2 (HSV-1 & 2), Varicella zoster virus (VZV), and Influenza A virus; (ii) persistent bacteria - *Helicobacter pylori* (HP), (iii) persistent parasite - *Toxoplasma gondii*, and (iv) pathogens targeted by vaccines - Measles, Mumps, Rubella, and Hepatitis B virus. Illumina Omni Express and HumanExome arrays were used for genotyping. After imputation with IMPUTE2, logistic or linear regressions were performed to detect associations between 5 million human polymorphisms, immune phenotypes and antibody responses. No genome-wide significant associations were found for serostatus. In contrast, genome-wide significant associations ($P < 5*10^{-8}$) were observed within the MHC locus on chromosome 6 for the levels of IgG mounted against EBV and Rubella. By imputing classical HLA alleles and amino-acids, we found that these associations correspond to variations in amino acid composition of HLA-DRβ1 and HLA-DPβ1 molecules respectively. Together, our results provide new possible insights into mechanisms determining response to persistent pathogens, and encourage further genetic and functional work.

F.57. Differential TCR Vbeta Usage in the Peripheral T Cells of Chickens Resistant and Susceptible to Marek's Disease

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Marek's disease (MD) is a herpesvirus-induced lymphoma in chickens with a significant economic impact to the poultry industry, costing the industry over $1 billion annually worldwide. MD is controllable by vaccination and improving genetic resistance in the host. Two inbred layer lines, matched at the MHC locus, have been bred for high (Line 6_3) and low (Line 7_2) genetic resistance, respectively, to MD. We identified large differences in TCR Vbeta usage in the peripheral T cells of these bird lines by flow cytometry. In the chicken, two families of Vbeta genes have been identified (Vbeta-1 and Vbeta-2). While TCR Vbeta-1+ T cells are more prevalent in both lines than TCR Vbeta-2+, MD-resistant birds used the Vbeta-2 TCR at very low rates. During infection with Marek's disease virus, Vbeta usage on CD4+ T cells was stable in both lines until day 21 post-challenge, when lymphomagenisis is occuring. Conversely, an increased bias towards Vbeta-1 TCRs was found as early as day 8 in CD8+ splenocytes in the MD-resistant chicken only. We hypothesize that differences in TCR repertoire may play a direct role in CD8+ T cell-mediated resistance to MD-induced tumors. We are comparing TCR usage in an additional MD resistance model which varies only at the MHC locus; as well as in a panel of Lines 6_3x7_2 recombinant strains, in order to further establish the importance of TCR usage in MD and to identify genomic regions which influence TCR expression in the chicken.

F.58. Role of STAT-1 and STAT-6 Factors in Susceptibility and Lung Pathology During Toxocara Canis Infection

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BACKGROUND: Toxocariasis is a worldwide zoonotic parasitic disease caused by *Toxocara canis*. Infection is caused by accidental ingestion of embryonated eggs, which hatch and the liberated larvae migrate to different organs. In murine models causes transitory hemorrhagic pulmonary lesions associated with strong TH2 responses and heavy parasite burdens. Alternatively, activated macrophages (aaM), through STAT6 signaling, are involved in tissue repair, but its role in *T. canis* infection has not been determined yet. On the other hand, STAT1 has been clearly related to protection in protozoan infections, but its role on helminths is poorly known.

METHODS: Here we tried to recognize the role of STAT1 and 6 molecules in this infection by using $STAT6^{-/-}$ and $STAT1^{-/-}$ BALB/c mice in determining the outcome of experimental toxocariasis. Mice were infected with 500 larvated eggs, and specific antibodies, serum cytokines, parasite burden and lung pathology were analyzed at different times.

RESULTS: STAT6+/+ and STAT1-/- mice displayed a Th2 immune response and their lung pathology were less evident compared to STAT6-/- mice, which displayed enhanced Th1 response but more damage in the lung. These differences in lung pathology were associated with the presence of aaM. In the other hand, parasite burden in STAT1-/- and STAT6-/- mice decreased as compared with wild type mice.

CONCLUSION: Our data suggest that the absence of one or another response would contribute to eliminate this parasite, but the presence of aaM is necessary to control tissue damage.

F.59. Increased Expression of Co-Inhibitory Receptor 2B4 (CD244) on CD4+ T cells in Human Septic Patients

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The SLAM family receptor 2B4 is mainly expressed on NK cells and memory CD8 T cells. In our previous research, we found that 2B4 was upregulated on both CD4+ and CD8+ T cells after sepsis induction in animals. Importantly, 2B4 on CD4+ T cells played the critical role in murine sepsis survival. However, the expression and functionality of 2B4 on human CD4+ T cells remain largely unclear. In this study, we investigate the expression, phenotype, and functionality of 2B4+CD4+ T cells isolated from septic patients and healthy donors. First, while no differences in 2B4 expression were observed on CD8+ T and NK cells, we found that CD4+ T cells isolated from human septic patients expressed significantly more 2B4 as compared to those obtained from healthy donors ($P < 0.01$). Notably, 2B4+CD4+ T cells isolated from septic patients were CCR7-CD45RA+ and displayed increased PD-1 and decreased ICOS and CD28 expression. Second, human 2B4+CD4+ T cells secreted less IL-2 and IFN-g following 6 hours of anti-CD3 anti-CD28 bead stimulation as compared to 2B4-CD4+ cells. Finally, 2B4-CD4+ and 2B4+ CD4+ subsets were sorted from healthy donor PBMC and stimulated with anti-CD3 and anti-CD28. 2B4+CD4+ cells were able to proliferate and maintained 2B4 expression, but had less proliferative capacity compared to 2B4-CD4+ cells. In conclusion, increased 2B4 expression was found on CD4+ T cells isolated from septic patients and these 2B4-expressing CD4+ T cells exhibited reduced cytokine secretion ability and proliferative capacity, suggesting 2B4 expression may compromise CD4+ T cell functionality during sepsis.

F.60. Core Transcriptional and Functional Signatures Define Human Tissue-Resident Memory T Cells in Lymphoid and Mucosal Sites

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Resident memory T cells (TRM) have been identified in mouse models as having rapid protective capacities to site-specific pathogens and are key targets for vaccine-mediated protection. TRM-phenotype cells are detected in human tissues but defining characteristics of human TRM for CD4 and CD8 T cells including how TRM differ from circulatory subsets and potential mechanisms for site-specific targeting are not defined. We performed whole transcriptome profiling of memory T

cell subsets from the lung, spleen and blood of 3 donors, for both CD4 and CD8 subsets, as well as in-depth phenotypic and functional profiling of memory T cells from 63 additional donors. Our results show that TRM are a transcriptionally distinct memory T cell subset in humans that can be identified by CD69 expression. We identify 30 core genes that define human TRM including the adhesion markers ITGA1 and ITGAE and homing receptors CXCR6 and CX3CR1, and we confirmed the surface expression of these genes and others by flow cytometry. TRM upregulated several cytokines transcriptionally and had a superior ability to produce IL-2, IL-17, and IFNg during stimulations compared with circulatory memory T cells. Interestingly, TRM showed enrichment of cell cycle inhibition pathways, a result which was supported by reduced Ki67 expression in TRM. TRM also upregulated IL-10, DUSP6, and PD-1, together suggesting that TRM may exist in an inhibited state to prevent inflammation-mediated tissue damage. Overall, our data comprehensively characterize CD4 and CD8 human TRM and provide new insights into how TRM establish residency and mediate protection in humans.

F.61. Tissue-Reservoirs of Anti-Viral T Cell Immunity in Persistent Human CMV Infection

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T cell responses to viruses are initiated and maintained in tissue sites; however, knowledge of human anti-viral T cells is largely derived from blood. Cytomegalovirus (CMV) persists in most humans, requires T cell immunity to control, yet tissue immune responses remain undefined. Here, we investigated human CMV-specific T cells, virus persistence and CMV-associated T cell homeostasis in blood, lymphoid, mucosal and secretory tissues of 44 CMV seropositive and 28 seronegative donors. CMV-specific T cells were maintained in distinct distribution patterns, highest in blood, bone marrow (BM), or lymph nodes (LN), with the frequency and function in blood distinct from tissues. CMV genomes were detected predominantly in lung and also in spleen, BM, blood and LN. High frequencies of activated CMV-specific T cells were found in blood and BM samples with low virus detection, while in lung, CMV-specific T cells were present along with detectable virus. In LNs, CMV-specific T cells exhibited quiescent phenotypes independent of virus. We observed differential responses of CMV-specific T cells in distinct tissues to stimulation by CMV antigen. Additionally, certain epitope-specific T cell populations in BM were tissue-resident in phenotype (CD69+) and localized to the BM, while others were TEM (CD69-) and circulatory. Polyclonal T cell differentiation was enhanced in sites of viral persistence with age. Together, our results suggest tissue T cell reservoirs for CMV control shaped by both viral and tissue-intrinsic factors, with global effects on homeostasis of tissue T cells over the lifespan.

F.62. Adenosine DeAminase (ADA) as an Adjuvant Molecule for Human HIV-1 Vaccine

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Follicular helper T cells (Tfh) play critical role in shaping, instructing, and initiating T-cell dependent antibody responses. Understanding the underlying mechanisms that enhance their function is therefore critical for vaccine development. Using a unique gene array analysis, we have identified adenosine deaminase (ADA), as a novel key molecule that drives Tfh helper program in proliferating Germinal Centers Tfh (GC Tfh) and circulatory Tfh (cTfh) cells following their interactions with B cells. In fact, our gene array analysis showed that CD26 and ADA were exclusively up-regulated within the less efficient cTfh1 (CD4+CD45RA-CXCR5+CXCR3+) and cTfh2-17 (CD4+CD45RA-CXCR5+CXCR3+CCR6+/-) subsets, respectively. ADA enzymatic activity is significantly higher, as well, in cTfh2-17 than in the less-efficient cTfh1 cells. Exogenous ADA enhances the ability of Tfh cells to provide B cell help while inhibition of ADA activity by specific inhibitors impeded Tfh function and blunted antibody response. Our results further demonstrated that enhancement of Tfh function by ADA pathway could be due to increase in IL- 6 and decrease in IL-2 production in the co-culture, and maintenance of low extracellular expression of CD26. Moreover, blocking IL-2 in cTfh2-17 co-culture from virally suppressed HIV subjects (ST) showed a significant decrease of CD26 expression associated with a rescued helper capacity. Finally, *in vivo* use of recombinant ADA as an adjuvant in a DNA based HIV vaccine enhanced Tfh cells differentiation and enhanced anti-Env

humoral response and isotype class-switch. Thus, ADA activity fine-tunes Tfh helper program and deciphering how ADA and CD26 regulate the function of the cTfh subsets would benefit future vaccine adjuvant design.

F.63. Novel Role for T cell intrinsic MyD88 in the Regulation of HIF1a Dependent Metabolism and Oxidative Stress Response

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Previously, we showed that TLR/IL-1R family signaling adaptor, MyD88, plays a crucial cell-intrinsic role in the survival of activated T lymphocytes. However, the underlying mechanisms by which this occurs remained unclear. Here, we demonstrate that MyD88-deficient T cells undergo p53 activation and ROS mediated DNA damage that results in their apoptosis by 72h post activation. This occurs despite downstream activation of NFKB and its anti-apoptotic targets Bcl-2 and Bcl-xl. Importantly, excessive ROS generation was accompanied by the destabilization of HIF1a, and the downregulation of its target, pyruvate dehydrogenase kinase 1 (PDHK1) that normally inhibits pyruvate dehydrogenase (PDH), the first rate-limiting enzyme of the TCA cycle. Interestingly, MyD88-deficient T cells showed redirection of glucose towards the pentose phosphate pathway, a process that generates nucleotides and reducing equivalents such as NADPH, a critical step that in-turn produces anti-oxidants to counterbalance this altered redox state. Nonetheless, despite increased compensatory anti-oxidant production, MyD88-deficient T cells remained sensitive to oxidative stress indicating *that the abnormal metabolic rewiring in the absence of Myd88 potentiates a "futile redox cycle" in T cells*. Furthermore, pharmacological intervention to either enhance HIF1a stability or block ROS generation by ETC complex-I inhibition rescued the survival of MyD88-deficient T cells. Conversely, silencing MyD88 in multiple tumor cell lines, that metabolically resemble T lymphocytes, destabilized HIF1a and prevented tumor growth *in vivo.* Together, these findings indicate a non-canonical role of MyD88 linking metabolism, redox balance and genomic stability, and highlight the therapeutic potential of MyD88 inhibition in sensitizing tumor cells to oxidative stress-induced apoptosis.

T.46. Distinct role of Estrogen Receptor α Agonist and Antagonist on Urinary Tract Infection Outcome in Bladder Versus Kidney

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Menopause induced hormonal changes in women increase their susceptibility to urinary tract infections (UTIs). FDA approved topical vaginal estrogen prophylaxis is effective in preventing recurrent UTIs but the underlying mechanisms are poorly understood. Using *in vitro* and *in vivo* UTI models, we found that estrogen via estrogen receptor α (ERα) alters host immunity in kidney in response to UTI by Dr fimbriae bearing Escherichia coli (E. coli). Time-point infection studies showed delayed interleukin-17 (IL-17) responses in kidneys of ERα gene knockout mice. The present study was conducted to determine the effectiveness of ERα agonist, propyl-pyrazole-triol (PPT), and ERα antagonist, methyl-piperidino-pyrazole (MPP), in bacterial clearance and IL-17 induction in bladder and kidney.

PPT-treated ovariectomized and MPP-treated ovary-intact C3H/HeJ mice were subjected to experimental UTI by E. coli. Bacterial load and IL-17 levels (ELISA and immunohistochemistry) in kidney and bladder were determined at 2 and 6 days post-infection. PPT induced significant decrease in bacterial load and significant induction of IL-17 in kidney (P) but on in the bladder. Blocking ERα by MPP compromised bacterial clearance and induction of IL-17 in kidney but showed significant bacterial clearance (P in bladder, however not via IL-17 induction. These studies confirm our previous findings for ERα in kidney where estrogen activates ERα to induce IL-17 production and bacterial clearance while bladder may

utilize other estrogen induced receptor mechanisms for protection that need further investigation. These results, showing differential effects of PPT or MPP in bladder and kidney, may have clinical implications for women undergoing hormonal therapy.

T.47. Metabolite Regulation of Human T Cell Immunity

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T lymphocyte activation is regulated by the metabolism of glucose, fatty acids and amino acids, allowing the cell to meet increased energetic and biosynthetic demands. We previously determined that exogenous nutrient availability regulates the terminal differentiation of murine CD4+ T cells into distinct effector fates. Activation of naïve murine CD4+ T cells under conditions of glutamine deprivation causes them to terminally differentiate into $Foxp3^+$ regulatory T cells (Treg) with potent *in vivo* suppressor function while blocking Th1 differentiation (Klysz et al., 2015). We now show that human CD4+ as well as CD8+ T cells are significantly more sensitive to extracellular glutamine than glucose, with deprivatio of the former resulting in a severely attenuated proliferation and defective upregulation of nutrient transporters. Furthermore, while the potential of T lymphocytes to secrete IL-2 is not markedly affected by glutamine deprivation, the secretion of IFNg is decreased by 5-10-fold. Notably, increasing intermediary glutamine metabolites in the tricarboxylic acid cycle significantly augments the potential of human T lymphocytes to transport extracellular nutrients, inhibiting Treg differentiation. Thus, nutrient availability and downstream metabolites can be used to modulate the T cell differentiation program and immune responsiveness.

T.48. Exacerbated immunodominance constitutes an important pathogenetic factor in CD4 T cell mediated diseases

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In murine schistosomiasis, previous observations identified that failure to modulate the anti-egg CD4 T helper response from a Th1-type to Th2-type, directly correlates with the disproportional immunodominance of the major egg antigen Sm-p40234-246 over the I-Ak MHCII antigen presentation pathway and severe liver immunopathology. To analyze the contribution of Sm-p40234-246 immunodominance to liver immunopathology, we developed a strategy to either enhance or reduce an epitope's immunodominance in the context of its I-A MHCII restriction, to verify the ensuing effects on immunopathology. This strategy revealed that the severity of the disease directly correlates with the degree of immunodominance of an epitope suggesting that exacerbated immunodominance constitutes an important factor in the genesis and severity of this, and maybe other murine and Human CD4 mediated diseases, which could be manipulated to prevent or modify their outcome.

T.49. Comparison of IgA+ Plasma Cells in the Intestinal Mucosa Compartment versus the Periphery in a Healthy Kenyan Cohort

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Little is known about the function of B cell lineages in the human intestinal mucosa, an important effector site for activated immune cells. Since cellular readouts on the peripheral blood mononuclear cells may not reflect the cellular phenotypes within the intestine, we analyzed IgA^+ plasma cells (PCs), the main B cell subset of the intestinal lamina propria, in a

healthy cohort of Kenyans with the goal of determining whether IgA$^+$PCs correlate with T helper cell subtypes in the intestinal mucosa versus in the peripheral blood. Peripheral blood, terminal ileum (TI) and sigmoid colon (SC) biopsies from healthy individuals under colonoscopy examination were collected and studied by flow cytometry. Compared with peripheral blood and TI, SC was found to be highly enriched in PCs, the majority of which were IgA$^+$PCs. We also observed that the frequency of IgA$^+$PC positively correlated with interleukin (IL)-17, interferon g, IL-22, and tumor necrosis factor a-producing CD4$^+$ T cells in the TI, but not in the SC or the peripheral blood. These results suggest that 1) in the SC and the peripheral blood, the T helper cell subsets we examined were not directly associated with the frequency of IgA$^+$ PCs; 2) in the TI, multiple cytokines produced by CD4$^+$ T cells have a significant impact on the abundance of IgA$^+$ PCs. Our next steps are to look at specific factors produced by IgA$^+$PCs including IL-10, Granzyme B and inducible nitric oxide synthase by immunofluorescence, and to correlate our FACS and immunofluorescence findings with gut permeability measurements.

T.50. CD8+CD28-CD127IoCD39+ Treg Expansion: a New Pathogenic Mechanism for HIV Infection?

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HIV-associated immunodeficiency is related to loss of CD4+ T cells. This mechanism does not explain certain manifestations of HIV disease such as immunodeficiency events in patients with >500 CD4+ T cells/ml or occurrence of non-AIDS tumours. CD8+CD28-CD127lowCD39+ T cells are regulatory T lymphocytes highly concentrated within tumor microenvironment. Here we show that HIV-infected patients have elevated circulating levels of functional CD8+CD28-CD127lowCD39+ T regulatory cells. These cells have antigen specificity against HIV proteins, suggesting their origin from HIV-specific T lymphocytes. Their frequency post anti-retroviral therapy (ART) correlates with HIV viremia, CD4+ T cell count and immune activation markers, suggesting their pathogenic involvement in AIDS- or non-AIDS related complications. Their increase after initiation of ART heralds a lack of virological or clinical response: hence their monitoring is clinically relevant.

T.51. SQSTM1/p62 influence the Macrophage and Splenic Autophagic-Proteasomal Degradation pathway with subsequent Immunological alterations during Cerebral Malarial Infection.

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Objective: Impact of *Plasmodium bergheei ANKA* infection on spleen and macrophages in mice model with special emphasis on the regulation of p62/sqstm1 molecule and it's impact on Nrf2-Keap1 pathway and subsequent effect on autophagy-proteasomal degradation pathways.

Methodology: Development of mice model of *Plasmodium bergheei ANKA*. Staining Spleen sections with Acridine Orange, MDC. Transmission electron Microscopy. Immunohistochemistry and immunofluorescence with LC3B, NRF2.

Quantitative Real time PCR, RT PCR & Western Blot analysis of the marker genes. DCFDA method of ROS measurement & GSH-GST assay, FACS for detecting autophagosome positive cells, immunoprecipitation. Proteasomal degradation assay by Promega luciferase assay kit.

Results: The parasitemia load in spleen is increased. The MDC punct formation and the acridine orange staining is positive. The ubiquitin level increased. Data suggests upregulation of LC3B and Beclin. The FACS with autophagic dye indicates autophagic process. The Proteasomal degradation levels are markedly higher. Upregulation ROS and change in the anti-oxidant enzyme level noticed. Change in Nrf2 – keap1 pathway is observed. Autophagic Flux flow is studied in every step of it. The autophagosome-lysosome fusion occurs and GFP-RFP-LC3B transfection experiment suggests a block in autophagic pathway. Macrophage cells also got affected. The phosphorylation status of p62/sqstm1 changed with subsequent impact on nrf2-keap1 pathway by binding with keap1 molecule.

Conclusion: The initial studies depicts experimental cerebral malaria definitely brings about change in the autophagic & proteasomal degradation status in mice spleen and macrophage. P62/sqstm1 plays a critical role in balancing the above two aspects and regulates nrf2-keap1 pathway.

W.30. Expression of Succinate Receptor and Glutaminolysis Enzimes in Pulmonary Tuberculosis

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Tuberculosis is an infectious disease that affects mainly the lungs, and represents a major health problem worldwide.

In recent years, the relationship between bioenergetics pathways and immune cell function has regained interest. It has been observed that glucolysis is enhanced during tuberculosis, nevertheless glucolysis alone is not sufficient to sustain metabolic demands, and other metabolic pathways have barely been studied during this infection.

We analysed the expression of key enzymes of glutamionlysis; glutaminase (GLS) and glutamatedehydrogenase (GLUD); as well as succinate receptor(GPR91) and succinilated proteins (lysine hydroxilase and carboxymethyl lysine) in experimental pulmonarytuberculosis and human postmortem lung samples.

In experimental tuberculosis, glutaminase (GLS) expression increases during both early and progressive infection, meanwhile glutamate dehydrogenase (GLUD) only increases during early infection, suggesting that glutamine is fully oxidized during early infection and partially oxidized during progressive infection. On the other side, succinate receptor GPR91 was overexpressed during early infection, and decreased on day 21 posti nfection, suggesting that succinate may act as a proinflammatory signal during early infection. It was also measured the expression of HIF1- α, which increased gradually during early infection until progressive infection.

In human postmortem lung samples, there is enhanced expression of GLS, GS, HIF-a, GPR91, lysine hydroxylase and carboxymethyl lysine in tuberculosis positive patients compared to controls.

The results from the experimental model suggests that during M. tuberculosis infection glutaminolysis increases during early infection and is associated with enhaced succinate signaling, probably through anaplerotic production of succinate, that may be partially explained byHIF-1α expression. Succinate may act as pro-inflammatory stimuli, that decreases during progressive infection when anti-inflammatory micro enviroment prevale, although partial glutamine oxidation is still present. These findings resemble to those in human samples.

W.31. Immune Responses in Acute and Convalescent Patients with Middle East Respiratory Syndrome During the 2015 Outbreak in Korea

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Understanding of immune responses against newly emerging acute respiratory infection, Middle East respiratory Syndrome (MERS), is important for the development of efficient treatment strategies and preventive measures. Here, we investigated several immune parameters using peripheral blood samples obtained from 26 hospitalized patients with different disease severities in their acute and convalescent stage. In general, the immune responses increased with enhanced disease severity. At acute stage, higher levels of inflammatory cytokines and chemokines were detected in the severe patients compared to mild cases. The increased percentages of CD8+ T cells were also detected to express the activation markers in severely ill patients. Moreover, when PBMCs were stimulated with overlapping peptides spanning whole virus structural proteins, distinctively high frequencies of MERS-CoV-specific CD8+ T cells were observed at acute stage of severe infection, while CD4+ T cell responses were not clearly detected at this stage. At the convalescent stage, antigen-specific cells were clearly identified in both T cells subsets, with the higher frequencies in severe diseases. Similar frequencies of CD8+ T cells directed against E, M, and N proteins as well as S protein. However, in CD4+ T cell subset, more cells responded to E, M, N proteins compared with S protein. Our findings highlight the potential contribution of human CD8+ T cell response to the pathogenesis of MERS-CoV induced respiratory disease. They also provide basic information for MERS-CoV vaccine design.

W.32. Pharmacological Induction of Heme Oxygenase-1 Activity Blocks Herpes Simplex Virus Infection

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Heme oxygenase-1 (HO-1) is a host inducible enzyme that responds to numerous stress-related stimuli, such as reactive oxygen species, hyperthermia, UV radiation and infections. HO-1 degrades iron-containing heme into ferrous iron (Fe2+), carbon monoxide (CO) and biliverdin. Noteworthy, these products elicit cytoprotective events that limit damaging oxidation within cells, as well as apoptosis. Moreover, recent studies reveal that HO-1 products also exhibit antiviral effects against several viruses such as Ebola, influenza and hepatitis C, among others. Here, we sought to assess the role of HO-1 over herpes simplex virus (HSV) infection *in vitro,* by pharmacologically modulating the activity of HO-1. Importantly, we found that inducing HO-1 activity with cobalt protoporphyrin (CoPP) significantly decreased HSV replication in human epithelial cells and neurons, even though equivalent amounts of virus bound to the surface of CoPP- and vehicle-treated cells and a similar quantity of virus entered the cytoplasm early after infection. Concomitant with reduced virus output, we observed decreased expression of HSV-encoded genes in CoPP-treated cells, as compared to untreated cells or cells treated with SnPP, an inhibitor of HO-1 activity. Remarkably, CoPP-treatment hampered viral capsid accumulation around the nucleus in HSV-infected cells, suggesting that HO-1 activity likely blocks viral genome delivery into the nucleus of virus-inoculated cells. These findings extend the antiviral effects of HO-1 onto HSV and suggest a novel strategy for treating infection with this type of viruses.

W.33. Immune Profiling of Vaccine-Specific Responses in Elderly Adults Using CyTOF

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A new high dimensional single cell mass cytometry approach called 'CyTOF', for Cytometry by Time-of-Flight, permits the simultaneous detection of ~ 40 phenotypic and functional immune markers in individual cells without the issues of spectral overlap seen in traditional flow cytometry. In this study we applied CyTOF to comprehensively characterize the circulating immune cell populations in elderly individuals both before and after administration of an exploratory adjuvanted protein vaccine in a Phase 1a trial. As previously reported, the vaccine cohort receiving the highest dose of soluble respiratory

syncytial virus (RSV) fusion (F) glycoprotein in a TLR4 agonist adjuvant emulsion formulation demonstrated antigen-specific cellular responses by IFNg ELISPOT. However, the CD4/CD8 contribution of the response was not determined and it was unclear why some subjects did not mount a detectable T cell ELISPOT response. Our approach was to use CyTOF to further interrogate the T cell response in this vaccine dose cohort. Participants showed both CD4 and CD8 IFNg responses following stimulation of patient PBMC with RSV(F) peptides. Several statistical techniques are being employed to analyze the differences between responders and non-responders. Using viSNE analysis, we found increased expression of CD4 and CD8 HLA-DR, CCR7, CD127 and CD69 in non-responders versus responders. Citrus analysis is currently underway to further explore differences between responders and non-responders. High parameter CyTOF can help profile immune components associated with differential vaccine responsiveness.

W.34. The Offspring Gestated in Hypothyroxinemia Develops a Poor Immune Response Against Respiratory Syncytial Virus

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Hypothyroxinemia (Hpx) is highly frequent condition in humans characterized by low levels of T_4 and normal levels of T_3 and TSH. We have shown that gestational Hpx significantly increases the severity of autoimmune encephalomyelitis experimental (EAE) increasing inflammatory cytokines in the blood, suggesting that the immune response could be altered in the offspring. We evaluated whether gestational Hpx could affect the immune response of the offspring against the infection with human respiratory syncytial virus (hRSV). We observed that the offspring gestated in Hpx and infected with RSV showed significantly weight loss, higher viral load and a higher infiltration of neutrophils correlating with a higher clinical score compared to the offspring gestated in euthyroidism. Moreover, it was detected a significant reduction in the infiltration of $CD8^+$ T cells into the lung, as well as in the production of IFN-g and cytotoxic capacity, but without changes in IL-4, IL-10 and IL-6 production. Finally, in *in vitro* cultures of splenocytes from mice gestated under hypothyroxinemia, we observed a lower secretion of IFN-g in response to virus N protein in comparison to splenocytes from mice gestated under euthyroid conditions.

The support the notion that the progeny gestated in Hpx has altered its immune response.

W.35. Studying the contribution of hRSV in the Susceptibility to Tuberculosis Development in Model Mycobacterium Bovis

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Human respiratory syncytial virus (hRSV) is the principal cause of childhood hospitalizations due to severe lower respiratory tract infection. HRSV infection alters the function of inmune cells that are primordial in the acquisition of immunity to pathogens, such as dendritics cells (DCs) and T cells. Consequences over the epithelial lung after immune response against hRSV infection and on the predisposition a susceptibility to a sub sequential infection still remain unknown. Here we evaluated the whether hRSV may generate a susceptible environment to sub sequential infection using as a model the infection *Mycobacterium bovis* which belongs to the *Mycobacterim tuberculosis* complex. Our data suggest the pre-infection with hRSV and then on tenth day post infection the mice is challenged with *M. bovis* increased the pathology in the lung. Importantly, this also observation also correlated with increased viral and bacterial loads, as observed by increased copies of *M. bovis* 16S rRNA gene, suggesting that clearance of infecting bacilli was also impaired due to hRSV pre-exposition. Futher, our results suggested that this poor anti-mycobacterial response is not due to a decrease in the T-cell response to mycobacterial antigens, but due to alterations in the functionality of APCs in the lungs of hRSV-infected mice.

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W.36. Characterization of the Human B Cell Response in Dengue Infected Children from India: The Massive Expansion of Plasmabalsts Parallels with the Timing of the Onset Of Severe Disease

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Epidemiological studies suggest that India has the largest number of dengue cases world-wide. B cell responses are important in dengue because they are implicated in both protection and immunopathology. However, plasmablasts, the major antibody producing B cells remain poorly characterized in dengue and virtually no information is available on human plasmablast responses in the patients from India. Here we provide a first comprehensive description of plasmablasts in dengue patients from India. Samples from a total of 108 children confirmed as dengue by dengue specific NS1, IgM and/or PCR assays were analysed by multicolour flow cytometry using CD19, CD20 CD38, CD27 surface markers; proliferation characteristics using intracellular Ki67 staining, and the dengue specific antibody response using functional ELISPOT assays. Our studies show that plasmablasts expand massively in these dengue patients which was associated with loss of surface IgD, down regulation of CD20, and expression of Ki67. A vast majority of these massively expanding plasmablasts secreted dengue specific IgG isotype antibody. In some patients plasmablast frequencies reached as high as 80% of the B cells, translating to more than a million plasmablasts/ml of blood. Kinetic analysis of the plasmabalsts and dengue disease severity was also performed by stratifying the patients based on the day of symptoms revealed that the timing of the peak of this massive plasmablast expansion parallels with the time when a majority of the patients present with severe dengue disease. These studies open novel avenues to understand the role of plasmablasts in human dengue

W.37. Recombinant BmHAXT Fusion Protein Vaccine Along with a Novel Adjuvant Confers Significant Protection Against Lymphatic Filariasis in a Mouse Model

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Currently there are no licensed vaccines to control lymphatic filariasis, a tropical parasitic infection that affects 120 million people in 73 countries worldwide. A trivalent r*Bm*HAT fusion protein vaccine developed in our laboratory gave 40% protection against challenge infections in rhesus macaques when given along with alum. In an attempt to improve the vaccine efficacy, in this study we constructed a tetravalent vaccine (r*Bm*HAXT) by combining *Bm* thioredoxin peroxidase 2 (*TPX2*) sequences to *Bmhat* and vaccine potential of r*Bm*HAXT were evaluated in a mouse model. Three different adjuvants formulations were compared to determine the best adjuvant for r*Bm*HAXT. Our results showed that following s/c immunization with four doses of the antigen, all immunized mice developed high titer of antigen-specific serum IgG antibodies. Isotype analysis revealed that alum adjuvanated vaccine promoted only IgG1 antibodies. However, TLR4 agonist (AL019) and mannosylated chitosan (MCA) adjuvanated r*Bm*HAXT induced significant levels of IgG1, IgG2a and IgG2b antibodies. Thus, AL019 and MCA appeared to promote a balanced Th1/Th2 response. Antigen recall response of splenocytes from vaccinated mice showed antigen-specific proliferation and secretion of significant levels of IFN-γ, IL-17 and IL-6. There was also significant increase in the antigen-specific T_{EM} cells in mice vaccinated with r*Bm*HAXT+AL019 and r*Bm*HAXT+MCA. Challenge studies showed that mice vaccinated with r*Bm*HAXT+MCA had maximum parasite killing (82%) compared to the controls (15%). Vaccination with r*Bm*HAXT+alum and r*Bm*HAXT+AL019 showed only 66% and

63.2% parasite killing respectively. These results thus showed that r*Bm*HAXT+MCA is an excellent and improved prophylactic vaccine against lymphatic filariasis.

W.38. Low Frequency HLA class II-Restricted CD8+ T cells in HIV-1 infection

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Conventional T cell paradigm dictates CD8+ T cell recognition of peptides in the context of MHC class I. However, a paradigm shifting study demonstrated that the induction of a novel subset of MHC class II-restricted CD8+ T cells was associated with clearance of SIV infection in rhesus macaques, whereas another study recently highlighted the presence of HIV-1-specific class II-restricted CD8+ T cells in a small subset of HIV-1 patients who naturally control infection (virus controllers; VCs). However, the distribution of these atypical CD8+ T cells in different HIV-1 disease states and their direct role in viral control are still unknown. In this study, we assessed the distribution and ability of MHC class II-restricted CD8+ T cells to suppress HIV-1 replication in autologous CD4+ T cells in different HIV-1 patient cohorts (VCs, chronic viremics and healthy donors) using MHC class II tetramer staining and MHC Blocking Viral Inhibition Assay (VIA), respectively. Six of the seven VC patients analyzed had memory Gag-specific MHC class II-restricted CD8+ T cells, with two of these six patients having persistent class II-restricted CD8+ T cells. Class II CD8+ T cell responses were also detected in chronic viremics at lower frequency than in VCs but were absent in healthy donors. The two VCs with persistent Gag-specific MHCII-restricted CD8+ T cells also exhibited MHCII-blockable antiviral activity, confirming the functionality of their $CD8^+$ MHCII-restricted cells. In summary, functional memory class II-restricted Gag-specific CD8+ T cells are present at low frequencies during natural HIV-1 infection.

W.39. Daptomycin Resistance Mutations in Staphylococcus aureus Affect Immune Recognition and Dendritic Cell Activation

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Methicillin-resistant *Staphylococcus aureus* (MRSA) represents an emerging public health threat, causing significant morbidity and mortality in both communities and the hospital setting. Resistance to last line therapeutic antibiotics is on the rise and correlates to an increase in both morbidity and mortality. Recently, whole genome sequencing revealed that mutations conferring resistance to last line antibiotics daptomycin and vancomycin affect the composition of the bacterial cell membrane and cell wall. Several of these mutations concomitantly conferred a dual resistance to host antimicrobial peptides.

Here we demonstrate that the acquisition of antibiotic resistance to daptomycin is associated with a decrease in immunological recognition by dendritic cells (DCs). As the chief antigen presenting cells, DCs form a critical adaptor linking the innate recognition of microbes with the induction of highly specific adaptive responses. Using a broad panel of soluble mediators and surface activation markers as indicators of DC activation, we show that daptomycin resistant strains of MRSA are less effective at activation of DCs than their isogenic clinical pair taken prior to commencement of antibiotic therapy. We further show that point mutations in genes responsible for phospholipid biosynthesis found in daptomycin-resistant MRSA strains play a critical role in modulating this immune recognition of resistant strains by DCs. These findings provide novel insight into the pathogenesis of MRSA infections and host immune interactions. Further work is underway seeking to identify the molecular mechanisms affecting DC activation as a consequence of genomic mutations in MRSA during the course of infection.

W.40. A Multivalent Fusion Protein Vaccine Confers Significant Protection Against Lymphatic Filariasis Infection in Rhesus Macaques

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Lymphatic filariasis currently affects 120 million people around the world and another 1.2 billion people are at risk. An effective prophylactic vaccine is needed to control and eliminate the infection from 72 countries that are endemic for this disease. In this study we compared and evaluated the immunogenicity and efficacy of two multivalent fusion proteins (r*Bm*HAT and r*Bm*HAXT) for their ability to protect rhesus macaques against challenge infections with *Brugia malayi.* A total of 40 macaques were divided into three treatment groups and one control group (N=10/group). Two different adjuvants, alum and alum adsorbed TLR-4 agonist (AL019) were used in this study at 2 mg/ml. vaccinated animals received four immunizations (days 0, 28, 56 and 84) with 1.5mg/ml of r*Bm*HAT+alum, r*Bm*HAT+AL019 or r*Bm*HAXT+AL019. Control animals received AL019 only. One month after the last dose, all macaques were challenged subcutaneously with 130-180 third stage larvae of *B. malayi*. Our results show that all vaccinated macaques developed significant ($p \leq 0.003$) titers of antigen-specific IgG antibodies (1:20,000) compared to controls. Specifically, levels of antigen-specific IgG1 and IgG2 antibodies were significantly ($p \leq 0.01$) increased. Analysis of the PBMCs, demonstrated increases in IL-4 and IFNγ secreting effector memory T cells in vaccinated animals. Challenge results showed that 60% of macaques vaccinated with r*Bm*HAT+AL019 and 50% of macaques vaccinated with r*Bm*HAXT+AL019 did not show any signs of infection or lymphatic pathology until week 18 after infection (end of the experiment). These studies demonstrate that *rBm*HATor *rBm*HAXT in combination with AL019 are promising vaccine candidates against lymphatic filariasis.

W.41. Testing the *in vivo* Efficacy of a Q Fever Vaccine using a Novel Tri-Agonist Compound Library

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Coxiella burnetii is a gram negative, obligate intracellular bacterium that is the causative agent of Q fever, a debilitating disease that can evolve into a fatal chronic infection resulting in endocarditis or neurological manifestations up to 20 years following the initial infection. *C. burnetii* is designated as a Category B pathogen due to its potential as a robust bioterrorism agent. Despite these facts, the only vaccine that currently exists is licensed in Australia and significant concerns about safety and efficacy necessitate the development of a safer and more effective vaccine. Our research group has developed a novel vaccine adjuvant system by conjugating three Toll-like receptor (TLR) agonists together to form a tri-agonist adjuvant. Using this tri-agonist adjuvant we have developed and tested novel *C. burnetii* vaccine candidates *in vitro* and *in vivo* for safety, immune stimulating activity and protection in a live *C. burnetii* infection challenge study. We have previously shown that our tri-agonist adjuvants elicit a more balanced Th1 and Th2 immune response and successfully increased antibody scope and diversity compared to non-adjuvanted vaccine candidates, suggesting downstream changes in immune signaling and adaptive immune activation. Here we show that our Q fever vaccination model, formulated with our tri-agonist adjuvant construct, elicits productive and effective adaptive immune responses in both a naïve vaccination model and a live *C. burnetti* infection. Our studies suggest that our novel *C. burnetti* vaccine could be an effective and safer alternative to the only vaccine currently available.

W.42. Sensing of Isoprenoid Metabolites by Human Vγ2Vδ2 T Cells is Critically Dependent on the Intracellular Coiled Coil Domain of Butyrophilin 3A1

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Vγ2Vδ2 T cells play important roles in human immunity to pathogens and in cancer immunotherapy by responding to isoprenoid metabolites such as (*E*)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate (HMBPP) and isopentenyl pyrophosphate. The immunoglobulin superfamily protein, butyrophilin 3A1 (BTN3A1), is required for this stimulation. HMBPP is not an antigen but instead is sensed by binding to the B30.2 domain of BTN3A1. How this binding is detected by Vγ2Vδ2 TCRs is unclear. BTN3A1 is a homodimer with extracellular IgV/IgC domains (ECD) linked to intracellular B30.2 domains by an intracellular coiled coil domain. HMBPP binding could alter the conformation of the BTN3 ECD through "inside-out" signaling allowing Vγ2Vδ2 TCR recognition. This could involve only BTN3A1 or all three BTN3 isoforms. We find that mutagenesis of 39 BTN3A1 ECD residues had no effect whereas mutagenesis of coiled coil residues either abrogated (mid-region) or decreased (proximal and distal regions) HMBPP stimulation. Thus, Vγ2Vδ2 TCRs do not appear to recognize BTN3A1 ECDs with intramolecular conformational changes. However, recognition of conformational changes in BTN3A2 and BTN3A3 ECDs induced by intermolecular interactions is possible. Alternatively, Vγ2Vδ2 TCRs may bind to the ECD of a protein recruited to the intracellular tail of BTN3A1 upon B30.2 binding to HMBPP. In all mechanisms, the coiled coil domain of BTN3A1 plays a critical role. Coiled coil regions are predicted for most butyrophilins with B30.2 domains and they likely function as essential rod-like helical scaffolds to allow sensing by B30.2 domains.

W.43. What Really is the Role of the Immune System?

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It is natural for investigators to round their perspectives around and through their subject of expertise, however, as useful as this can be to create inroads into a new field of exploration, in time, this practice tends to create a closed universe of restricted debate where concepts and conclusions self-support each other and progressively court researchers to look only towards the inside, not the outside. Thomas Kuhn called these closed "universes", paradigms, and stated that from time to time, they need to either fall under the weight of their own contradictions or an unsurmountable challenge so evolution to new directions can happen.

In the case of immunology and its bearing on health and science, the tendency has been towards the micro. Voluminous articles about transcription cascades, new subgroups of cells, new SNP associations, and mountains of genomic data have been published, and challenged, but still, regardless of this enormous amount of data, sometimes the feeling is that progress is minimalist. Very little actually changes. No new big questions arise.

Recently, an attempt to solve a research problem led to an interesting observation that resulted in a line of theoretical research that questions the tenets of what we currently teach about the immune system, for instance, that its role is to defend the organism against foreign invaders. What about its role in defending against tumors, which certainly may not be foreign? Or healing from autoimmune aggression? Or how to define disease or an autoimmune aggression? Do we have clear answers for that?

If one takes one step behind and states the obvious: that our organisms are matter and as such, are subject to the same laws that govern the traffic of matter and energy within this universe, we realize that biology is actually only a transvestite for what really defines life, death, health and disease: thermodynamics, and in this context, it becomes necessary redefine all our concepts under this perspective. In doing so, one gains new insights of what is Life, disease, why life exists, what exactly can a Doctor do for a patient and what really is the role of the immune system?

We propose a parallel thermodynamic approach to life sciences, and immunology in particular, where all definitions stem from the laws of thermodynamics. We believe this added perspective will significantly impact education, research and Medicine and/or, at the least, make the debate more interesting.

W.44. IFN-γ-based Stratification of Latent Infection and Active Tuberculosis Disease Using a Novel Whole Blood Stimulation System

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Tuberculosis (TB) is a global public health crisis with an estimated 1.7 billion latently infected individuals worldwide. An effective blood based biomarker test to diagnose patients with active TB disease is urgently needed. Current diagnosis depends on detection of bacteria by microscopy, culture or PCR in sputum. We tested the capacity of TruCulture, a novel whole blood collection and stimulation system, to stratify patients with active disease from latently infected individuals. Using *Mycobacterium tuberculosis* (*M.tb)* antigens, we stimulated whole blood from 25 active TB and 25 latently infected individuals. Supernatants were assessed by Luminex multi-analyte profiling and ultrasensitive digital Simoa ELISA, and RNA by Nanostring gene expression arrays. Utilizing *M.tb* antigen-induced IFN-γ as a diagnostic readout ROC curve analysis showed a better stratification of diseased from latently infected individuals using TruCulture (AUC=0.814) as compared to QuantiFERON-TB Gold (AUC=0.558). TruCulture resulted in significantly lower background IFN-γ secretion, which we interpreted as the reason for improved signal detection of antigen-specific responses. Utilizing Simoa (limit of detection = 11fg/ml) the majority of latently infected donors secreted less than 14 pg/ml of IFN-γ (QuantiFERON cutoff) following TruCulture *M.tb* antigen stimulation. Interestingly, hierarchical clustering analysis of gene expression data identified a subset of latently infected donors with high IFN-γ expression. Current investigations are examining whether this signature is indicative of near-term conversion to active disease. In sum, our study demonstrates the enhanced specificity of TruCulture versus the current gold-standard QuantiFERON test for diagnosis of active TB in areas where latent infection is highly prevalent.

W.45. Dendritic cells Challenged with a Glycoprotein D-deficient Herpes Simplex Virus Type 2 activate CD4+ and CD8+ T cells

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Herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) are prevalent in the human population and produce lifelong infections with frequent reactivations. Persistence in the host relies, within others on the evasion of innate and adaptive immunity and may result in pathology. Noteworthy, HSV interfere with the maturation and viability of dendritic cells (DCs), likely affecting the capacity of the host to mount effective adaptive immune responses against these viruses. An HSV-2 mutant that has the US6 gene deleted (encoding glycoprotein D, gD) and is phenotypically complemented with the gD protein on its surface, was previously reported to be safe, immunogenic and induce protective immunity against skin and genital challenges with HSV-1 and HSV-2 in animals (eLife;4:e06054,2015, doi: http://dx.doi.org/10.7554/eLife.06054). Here, we sought to evaluate whether this HSV-2 gD-mutant was attenuated in murine DCs and whether it was able to license these cells to activate T cells. We observed that, unlike wild-type (WT) HSV-2 and other glycoprotein-mutant viruses, the gD-deletant was innocuous to DCs and promoted both, their maturation and capacity to activate naïve CD8+ and CD4+ T cells. Furthermore, DCs exposed to the WT and gD-mutant virus experienced different unfolded protein responses. Taken together, these results suggest that the attenuated phenotype of the gD-mutant virus in DCs is particular to this virus and likely accounts for its significant immunogenicity *in vivo*, as well its vaccine-like properties. Assessing the virulence of HSV mutants in DCs may provide grounds for identifying novel attenuated viruses that are protective *in vivo*.

W.46. Low Level of Serum NGAL in MGUS Patients Could Contribute to Higher Risk of Infections

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MGUS is diagnosed in the patients when monoclonal protein is present in the blood with the concentration under 3 g/dL, clonal bone marrow plasma cells are under 10%, and other symptoms are absent. Despite the lack of any organ damage, and independently from the transformation to MM, MGUS patients have lower life expectancies and higher risks of bacterial infections and other condition like vein thrombosis, renal, heart and liver disorders.

NGAL is synthetized in the bone marrow and stored in the neutrophils in complex with gelatinase. The main function of NGAL is to bind to bacterial siderophores and by this way limit its growth. Therefore NGAL is recognized as the factor that mediates innate immune response and protects from infections.

The goal of the study was to assess serum and urine NGAL in 46 MGUS patients. The control group consisted of 23 healthy subjects matched for age and sex.

Serum and urine NGAL were measured with an immunoenzymatic method.

Serum level of NGAL in MGUS patients was lower than in healthy patients. This was not observed in case of urine NGAL. There was also no correlation between serum and urine NGAL.

Low serum level of NGAL could result in impaired innate immunology response and contribute to well-known higher risk for infections in MGUS patients. Obtained results do not allow to determine the cause of this phenomenon, however the lack of correlation between serum and urine levels of NGAL indicated that it is likely not caused by renal escape.

W.47. Recombinant BCG strain induces Protective Humoral Immune Response Against the Human Respiratory Syncytial Virus (HRSV) and Human Metapneumovirus (HMPV)

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Human Respiratory Syncytial Virus (hRSV) and Human Metapneumovirus (hMPV) are two of the leading etiology agents of acute lower respiratory tract infections (ALRTIs) worldwide affecting young infants, elderly and immunocompromised patients. Currently, many approaches for the licensing of a safe and effective vaccine have been unsuccessfully tested. We have previously described that immunization with recombinant Mycobacterium bovis Bacillus Calmette-Guérin (BCG) strains expressing the HRSV Nucleoprotein (rBCG-N) and HMPV Phosphoprotein (rBCG-P) are able to induce protection through a T helper 1-induced cellular immunity. We show that upon viral challenge, immunization with these recombinant BCG strains promotes protective humoral immunity, characterized by the production of antibodies against several viral proteins. We detected a consistent isotype switching from IgG1 to IgG2a, which correlated with an increased viral clearance. Sera obtained from animals immunized with the BCG vaccines showed virus neutralizing capacity *in vitro* and protected naïve mice from pathology, by preventing viral replication and pulmonary disease. Finally, we found an increase in the plasma cells subset and in other B cells types in the immunized animals. These results support the notion that the use of recombinant BCG strains could be considered as potential vaccination approach against respiratory viruses, such as hRSV and hMPV.

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W.48. Systemic lupus Erythematosus Patients with Infections Present Lower Expression of TLR2 In CD14+, CD16+ Monocytes: Preliminary Results of a Prospective Cohort Study

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Introduction: The immune system abnormalities in patients with systemic lupus erythematosus (SLE) may predispose them to the development of infections, which is one of the leading causes of morbidity and mortality. The objective of this study is to describe the immunologic factors related to infections development in patients with SLE.

Patients and methods. Fifty-two patients with SLE according to the ACR or SLICC criteria were prospectively followed-up during a median time of 6 (1-9) months. Clinical features were assessed at baseline, one and three months later. With multiparametric flow citometry we measured the percentage and absolute numbers of CD4+, CD8+, NK cells, monocytes subsets, T helper subpopulations, regulatory T cells and B lymphocytes as well as TLR2 mean fluorescence intensity and percentage and absolute number of monocytes subsets expressing TLR2.

Results: Eight patients (15.3%) presented an infection after a median time of 4.5 months. At baseline, patients with infections presented higher percentage of B cells (43.8 (21.2) vs 17.9 (16.3), p=0.029) and it correlated moderately with the development of infection ρ 0.5, p=0.028). Likewise, they had lower baseline percentage of CD14+/CD16+ monocytes expressing TLR2 (78.9 (29) vs 97.4 (4.2), p=0.007). In the following three months, patients with infections had higher percentage of Th17 cells (0.33 vs 0.09%, p=0.002).

Conclusions: Immunologic abnormalities such as low percentage of TLR2 positive CD14+/CD16+ monocytes, higher percentage of B-cells at baseline and higher Th17 cells three months later are more frequently present in lupus patients that developed infections during prospective follow-up.

W.49. Ratio between HBHA- and ESAT-6-induced Polycytotoxic CD4+ T cells, a New Biomarker for LTBI

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Tuberculosis (TB) remains the leading cause of death from a curable infection with 1.4 million TB deaths reported in 2015. *Mycobacterium tuberculosis* (Mtb) is so infrequently cleared from its host that an estimated one-third of the world population is latently infected (LTBI), most often for life. Five to 10% of LTBI individuals will, at some point, convert to active TB (aTB). Finding a biomarker that can discriminate LTBI from aTB is absolutely essential if the WHO's End TB Strategy target of a 90% reduction in TB deaths by 2030 is to be achieved.

We developed a 7-day *in vitro* PBMC stimulation assay in which we used lymphoblast formation, measured as FSC/SSC changes observed by flow cytometry, as a measure for T lymphocyte activation and proliferation. Such 7 days stimulation of PBMC from Mtb infected individuals (n=27), with the mycobacterial antigen heparin-binding hemagglutinin (HBHA) induced degranulation of $CD4^+$ lymphoblasts, measured by the surface expression of CD107a, whereas PBMC from healthy, uninfected individuals (n=10) did not ($p < 0.001$). Furthermore, we demonstrated that HBHA stimulation induced proliferation of $CD4^+$Polycytotoxic T cells (PCTL), releasing granulysin, granzymes, perforin and IFN-gamma simultaneously, in LTBI individuals (n=9), while significantly less PCTL proliferation was observed in aTB individuals (n=8). Conversely, the 6 kDa early-secretory-antigenic-target (ESAT6) induced PCTL proliferation in aTB individuals but not in LTBI. In fact, the ratio of PCTL induced by HBHA to PCTL induced by ESAT6 allows for the discrimination of LTBI and aTB individuals ($p < 0.001$) , representing, thus, a novel LTBI biomarker.

W.50. Phenotypic Changes in CD4+T cells Associated with Antiretroviral Initiation in HIV associated Cryptococcal Meningitis.

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Initiation of ART has been associated with immune recovery, however without a clear understanding of its immunological effects on the $CD4^+$T cell repertoire. Our aim was to determine phenotypic changes in the $CD4^+$T cell subsets following ART initiation in HIV associated Cryptococcal Meningitis (CM). We hypothesized that ART alters the clonotypic phenotype and structural composition of $CD4^+$T cells in HIV-1 patients with CM.

Phenotypic characterization of $CD4^+$T cells isolated from a pool of PBMC samples from 29 HIV-1 participants was done by flow cytometry after *in vitro* stimulation using relevant antigenic preparations.

Our results showed a variation in the expression of immunophenotypic markers over the time points delineating an equipoise in the central memory ($CD27^+CD45R0^+$ p=0.340), reduction in immune activation ($CD38^+$,HLA-DR^+ p=0.113), effector memory markers ($CD45R0^+$,$CD27^-$ p=0.02) and exhaustion (PD-1, p=0.08). In comparison to the $CD8^+$T cells, markers of central memory declined gradually with trivial increases in the effector memory markers. Immune exhaustion and activation markers remained elevated throughout the time points.

The relative surge and decline in the expression of T cell surface markers outlines that the effect of ART on the differentiation state of $CD4^+$T cells. We noted that ART maintains the central memory pool while suppressing the virus using the effector memory subsets. We concluded that complete $CD4^+$T cell recovery after ART is a process that typically requires many years. Meanwhile, the $CD8^+$T cell pool does not seem to be influenced by the homeostatic forces associated with $CD4^+$T cell depletion.

W.51. In situ Determination of MIF and CD74 in Skin Lesions of Lepromatous Leprosy Patients

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Leprosy is a chronic disease caused by Mycobacterium leprae that affects the skin and peripheral nerves; it may present as one of two distinct poles: the self-limiting tuberculoid leprosy and the highly infectious lepromatous leprosy (LL) characterized by M. leprae-specific absence of cellular immune response. The proinflammatory cytokine Macrophage Migration Inhibitory Factor (MIF) enhance the bactericide activities of macrophages after interaction with its receptor, CD74. MIF-mediated activation of macrophages is a key process for elimination of M. tuberculosis; however, its participation for the clearance of M. leprae is unclear. Particularly, it has not been studied in skin lesions of LL. The aim of this study was to evaluate MIF and CD74 in skin lesions of LL and compare it with healthy skin (HS) taken from subjects attending to dermatological consult. 37 biopsies of LL and 10 biopsies of healthy skin were analyzed by immunohistochemistry. Smears were observed in twelve 100X microscopic fields, in which percentage of stained cells and staining intensity were evaluated. Both variables were used to calculate a semiquantitative Score that ranged from 0 to 3+. Differences between groups were analyzed using Kruskar-Wallis test and Dunn-Bonferroni as post-hoc. We found no differences in MIF expression between the skins of LL and HS. We found CD74 Score statistically higher in LL skin than HS ($p < 0.001$); this was the result of a higher percentage of cells positive for CD74 ($p < 0.001$). As a conclusion, CD74-positive cells are intensely recruited to skins where lesions due to LL are observed

W.52. Immunological Aspects of the Periapical Lesions

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The therapy of the periapical lesions is a complex intervention, aiming to stimulate the reparatory processes of the periapical tissues. When the periapical lesions are treated surgically, the level of the immunosuppression depend on the complexity of the surgical intervention. The aim of this paper is to prove that there is a presence of the cytokines in the

serum after the surgical and the endodontic treatment of these lesions. The research is conducted at 30 patients of different gender, with different scale of periapical lesions. At 15 of the patients is conducted the endodontic, and at the other 15, the surgical therapy. Before the therapy, analysis are made of the serum levels of the cytokines. 24 hours after the conducted treatment, the patients of both groups are tested, in order the serum levels of the cytokines to be registered. We determine the level of the cytokines with the use of a serum, by the method Elisa (Enzyme Linked Immunosorbent Assay). The acquired results of the endodontic treatment has shown statistically significant increase. After the surgical treatment, their values were increased too. This points to the fact that the tissue trauma is a cause for increasing of the levels of the cytokines, which are responsible in the systemic immunological response. It is an imperative that the decision of this issue should favor the endodontic procedure, and to minimize the invasive surgical method.

W.55. Contribution of Natural Killer T cells to the Pathology Caused by the Respiratory Syncytial Virus and the Metapneumovirus

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Natural killer T (NKT) cells are unconventional T cell lymphocytes. The NKT cell receptors recognize glycolipids bound to the MHC-I-like CD1d molecule. The synthetic α- Galactoceramides (α-Galcers) loaded onto CD1d has been shown to be a potent NKT cell activator, thereby these glycolipids have been explored as modulators of the immune response. Here, we describe the effect of α-Galcer on anti-viral immunity during the infection by the Human Respiratory Syncytial Virus (hRSV) and the Human Meptaneumovirus (hMPV). BALB/cJ mice were treated with α-Galcer and challenged either with hRSV or hMPV. α-Galcer treatment induced: 1) NKT proliferation in the lungs, spleen and LNs, 2) a significant reduction of neutrophil cells infiltration and loss weight amelioration in hMPV- but not in hRSV-infected mice and 3) a $CD8^{+}$T cells recruitment to the lungs in the hRSV-infected mice. Preliminary data with BALBc CD1d-KO mice suggest that NKT cells can be important for the immune response against hMPV infection. To better understand as to how NKT cells modulate T cell immunity during in these infections, DCs were infected with hRSV or hMPV, pulsed with different concentrations of α-Galcer and co-cultured with NKT cells. While stimulation by hRSV-infected DCs led to reduced levels of IL2 secretion by NKT cells, hMPV-infected DCs promoted a significant IL2 secretion by NKT cells as compared to mock-treated DCs. Further, the observed increase of IL-2 by NKT cells stimulated with hMPV-infected DCs was α-Galcer dose dependent. Our data suggest that α-Galcer modulates NKT cell function, decreasing the exacerbated inflammation associated with hMPV but not for hRSV infection and modulating the IL-2 production in NKT cells co-cultured with hMPV-infected DCs.

W.56. IgA Deficiency Induces Spontaneous Inflammation in the Ileum

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Background & Aim: Immunoglobulin (Ig) A is known to be involved in the maintenance of mucosal homeostasis. It has been reported that deficiency of activation-induced cytidine deaminase results in aberrant composition of commensal flora in the gut. However, it is still unclear whether such phenotype was directly caused by the lack of secreted IgA. Therefore we created an animal model of IgA deficiency ($IgA^{-/-}$) by using the CRISPR/Cas9 system.

Methods & Results: The guide RNAs specific for IgE and IgA cytoplasmic domains and Cas9 mRNA were injected into C57BL/6 zygotes. PCR products corresponding for the region between IgE and IgA tails from the offsprings were applied for sequencing. Subsequently we found that one of them had a deletion of over 5kb region coding the entire IgA allele and

thus identified as $IgA^{-/-}$. To characterize this line, we analyzed the histopathology at 12 weeks old. Although there were no significant changes in most of organs, we found irritated mucosa at the ileum compared to the littermate control. Flow cytometry analysis showed increased $CD4^+$ T cells in the intestinal lamina propria associated with increased production of IFN-γ and IL-17 in the $IgA^{-/-}$. Furthermore, scanning electron microscopy revealed an increase of segmented filamentous bacteria on the ileal epithelia in the $IgA^{-/-}$ compared to the control.

Conclusion: Our current results imply that the lack of IgA may induce disregulated mucosal homeostasis in the gut and altered commensal flora in the intestine. Additional experiments are currently ongoing.

Immunodeficiency: primary or acquired

F.64. Phenotypic and Functional Defects Underlie Ineffective Control of EBV in Patients with Distinct Primary Immunodeficiencies

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$CD8^+$ T-cells are largely responsible for controlling infection caused by the common herpes virus EBV. Indeed, due to the efficacy of $CD8^+$ T-cells, primary EBV infection in healthy individuals is often asymptomatic. However, individuals with primary immunodeficiencies characterised by uncontrolled EBV viremia and EBV-induced disease such as fulminant infectious mononucleosis, hemophagocytic lymphohistiocytosis and/or lymphoproliferative disorders have been identified. The molecular lesions affecting some of these individuals include heterozygous gain-of-function mutations in *PIK3CD,* and autosomal recessive biallelic mutations in *CD27* or *CD70.* In order to understand how mutations in these genes contribute to EBV susceptibility, we have utilized polychromatic flow cytometry to enumerate the functional and phenotypic attributes of total and EBV-specific $CD8^+$ T-cells in immunodeficient patients compared to healthy controls. Results show that total and EBV-specific $CD8^+$ T cells are skewed towards T_{EM}/T_{EMRA} phenotype, at the expense of naïve $CD8^+$ T-cells in patientswith mutations in *PIK3CD* or *CD27*, with the converse demonstrated in *CD70*-deficient individuals. In addition, $CD8^+$ T-cells in these patients exhibit altered expression of regulatory molecules including 2B4 and NKG2D, as well as the senescence marker CD57 coupled with aberrant expression of cytolytic molecules granzyme and perforin. Preliminary experiments suggest defective activation and effector function of $CD8^+$ T-cells from affected individuals, and by extension aberrant recognition of EBV-infected targets. Further investigation will give insight into the contribution of these pathways in the generation and function of EBV-specific $CD8^+$ T cells, which will be important in the treatment of EBV-associated diseases and the development of a vaccine against EBV.

F.65. DOCK8 deficient germinal center B cells rescued by a Vav-Bcl2 transgene undergo affinity maturation

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Analysis of the GC B cells in mice receiving Fas deficient wild type or DOCK8pri/pri SWHEL B cells showed similar results to Fas sufficient cells with a loss of DOCK8pri/pri GC B cells at day 9. By contrast, DOCK8pri/pri GC B cells carrying the VavBcl2 transgene were present at both day 9 and 15 after immunization, although there remained a significant decrease in GC B cells compared to wild-type. Sequencing revealed an overall acquisition of the Y53D mutation (indicating affinity maturation) in 34% of 126 DOCK8pri/pri VavBcl2 transgenic SWHEL GC B cells sequenced, and in 45% of 114 wild-type cells. This indicates that when the VavBcl2 transgene allows for GC B cell survival, mutations in DOCK8 do not prevent affinity maturation.

F.66. Hypoplastic Thymii from Mouse Models of DiGeorge-22q11.2 Deletion Syndrome Have a Signature Gene Expression Pattern

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Chromosome 22q11.2 deletion syndrome (22q11.2 DS) is the most frequent chromosomal microdeletion disorder reported (1/4000). Individuals with this deletion often present with multi-system disorders including a thymic hypoplasia, cardiac anomalies, hypoparathyroidism, and/or dysmorphic facial features. Over 90% of individuals have a deletion of 2.4 Mb, which comprises 90 genes, 50% protein coding and the remainder microRNAs, long noncoding RNAs and pseudogenes. The principal cause of the development defects is a haploinsufficiency of T-box 1 transcription factor (*Tbx1*). Between 40-60% of patients have some degree of thymic hypoplasia, resulting in their peripheral T cell lymphopenia. Defects in the thymic stromal tissue is the underlying cause of the hypoplasia. We analyzed the thymic tissue in a mouse models of 22q11.2 deletion syndrome, termed the Df1/+ line. Comparative transcriptome analyses of hypoplastic and normal-sized lobes from matched Df1/+ embryos revealed a signature mRNA expression pattern unique to hypoplastic tissue. Ingenuity pathway analysis uncovered selective pathways compromised in these lobes. Immunohistochemistry and fetal thymic organ culture assays are currently being used to characterize the molecular defects of the thymic stroma at the onset of thymic tissue specification in embryos. Inflammatory conditions are being introduced in pregnant Df1/+ mice to determine if the penetrance and severity of clinical phenotypes associated with 22q11.2 DS is affected by stress *in utero*. Findings from our studies may lead to better strategies for improving human thymopoiesis, particularly for patients with 22q11.2 and 10p deletion syndromes, as well as those undergoing chemo ablative treatments that impact the thymus.

F.67. ICF Syndrome Due to a Homozygous Mutation in DNMT3B Gene in Two Patients with Profound Hypogammaglobulinemia Without Facial Dysmorphism

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Immunodeficiency, centromeric instability, and facial dysmorphism (ICF) syndrome is a rare autosomal recessive disease characterized by facial dysmorphism, immunoglobulin deficiency, and branching of chromosomes 1, 9, and 16 in activated lymphocytes. The two main entities of this syndrome, ICF1 and ICF2, are caused by mutations in *DNMT3B* and *ZBTB24* genes and are very rare. In this study, we describe a 4 year-old patient from a consanguineous family of Gambian origin. The patient was referred to our hospital because of growth delay, recurrent pulmonary infections, several episodes of sepsis and hypogammaglobulinemia. At the age of 7, a sister was born prematurely with intra- and extra-uterine growth delay. The karyotype of this sister suggested ICF. Both the sister and the patient lacked memory B cells. The karyotype detected chromosomal instability. Genetic studies in both the patient and his sister revealed a homozygous mutation in

DNMT3B gene consisting in one base change in exon 16 (c.1747G>A) leading to an amino acid change (p.Gly583Ser) predicted as pathogenic. Interestingly, this mutation was recently found by another group in two patients also from Gambia, suggesting the possibility of a "founder effect" of this mutation in this region. We describe two new cases of ICF revealing that facial dysmorphism is not always present in ICF patients and consequently the disease might be under diagnosed.

F.68. Serum Free Immunoglobulins Light Chains. New Tool of Diagnostic and Prognostic Value in Common Variable Immunodeficiency

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Introduction: Quantification of serum free light chain (sFLC) was reported recently to help in the diagnosis of primary immunodeficiencies (PID) and secondary immunodeficiencies (SID) to B lymphoproliferative disorders. Moreover, PID patients are more prone to develop lymphoproliferative malignancies.

Objectives: We sought to compare sFLC in PID and SI; and to determine correlation of sFLC with clinical and B cell phenotype.

Methods: Clinical and immunological data were collected from 33 PID patients (13 Common Variable Immunodeficiency, CVID and 20 with specific antibody production deficit), 34 SID patients to malignant lymphoproliferation and 13 healthy controls (HC). sFLCs were quantified by nephelometry (FREELITE, Binding-Site, UK).

Results: CVID showed significantly lower kappa/lambda values than SID and HC groups ($p\text{-}l^+$ pattern, 2/13 patients; k^+l^- pattern, 1/13 patients; and k^-l^- pattern, 10/13 patients. CVID patients with bronchiectasis were more frequent in the k^-l^- pattern(5 out of 13). 2 CVID patients with detectable sFLC had history of lymphoproliferative disorders. CVID showed lower sFLC than other PIDs, although not significant. Commercial IgG preparations contained detectable sFLCs (mean Ã‚Â k: 41.5 and l: 15.9, respectively), which did not interfered on trough CVID sFLCs. We observed a correlation between sFLC and memory B cell phenotype, the lower the sFLC the lower the class-switched B subset.

Conclusion: sFLC quantification was a useful tool in the diagnosis of CVID over other PIDs, SID and HC. Further studies are necessary to ascertain their potential value as biomarker of malignant lymphoproliferation in CVID.

F.69. Humans with Compound Heterozygous Mutations in Forkhead Box N1 (Foxn1) Have a Severe Immunodeficiency with Normal Skin and Hair Development

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Patients with mutations in the *Forkhead Box N1* (*Foxn1*) transcription factor are born with a severe T-cell lymphopenia in combination with alopecia and nail dystrophy (OMIM # 600838). The T-cell lymphopenia results from the impaired development and/or function of the epithelial cells in the thymus. All the known *Foxn1* mutations in humans are homozygous and affect the thymus, skin, and hair, replicating the phenotypes of the nude mouse strain harboring mutations in *Foxn1*. We report on 3 independently identified patients with distinct clinical presentations compared to previously reported cases. Of significance, each patient had compound heterozygous mutations in *Foxn1*, leading to low T cell numbers. All 3 patients had normal hair, skin, and no clinical signs of nail dystrophy. To better define the molecular basis of this novel clinical presentation, we used CRISPR/Cas techniques and generated the corresponding mutations in the mouse *Foxn1* sequence. We will present data on the phenotypes of these mice, using intercrosses between individual mutant mice. Comparative transcriptome analyses of fetal thymii from these mice and epithelial cell lines will be

undertaken. These studies will reveal how the *Foxn1* mutations impact thymic epithelial gene expression and function compared to normal *Foxn1*. Our findings may lead to a better understanding of the role of Foxn1 in epithelial cell development and function for both the thymus and skin.

F.70. ImmunoHUB: A Comprehensive Web-Based Teaching and Diagnositic Support Tool for Human Primary Immunodeficiency Diseases

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The tempo of discovery continues to increase since the first deficiencies of immunity were described 60 years ago and is particularly rapid since the sequencing of the human genome in 1999. This module is updated monthly, which permits integration of newly described diseases into the growing list of deficiencies. People with similar primary Immunodeficiencies are likely to exhibit patterns of characteristics that are different from a sample of a population of individuals without primary Immunodeficiencies. Characteristics in this module include findings that are part of the medical history, physical examination and laboratory evaluation. The differential diagnosis of patients who present primarily with susceptibility to infection (frequent, severe, prolonged, unusual) includes noninfectious entities, including autoimmune, auto-inflammatory and atopic conditions. Molecular genotypes are associated with a range of human phenotypes; conversely individuals with similar genotypes display similar but not identical phenotypes. This diagnostic evaluation is personalized by directing inquiry to the most relevant immune cell(s), serological component, immunophysiological process or pathway/circuit, which facilitates efficient and cost-effective usage of confirmatory molecular diagnostic tests. Once a primary immunodeficiency is suspected, an optimal choice for care, if feasible, is efficient referral to a center that has particular expertise and experience in the diagnosis and management of patients with primary Immunodeficiencies. Given that the time to diagnosis is an important factor for optimal patient care, our primary objective in developing the Diagnostic Support Tool is to increase the tempo of the diagnostic process by provision of an organizational assistant to the user.

F.71. Phosphatidylinositol 3-kinase Delta Gain of function (GOF) Mutations Cause Impaired B cell Development and Function

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Background: Humans with gain of function (GOF) mutations in *PIK3CD*, encoding the p110d subunit of phosphatidylinositol 3-kinase, have an immunodeficiency characterized by clinical features consistent with a significant defect in the function of their humoral immune response. These include increased susceptibility to sinopulmonary infections and poor antibody responses to common encapsulated respiratory pathogens. In addition they have strikingly increased peripheral blood transitional B cell numbers pointing to a developmental abnormality in B cells.

Methods: To explore the consequences of Phosphatidylinositol 3-kinase GOF on both B cell development and function, we analyzed B cells from both mouse and humans with GOF mutations in the gene encoding PI3 kinase δ. Mice were generated using CRISPR/Cas9 gene editing to introduce the most-common disease-causing mutation (E1021K) into *Pi3kcd.*

Results: Analysis of bone marrow from both mice and humans revealed disrupted B cell development. Investigation of antigen-specific T-cell dependent B cell responses using mice expressing a transgenic BCR specific for hen egg lysozyme (HEL) combined with *Pi3kcd* GOF showed defects in Ig class switching and specific antibody generation that recapitulate the humoral immune defects seen in humans with *PIK3CD* GOF mutations.

Conclusion: This study provides new insights into the molecular and cellular defects underlying the humoral defects in patients with *PI3KCD* GOF mutations.

T.52. Molecular and Cellular Mechanisms for B Cell Defects in Elderly and Obese Subjects

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B cell function is decreased with age and obesity and associated with increased chronic low-grade systemic and metabolic inflammation. We have previously shown that obesity decreases the influenza vaccine response in young and elderly individuals and this is associated with a decrease in switched memory (swIg) B cells (CD19+CD27+IgD-) and an increase in late exhausted memory (LM) B cells (CD19+CD27-IgD-). We show here that elderly have more inflammatory cytokines (e.g.TNFa, IL-6) in their LM B cells and B, T, and monocytes from the obese subjects have significantly elevated IL-6, IL-17a, and TNF-a respectively as compared with their lean controls. Molecular markers of class switch recombination [activation-induced cytidine deaminase (AID), the transcription factor E47, and IgG in culture supernatants] are decreased in obese young and elderly as compared with their lean age-matched controls. To query possible mechanisms for these differences we have measured conditions and effects of visceral adipose tissue (VAT) on B cell phenotype and function. We found significantly more LM B cells in the VAT than in the blood. Moreover, adipocytes make pro-inflammatory chemokines (e.g. CXCL10, IL-8, CCL2, and CCL5) which could recruit B cells into the VAT and for which B cells have chemokine receptors. B cells from the VAT also express more pro-adipogenic markers (e.g. more Hormone-sensitive lipase and PPAR gamma and less SOD1). We have also shown that B cells from human blood co-cultured with adipocytes induce more LM B cells. These results show a direct effect of adipocytes on pro-inflammatory B cells

T.53. Aging of Adaptive Immunity Driven by Chronic Antigen Exposition has a Major Impact on the Course of Regenerative Processes Which Are Overlooked in Our Commonly Used Mouse Models

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Antigen exposition over the life drives the adaptive immune system to an aged state characterized by a shift to effector-T cells (Teff). In contrast to naïve/central memory T cells, Teff can migrate to any inflamed site in the body and modify the intratissue inflammation. Recently, we could show that immune aging, defined by enhanced Teff levels, is associated with poorer outcome of a sterile bone fracture in trauma patients. Accumulation of those cells and release of inflammatory cytokines at the fracture hematoma blocks the osteoblast differentiation. In normal SPF mice, only few Teff are detectable and the impact on bone fracture healing is negligible. However, change of environmental housing conditions to non-SPF, induces an "aged" immune system within few weeks without signs of pathological infections. Remarkably, bone fracture healing is delayed compared to SPF animals and the level is related to the ratio between effector and regulatory T cells (Teff/Treg), as in patients. Consequently, depletion of CD8+ T-cells or adoptive transfer of Treg promote fracture healing if the ratio was successfully converted, supporting the causative role of adaptive immunity. Translating to other disease models, we confirmed the important impact of Teff/Treg on regenerative processes. Non-SPF but not SPF-mice develop NASH-like liver pathology under high-fat diet associated with accumulation of Teff in liver and fat. Teff also showed negative impact on muscle injury, transplantation, myocarditis. In summary, mice kept under well controlled non-SPF housing conditions and immune monitored are better disease models reflecting the impact of intratissue accumulation of Teff.

T.54. INSIGHTS: A Quality Improvement Project

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Objective: Immune globulin (IG) is indicated for the prevention of recurrent infection in primary immunodeficiency diseases (PIDD) but is occasionally used off-label with uncertain benefit. We performed a retrospective systematic review of clinical documentation for patients treated with IG for a diagnosis of PIDD to determine the quality of supportive evidence for the diagnosis and treatment.

Methods: We collected clinical and laboratory data submitted for insurance approval for patients prescribed IG. Anonymized data was reviewed by a panel of immunologists with expertise in PIDD. Panelists adjudicated diagnosis and appropriateness of therapy.

Results: 147 cases were reviewed: 112 female and 35 male, age range 19 - 81.Ã‚Â Mean dosing was 0.49gm/kg monthly (STD 0.23), range 0.12-2 gm/kg. Reviewers found clinical data was frequently lacking detail to support the diagnosis or indication for IG therapy, specifically laboratory data or documented evidence of infection. Reviewers agreed 91 of 147 (62%) patients reviewed had a confirmed PIDD diagnosis with IG therapy appropriate for 61 (41%). Diagnostic concordance between prescribers and reviewers was most discrepant for common variable immune deficiency (CVID) patients, with reviewers identifying 28 cases of CVID compared to 86 identified by prescribers (32%).Ã‚Â

Conclusions: Based on case review, expert immunologists found insufficient clinical and laboratory evidence to support IG therapy in 58% of cases reviewed. Improvements in diagnostic evaluation and clinical documentation may ensure IG therapy is secured in patients who require the life-saving therapy while reducing use in patients without PIDD, where the benefit is unproven.

W.57. Biological Efficacy and Safety of Intranasal GnRH-analogue in Treatment-naïve HIV1 Infected Male Patients: An Open Phase IIa, Proof of Concept Trial

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Background Gonadotrophin releasing hormone (GnRH) can affect the immune system by binding to GnRH receptors on immune cells. We wanted to evaluate safety and efficacy of a novel treatment of HIV with the GnRH analogue Buserelin. The aim was to assess the ability of Buserelin to increase antigen presentation of HIV and cause specific immune activation.

Methods Twenty-six asymptomatic, treatment-naïve HIV infected adult men were included in a Phase IIa clinical trial. Patients were treated with intranasal Buserelin (1.2 mg/day) for four weeks. After seven days, the patients were injected with a single intramuscular testosterone depot injection (150 mg) to prevent known adverse effects. Patients were followed by weekly laboratory assessments, Testosterone, FSH, LH, HIV viral load, CD4 and CD8 cells counts, immune cell activation and HIV specific tetramers. After 36-40 months, an ad hoc follow-up study was conducted in thirteen of the patients.

Results No severe adverse events were reported. Expression levels of HLA class I and CD69 were significantly increased on $CD4^+$ and $CD8^+$ T cells after treatment. Also, the frequency of $CD25^+CD8^+$ T effector cells and HIV specific $CD8^+$ T cells was increased. Furthermore, the patients displayed a reduction in HIV viral load that further decreased four weeks after discontinuation of treatment. In the follow-up study six of the thirteen patients showed no progression of HIV viral load, indicating that GnRH may have positive long-term effects.

Conclusions GnRH treatment of HIV infected patients is safe and well tolerated and leads to immune activation and reduced HIV viral load.

W.58. CD4 T Cell-Restricted IL-2 Signaling Defect in a Patient with a Novel IFNGR1 Mutation

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We identified a patient who is a compound heterozygote for mutations in the interferon-gamma receptor-1 gene [a maternally-derived frame-shift mutation in exon 4 and a paternally-derived premature stop-codon in exon 5]. Clinically, the patient has experienced pulmonary infections with mycobacterium fortuitum, mycobacterium-avium complex that were controlled with medications targeting rapid and slow-growing mycobacteria. In addition, the patient developed chicken-pox following Varivax administration and also experienced a prolonged episode of severe eczema starting right after birth that was controlled at 6 months of age with topical medication. The patient who is now ~ 4.5 years old continues to experience intermittent episodes of HSV-induced gingivo-stomatitis but is clinically-stable and awaiting a peripheral blood stem cell transplant.

Immunologically the patient had slightly depressed CD8 T cell and NK cell counts at initial analysis and mildly elevated frequencies of both isotype-switched and unswitched circulating B cells and normal mitogen-induced lymphocyte proliferation. We also observed a loss of IFNgR1 expression on the patient's lymphocytes and complete abrogation of STAT1 phosphorylation following treatment with recombinant human IFNg. In depth analysis of immune function additionally identified an IL-2 associated signaling deficiency that was restricted primarily to the circulating CD4 T cell subset.

W.59. Lentiviral-Mediated FOXP3 Gene Transfer and FOXP3 Gene Editing: Developing Novel Treatment Options for IPEX Syndrome

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FOXP3 is a key transcription factor for the maintenance of immune tolerance. *FOXP3* mutations result in dysfunction of FOXP3+ regulatory Treg cells (Tregs) causing Immune dysregulation, Polyendocrinopathy, Enteropathy, X-linked (IPEX) syndrome, a severe autoimmune disease. Current therapies for IPEX syndrome are limited to immunosuppression and allogenic hematopoietic stem cell (HSC) transplantation, both with side effects and limited efficacy. To develop better treatments for IPEX patients we have investigated lentiviral FOXP3 gene transfer (LV-FOXP3) in T cells and FOXP3 gene editing in HSC.

LV-FOXP3 successfully converts IPEX patients-derived CD4+ effector T cells (Teff) into Treg-like cells ($CD4^{LV\text{-}FOXP3}$ T cells) with stable suppressive capacity *in vitro* and *in vivo* (Passerini, Sci Transl Med, 2013). Recently, we could generate nominal- and allo-antigen-specific $CD4^{LV\text{-}FOXP3}$ T cells. These results clearly demonstrated the potential clinical benefit of $CD4^{LV\text{-}FOXP3}$ T cells, which could be applied not only in IPEX but also in immune mediated diseases of different origin.

We are currently pursuing a CRISPR-Cas9 based gene editing approach in HSC. The site-specific gene correction of FOXP3 would permit the regulated expression of the functional FOXP3 protein not only in Treg but also in Teff cells, which

transiently express FOXP3 upon activation. To edit FOXP3, we used CRISPR-Cas9 protein combined with an AAV6 packaged donor DNA template, and showed that the system effectively targets FOXP3 in HSCs. Moreover, gene-edited HSPCs can be transplanted into NSG mice for long-term multilineage reconstitution.

Our data indicates that both LV-FOXP3 and FOXP3 gene editing hold promises for the cure of IPEX syndrome.

W.60. Prevalence of Secondary Hypogammaglobulinemia and Risk of Severe Infection following Rituximab Treatment

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Introduction: Rituximab is a chimeric monoclonal antibody for treating hematologic and autoimmune diseases by depleting CD20-expressing B-cells. Hypogammaglobulinemia following rituximab treatment has been reported. Risk stratification for developing severe infection would identify patients who may benefit from immunoglobulin replacement therapy. The aim of this systematic review was to describe the prevalence of hypogammaglobulinemia and its risk for developing severe infection.

Method: We performed an electronic search of Ovid Medline, Embase, Web of Science, Scopus and the Cochrane Library identifying studies describing the prevalence of hypogammaglobulinemia following initiation of rituximab.

Results: 16 studies met eligibility criteria. Study sample size was between 12 and 3595 patients (median 177). Cohort studies included lymphoma patients receiving maintenance rituximab, with combination chemotherapy or in association with autologous bone marrow transplant (6 studies). 8 cohort studies described prevalence of rituximab-induced hypogammaglobulinemia following treatment for multisystem autoimmune disease including rheumatoid arthritis (3), systemic lupus erythematosus (2), systemic autoimmune disorders (2) and granulomatosis with polyangiitis (1). Hypogammaglobulinemia prevalence was between 3.5 and 45%; lowest prevalence in on maintenance rituximab in follicular lymphoma (0.9%) and highest in granulomatosis with polyangiitis (45%). Prevalence of severe infection ranged from 3.9 to 46%. Risk factors for infection included low baseline immunoglobulin, female gender and treatment with cyclophosphamide, fludarabine or glucocorticoids.

Conclusions: Prevalence of hypogammaglobulinemia is widely variable owing to the heterogeneity of patient cohorts with multifactorial disease and treatment-related etiologies for hypogammaglobulinemia. Monitoring baseline serum immunoglobulin and B-cell levels is essential to identify patients at risk of developing severe and recurrent infection.

W.61. Antibiotic Prophylaxis in Primary Antibody Deficiency Patients: Study Design

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Background: At now, data on antibiotic prophylaxis in primary antibody deficiency patients are uncertain. We are studying the role of azithromycin on primary antibody deficiency patients.

Methods: We are conducting a multi-center randomized placebo-controlled-double-blind trial on 89 patients with COPD and exacerbations. The aim of the study is evaluating efficacy and safety of azithomycin low-dose (250 mg 3 consecutive days a week) for 24 months *vs* placebo. In patients under azythomycin we expect a decrease of COPD exacerbations (reduction of dyspnea, cough, sputum), no use of additional antibiotics, an increase of respiratory volumes, an improvement of the Health Related Quality of Life measures.

Results: The study started on June 2014 and will last 30 months (therapy: 24 months, follow-up: 6 months). Monthly evaluations: lung function, St. George's Respiratory Questionnaire, sputum sample for microbiological assessment, blood test, diaries for use of additional antibiotics, SF-36 Questionnaire for quality of life, report of adverse events. Our study will end on December 2016. During the study we observed 14 drop out (9 patients withdrew informed consent; 5 patients died: 2 for respiratory distress; 1 for cancer, 1 for Parkinson disease, 1 for stroke).

Conclusion: To our knowledge our study is the first one on antibiotic prophylaxis in primary antibody deficiencies patients.

Immuno-dermatology

W.12. Case Report: Acrodermatitis Continua of Hallopeau (ACH) with Acro-Osteolysis Progressing to Generalized Pustular Psoriasis

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Introduction: Acrodermatitis Continua of Hallopeau (ACH) is a recurrent painful form of localized pustular psoriasis occurs on the fingertips and nail folds mainly in females. In each episode only one or two digits are involved. It may be associated with nail disorders, distal phalangeal atrophy and osteolysis. Rarely ACH progresses to generalized pustular psoriasis (GPP) in elderly.

Case presentation: We present a 63-year-old woman with painful pustular lesions on three fingertips, great toes tips and plantar areas which became gradually generalized after a febrile attack and discontinuing oral retinoid. She had no distal phalanx on the right index finger. Other distal phalanxes were atrophic with onychodystrophy and deformed interphalangeal joints. The first episode of ACH occurred during pregnancy, 40 years ago but sequential episodes happened afterwards. She had geographic tongue and ear cholesteatoma. Laboratory data showed elevated erythrocyte sedimentation rate, leukocytosis, positive C-reactive protein, bacteriuria with positive culture. Imaging showed acro-osteolysis and right index distal phalangeal bone absorption. Biopsy confirmed pustular psoriasis. She was treated with methotrexate, acitretin and ciprofloxacin.

Discussion: ACH is a rare and recalcitrant type of pustular psoriasis. Trauma, infection, steroid withdrawal, sun light, stress, hypocalcemia and pregnancy are inducing factors. Interleukin 36RN gene mutations have a pathogenic role in pustular eruptions including ACH, palmoplantar pustulosis and acute GPP which explain this co-occurrence.

Conclusion: ACH is treatment resistant but therapies should be considered as soon as possible. Early management may prevent progressive disabilities. Better understanding of pathogenesis of GPP might bring new treatments such as biologic agents.

W.13. A novel Population of Skin-Tropic T Cells with a Potential Role in Wound Healing

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The skin functions as the primary barrier between an organism and the outside world. Although it is home to a robust and diverse community of commensal microorganisms, it must also prevent entry of environmental toxins, irritants, and numerous viral, bacterial and parasitic pathogens. To effectively maintain its barrier function, the skin must undergo rapid and highly efficient tissue repair when damaged or compromised, and this is associated with local antimicrobial and inflammatory responses that help prevent or combat concurrent infection. Consistent with its functions in maintaining barrier integrity and preventing infection, the skin is home to a number of specialized T cell populations. Using 33-parameter CyTOF (Cytometry by time-of-flight) analysis and standard flow cytometry we have identified a novel population of CLA^+CD4^+ T cells in the peripheral blood of healthy subjects that expresses the CD103 integrin and produces the novel cytokine combination of IL-22, GM-CSF and IL-13 upon activation ($CD103^+CLA^{hi}$ cells). Interestingly, this cytokine combination is also produced by epidermal $CD103^+CLA^{hi}CD69^+$ T_{RM} in the skin itself. Based on the ability of IL-22 to promote keratinocyte proliferation, migration and production of antimicrobial peptides, and GM-CSF and IL-13 to act on fibroblasts, and to mediate the differentiation of monocytes and macrophages into 'alternatively activated' cells that promote tissue repair, we are currently testing the impact of $CD103^+CLA^{hi}$ cells on skin repair functions. In summary, we have identified a circulating population that resembles skin T_{RM} and might be useful in studying (recirculating?) skin T_{RM}.

W.14. Omalizumab Therapy for Bullous Pemphigoid Despite Transient Reaction

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Bullous pemphigoid (BP) is a life-threatening blistering skin disease. Urticaria and trauma to the skin are common underlying causes of bullae, especially in elderly patients. In certain cases, morbidity and mortality may increase due to infected bullae. We present an 82-year-old woman with severe BP after a fractured left leg. Bullae and toenail deformity occurred on both lower extremities. Biopsy direct immunofluorescence showed subepidermal blistering with numerous eosinophils. The dermal-epidermal junction showed 2+ IgG, C3 and granular IgM.

We started her on a regimen of omalizumab 300 mg subcutaneously every four weeks. Within a week, she reported significantly decreased pain and healing time of lesions. Incidentally, bilateral erythematous, non-blistering dermatitis developed five centimeters distal to the injection sites within a week of her first injection and resolved spontaneously. Nail and skin tissue healed and grew back within a two days of drug administration. In the fourth week of each treatment cycle, she develops new blisters in the legs, in the usual areas. She continues to tolerate the omalizumab injections well after four months of treatment and has not developed the injection site dermatitis since the first administration.

Omalizumab is an IgE-targeting monoclonal antibody commonly used to treat severe asthma or chronic idiopathic urticaria. After failing cytotoxic and immunosuppressant agents, few other treatment options exist for patients with refractory BP. Omalizumab is a novel treatment that has proven effective in our patient despite a transient injection site reaction.

W.15. Deciphering the role of Dendritic Cells and its co-stimulatory markers in the immunopathogenesis of Pemphigus Vulgaris

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Pemphigus vulgaris (PV) is an autoimmune blistering disease of skin and mucous membranes characterized by auto-antibodies against Desmoglein3. The breach in immune tolerance, atypical T cell function and flawed antigen presentation are the predisposed factors in this disease. Dendritic cells (DCs) are crucial antigen presenting cells in maintaining tolerance and playing role in autoimmunity. The exact role of DC has not yet been explored in PV. In this study, DC frequency and phenotype was estimated using HLA-DR, Lin^-, CD11c and CD123 markers by flowcytometry. A significant

decrease in Myeloid DC (mDC) & Plasmacytoid DC (pDC) frequency in circulation of PV patients (n=20) *vs.* controls (n=20) was observed. Further, DC associated molecular markers were assessed in cultured DCs that were differentiated from monocytes and sorted by FACS Aria-III from the PBMCs of patients and controls. The mRNA levels of co-stimulatory/inhibitory molecules of cultured DCs and in circulation in PV patients were estimated. Expression levels of stimulatory molecules (CD40 & CD80) was found to be significantly increased, while a significant decline in inhibitory markers (PSGL1 & ILT3) was observed in sorted and cultured DCs from patients. Infiltrating Langerhans cells by IHC in perilesional tissue of PV patients suggests their involvement in this disease. The Lower frequency of mDC and pDC and increased expression of stimulatory molecules and decreased level of inhibitory molecules further strengthens the defect in functional status of DC. Thus, these co-stimulatory molecules might prove to be potential targets for novel therapeutics in PV.

W.16. Characterization of Immune Responses in Nevirapine-Induced Stevens-Johnson Syndrome/Toxic Epidermal Necrolysis

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Nevirapine is used globally in the combination treatment of HIV. Its current use in first-line therapy is limited by immune-mediated adverse drug reactions (IM-ADRs) that significantly impact patient outcome and healthcare cost. Stevens-Johnson Syndrome/Toxic epidermal necrolysis (SJS/TEN) is the severest nevirapine-induced IM-ADR. SJS/TEN is a HLA class I restricted, CD8+ T-cell dependent hypersensitivity syndrome, which presents as a severe blistering skin rash that can result in extensive epidermal necrosis. We have recently identified HLA-C*04:01 as a significant class I risk allele in South African HIV+ patients with nevirapine SJS/TEN. The feasibility of preventive HLA screening for nevirapine is hampered by the fact that only a small percentage of those carrying a risk allele develop an IM-ADR. To understand why hypersensitivity occurs in only a small proportion of those carrying an HLA-risk allele, we have immunophenotyped the nevirapine-specific pathogenic CD8+ T cells based on their cytokine outputs, cell surface and activation markers (CD137+/CD69+) using ELISpot assays, intracellular cytokine staining and flow cytometry following *ex vivo* stimulation with nevirapine. To identify the TCR specificities of the T-cell repertoire in patients with HLA-C*04:01+ nevirapine SJS/TEN, single cell TCR sequencing for paired TCR alpha and beta genes and phenotypic markers will be performed to compare T cell populations in the presence and absence of drug. Candidate TCRs will be confirmed with a Jurkat TCR reporter assay. It is expected that these approaches will lead to a sensitive predictive assay to identify the dominant cross-reactive TCRs in HLA-C*04:01+ patients who are destined to develop nevirapine SJS/TEN.

Immunology of the eye

F.72. Importance of Checking for Active Autoimmunity in Gene-Defined Retinal Dystrophy Patients

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Purpose: Anti-retinal antibodies (ARA) have been found in some patients with retinal dystrophies indicating an autoimmune component. Immune suppressive treatment in these patients can slow progression of vision loss but does not usually reduce ARA. We have set out to define more robust criteria for active retinal autoimmunity and discover clues to the mechanisms of autoimmunity in these patients.

Methods: A set of 49 patients with defined dystrophy-associated genetic mutations were consented. An extensive immune profile consisting of ARA Western blot, anti-recoverin IgG and IgM ELISA, recoverin-induced cytokine (IFNg, TNFa and IL-

10) production, four 10-color flow cytometry panels to define lymphocyte subsets, and gene expression analysis by NanoString™ was performed on blood samples. Data were compared to non-dystrophic, and non-autoimmune controls.

Results: Several dystrophy patients were classified as autoimmune by: 1) three or more ARA; 2) a high titer of recoverin-specific IgG or IgM; and/or 3) very high production of IFNg or TNFa in response to recoverin. We found that 28/49 (57%) of the dystrophy patients had elevated anti-recoverin antibody titers or inflammatory cytokine responses. These parameters closely correlated with disease activity and were sensitive to treatment with immune suppression. High levels of natural killer cells (>30%) and B lymphocytes (>20%) were frequently found in the blood of autoimmune patients but not the non-autoimmune groups. Cell activation and gene expression in the patients were also affected by treatments.

Conclusions: Gene-associated retinal dystrophies can be exacerbated by autoimmunity. Cellular and humoral immune responses against recoverin mark disease activity and treatment response.

F.73. IL-17A dampens Autoimmune Disease by Inhibiting the Expression of IL-17 Lineage Cytokines Through a Negative Feedback Loop involving IL-24

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The Th17 response has been associated with autoimmune diseases in patients and in animal models, including autoimmune uveitis and its experimental counterpart, EAU. Paradoxically, however, IL-17A treatment given to EAU-challenged mice was reported to ameliorate the disease (PMID: 19234216) and clinical trials targeting IL-17A in uveitis have been largely disappointing (PMID: 25648267). Here, we investigated susceptibility to uveitis of spontaneously uveitic R161H mice (IRBP T cell receptor transgenic, PMID: 23810578) crossed onto the IL-17A$^{-/-}$ background. Surprisingly, IL-17A$^{-/-}$ R161H mice developed essentially undiminished uveitis. Moreover, IL-17A$^{-/-}$ R161H T cells, polarized to Th17 and infused into wild type recipients, induced similar disease to IL-17A-sufficient R161H T cells. These Th17-polarized IL-17A$^{-/-}$ R161H T cells produced elevated amounts of other Th17 lineage cytokines, namely, IL-17F, GM-CSF and IL-22, and this was reversed by supplementing the cultures with recombinant IL-17A. RNAseq analysis revealed that the IL-17A$^{-/-}$ T cells expressed lower levels of IL-24 compared to their IL-17A sufficient counterparts. Mechanistic studies *in vitro* indicated a negative feedback loop where IL-17A induces Th17 cells to produce IL-24, which subsequently suppresses production of Th17 lineage cytokines. Studies *in vivo* showed that injection of recombinant IL-24 ameliorated adoptive Th17-induced EAU, and conversely, silencing IL-24 expression in the adoptively transferred Th17 cells increased their pathogenicity and enhanced disease severity. Our data suggest that IL-17A exerts a negative feedback on Th17 cells by inducing IL-24, which limits the expression of Th17-related cytokines and dampens Th17 effector pathogenicity.

Immuno-oncology

F.74. Modulation of Cbl-b in CD8+ T Cells Induces IFN-γ Dependent Resistance Against Regulatory T Cell Immune Suppression

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Adoptive cell therapy (ACT) utilizes patient-derived $CD8^+$ T cells to mount anti-tumour response, leading to tumour regression. Despite recent advances, ACT is limited by the presence of immunosuppressive regulatory T cells (Treg) within solid tumours. We are investigating the adoptive transfer of genetically modified $CD8^+$ T cells to enhance anti-tumour immune response. Cbl-b is an E3 ubiquitin ligase that negatively regulates TCR signalling. Ablation of Cbl-b in $CD8^+$ T cells have been shown to enhance proliferation and induce an inflammatory phenotype. The primary objective of this study was to investigate the mechanism of Cbl-b deficient $CD8^+$ T cell resistance against Tregs. Cbl-$b^{-/-}$ CD8 T cells were characterized by surface marker expression and cytokine secretion profile. Furthermore, Treg suppression assays (TSA) were conducted with $CD8^+$ T cells from either C57BL/6 or Cbl-$b^{-/-}$ mice to determine the role of Cbl-b dependent resistance against Tregs. We demonstrated that Cbl-$b^{-/-}$ $CD8^+$ T cells had an increased expression of activation markers, hyper-secretion of pro-inflammatory cytokines, and were unresponsive to Treg immunosuppression. Exogenous introduction of IFN-γ was sufficient in inducing Treg resistance specifically in WT $CD8^+$ T cells. Finally, TSA performed using $CD8^+$ T cells or Tregs from IFN-γ$R1^{-/-}$ mice demonstrated that IFN-γ confers Treg resistance in an autocrine-dependent manner. We report that ablation of Cbl-b results in IFN-γ dependent $CD8^+$ T cell resistance against Treg cells. While IFN-γ has previously been shown to enhance anti-tumour immunity by enhancing T cell activation, this study is the first to demonstrate its role in resistance of immune suppression.

F.75. Identification of Unconventional Immunosuppressive CD4+Foxp3-PD-1hi T Cells as a Biomarker of Immune Checkpoint Blockade Activity

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Despite the clinical successes of immune checkpoint blockade, a significant proportion of cancer patients relapse or are refractory to this intervention. This underscores the need to better clarify the biologic activity of checkpoint blockade and identify effective biomarkers for improving patients' selection and utilization of these strategies. Here, we aimed to determine the biologic and therapeutic significance of modulation of $CD4^+Foxp3^-PD1^{hi}$ T cells (abbreviated as $4PD1^{hi}$) during immune checkpoint blockade. We found that anti-CTLA-4 increased intratumoral and peripheral $4PD1^{hi}$ in a dose-dependent manner, while combination with anti-PD-1 mitigated this effect and significantly improved anti-tumor activity. Furthermore, on-treatment increases in $4PD1^{hi}$ during PD-1 blockade were associated with a poor prognosis in cancer patients, pointing to $4PD1^{hi}$ as a potential resistance mechanism. $4PD1^{hi}$ accumulated intratumorally as a function of tumor growth in untreated mice. Functional analyses revealed that both mouse and human circulating and intra-tumor $4PD1^{hi}$ suppressed T-cell activation in a PD-1/PD-L1 dependent fashion. In addition, $4PD1^{hi}$ from naïve and tumor-bearing hosts (mice and humans) resembled follicular-helper-T-cell(T_{FH})-like cells. Accordingly, immunization with sheep red blood cells increased this unconventional suppressive T-cell pool, while Bcl6 inhibition exerted the opposite effect and delayed tumor growth selectively in immune competent mice. These findings indicate that disinhibited T-cell priming promoted by anti-CTLA-4 may boost immunosuppressive $4PD1^{hi}$ with T_{FH}-like features in a dose dependent manner, and PD-1 blockade can counteract this effect. This contributes to understanding the incremental activity of combination checkpoint blockade and provides a new pharmacodynamic and prognostic biomarker for the design of optimal combination schedules.

F.76. Low Natural Killer Cells Counts Are Associated with Relapse After Imatinib Discontinuation in Chronic Myeloid Leukemia patients: the IMMUNOSTIM Study

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Despite leukemic stem cell persistence, patients with chronic myeloid leukemia (CML) who achieve and maintain deep molecular responses can successfully stop treatment with the tyrosine kinase inhibitor (TKI) imatinib. However, questions remain unanswered regarding the clinicobiological basis of relapse after imatinib cessation. In the IMMUNOSTIM study, we evaluated 51 patients from the prospective STop IMatinib (STIM) trial for peripheral blood T- and natural killer (NK)-cells phenotypes and *in vitro* functional assays at the time of imatinib cessation and 6 months later. We found that non-relapsing patients displayed significantly higher numbers of circulating NK-cells of the cytotoxic $CD56^{dim}$ subset at imatinib discontinuation than relapsing patients. We identified the $CD56^{dim}$ NK-cell count as an independent prognostic factor of molecular-relapse free survival in a multivariate analysis. However, compared with healthy individuals, NK activating receptors, degranulation in response to leukemic targets and cytokine-induced IFN-γ secretion were impaired in non-relapsing and relapsing patients. After imatinib cessation, NK-cells significantly increased and remained higher in non-relapsing patients with higher levels of the mature CD57+ NK subset. In conclusion, we provide evidence that NK-cells are associated with outcome after imatinib discontinuation in chronic phase CML patients in deep molecular response. Higher amounts of cytotoxic $CD56^{dim}$ NK-cells in non-relapsing patients may play an important role in controlling residual CML-initiating cells and their progeny soon after cessation of imatinib treatment while a reduced $CD56^{dim}$ compartment in relapsing patients may leave less chance to counterattack in an efficient manner.

F.77. Exhaustion-Associated *De Novo* DNA Methylation Restrains PD-1 Blockade-Mediated T-Cell Rejuvenation

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Immune-checkpoint blockade (ICB)-mediated rejuvenation of exhausted CD8 T cells has emerged as a promising frontier for treating cancer and chronic infections. However, antigen-specific T cells that have differentiated to a terminal state of exhaustion remain refractory to ICB-mediated rejuvenation and currently have limited potential for contributing to this promising therapeutic approach. Given that many of the impaired effector-properties of terminally exhausted CD8 T cells appear to be heritably maintained even in the absence of antigen, we investigated the role of *de novo* DNA-methylation programming as a cell-intrinsic mechanism for establishing the ICB-nonresponsive state of T-cell exhaustion. Combining whole-genome bisulfite sequencing with a TCR-inducible system to conditionally delete the *de novo* DNA methyltransferase, Dnmt3a, in recently activated CD8 T cells (cKO), we mapped genome-wide changes in epigenetic programming that regulate the progressive development of T cell exhaustion. PD-L1 blockade was unable to erase the exhaustion-associated *de novo* methylation programs in WT CD8 T cells and resulted in expansion of a clonally-restricted, functionally-stunted subset of T cells. In contrast, PD-L1 blockade treatment of chronically infected Dnmt3a cKO mice resulted in amplified expansion and retained TCR repertoire diversity of LCMV-specific T cells, that enhanced viral control. Extending our findings to the tumor setting, we confirmed that exhaustion-associated DNA methylation programs are acquired in tumor-infiltrating PD-1hi CD8 T cells. These data establish Dnmt3a as a critical regulator in the development of T-cell exhaustion, and the resulting *de novo* methylation programs as a primary and stable barrier of ICB-mediated T cell rejuvenation.

F.78. Correlation of Cancer Pathways and Mutational Load with Immunephenotype Pan Cancer

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Further advances in the revolution that cancer immunotherapy is providing are hampered by a lack of understanding of the underlying genetics determining the pre-treatment tumor immune environment. Consensus clustering on 32 TCGA cancer datasets consisting of 9219 samples, was performed using an immune signature consisting of a combination of Th1 orienting genes and regulatory immune genes that associates with improved prognosis. A high degree of correlation was found between these genes across the different cancer sets. In order to elucidate the mechanisms underlying the unfavorable, non-inflamed tumor phenotype we investigated the tumor load and inverse correlations between this signature and a large set of published cancer pathways. The Th1 phenotype was associated with higher number of mutations in some but not all tumors. This association was especially evident in cancers where micro satellite instability plays a role. However, this association cannot explain the difference in immune phenotype on its own. We recently showed the association of MAPK pathway perturbations, originating from MAP3K1 or MAP2K4 mutations, with a poor immune phenotype in breast cancer and are now trying to identify other pathways that associate pan-cancer with the unfavorable phenotype. Inversely correlating pathways are the WNT/B-Catenin, Hedgehog and KRAS signaling. The inverse correlation of some other pathways involved in DNA repair, G2M checkpoint or mismatch repair can be associated with the increase in mutational load observed in these samples before. Therapeutic targeting of these pathways might expand the patient cohort responsive to immunotherapy and needs further *in vitro* and *in vivo* experimentation.

F.79. Modular Repertoire Analysis Framework for Breast Cancer

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Transcriptional modular repertoire analysis has been developed and successfully used as a basis for the selection of biomarkers and development of multivariate transcriptional indicator of disease progression in patients with systemic lupus erythematosus or infectious diseases. In the context of cancer, understanding the molecular mechanisms underlying disease is an important step to improve diagnostic accuracy and to guide therapeutic decisions. Here, we employed our modular repertoire construction algorithm to identify coordinately expressed gene sets in 7 public breast cancer microarray datasets consisting of 1,860 transcriptome profiles. The analysis yielded a total of 40 modules. Functional characterization identified modules comprised of membrane receptors (M1.1), molecules involved in nucleic metabolic processing/DNA, RNA binding (M1.2, M2.3, M2.6, M4.3, M3.11), cell cycle/division (M1.3), cell adhesion/migration (M1.5, M5.3), protein localization (M2.4, M3.10, M5.1, M5.4), blood vessel development (M3.3), transmembrane transport activity (M2.9, M3.1, M3.4), immune response (M1.6, M2.5, M3.5), antigen presentation/processing (M5.2) and interferon response (M7.1), among others. This novel analytic framework will be employed to derive modular fingerprints of individual tumors, enabling in turn to refine the molecular classification of breast cancer and to develop more effective predictive and prognostic algorithms.

F.80. **Enhancing Adoptive Cancer Immunotherapy with Vγ2Vδ2 T Cells Through Pulse Zoledronate Stimulation**

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Human γδ T cells expressing Vγ2Vδ2 TCRs monitor foreign- and self-prenyl pyrophosphate metabolites in isoprenoid biosynthesis to mediate immunity to microbes and tumors. Vγ2Vδ2 cells have been used for adoptive cancer immunotherapy with some partial and complete remissions. Most trials have used continuous zoledronate exposure to expand Vγ2Vδ2 cells. Zoledronate inhibits farnesyl pyrophosphate synthase causing isopentenyl pyrophosphate to

accumulate that then stimulates Vγ2Vδ2 cells. Because zoledronate exposure is toxic, we hypothesized that a short period of exposure would reduce T cell toxicity but still be sufficient for monocytes uptake. Supporting this hypothesis, pulse zoledronate exposure with IL-2 resulted in more uniform expansion of Vγ2Vδ2 cells with higher purity and cell numbers as compared with continuous exposure. These Vγ2Vδ2 cells had higher levels of CD107a and perforin and slightly increased tumor cytotoxicity. Importantly, adoptive immunotherapy with Vγ2Vδ2 cells derived by pulse stimulation controlled human PC-3 prostate cancer tumors in immunodeficient NSG mice significantly better (halting tumor growth) than those derived by continuous stimulation. Pulse zoledronate stimulation of Vγ2Vδ2 cells with IL-15 also resulted in higher purity and cell numbers. Like with CD8 αβ T cells, IL-15 preserved early memory Vγ2Vδ2 T cell subsets better than IL-2. However, adoptive immunotherapy with Vγ2Vδ2 cells derived with IL-15 showed similar inhibition of PC-3 tumor growth as those derived with IL-2. Thus, pulse zoledronate stimulation maximizes the purity, quantity, and quality of expanded Vγ2Vδ2 cells. This simple modification to existing protocols would likely enhance the effectiveness of adoptively transferred Vγ2Vδ2 T cells.

T.55. Aberrant Form of MUC1 Promotes an Inflammatory Microenvironment Aggravating Colitis-associated Tumorigenesis

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MUC1 mucin, a transmembrane glycoprotein is overexpressed and hypoglycosylated in human adenocarcinoma and chronic inflammatory conditions compared to healthy tissues. Using azoxymethane/dextran sulfate sodium (AOM/DSS) murine model of inflammation driven colon carcinogenesis, we explored the consequence of this abnormal expression of MUC1 in colitis-associated colon cancer (CAC). MUC1.Tg mice showed higher tumor incidence, decreased survival and greater body weight loss. High expression levels of pro-inflammatory cytokines, including TNF-α and IL-6, were found in MUC1+ inflamed colon tissues and exogenous TNF-α promoted the transcriptional activity of MUC1 as well as over-expression of its hypoglycosylated form. We also discovered that MUC1/p65 complex up-regulated IL-6 and TNF-α gene expression in the intestinal epithelial cells (IECs). Therefore, the presence of human MUC1 established a positive feedback circuit of inflammatory cytokines in CAC. To understand the significance of MUC1/p65-modulated cytokines in progressive colitis that gives rise to colon cancer, we analyzed infiltration of inflammatory cells into the inflamed colon tissues. Immunofluorescence assay indicated increased presence of CD206+ type 2 macrophages (M2) in colon tissues of AOM/DSS-treated MUC1.Tg mice compared to WT mice. We are currently analyzing the abundance of M1- and M2-macrophages in colon tissues from inflammatory bowel disease patients. Preliminary data showed expression of hypoglycosylated MUC1 on the inflamed tissues and the presence of macrophages expressing CD163+ a human M2 marker. We are currently exploring *in vitro* the direct role of MUC1 in the recruitment and polarization of macrophages. Thus our findings suggest a novel pro-inflammatory role for MUC1 in CAC.

T.56. T Cell Immunotherapies Trigger Innate Immunity and Aseptic Inflammation Leading to Potent Anti-Tumor and Off-Targets Effects

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Mobilizing the immune system to treat advanced cancers is now a clinical reality. Successful immune-based therapies that treat tumors are often accompanied by immune-related adverse events (irAE) including toxicities that can occasionally

present with severe and lethal symptoms. The primary immunotherapies currently in clinic include agents that activate T cell responses such as checkpoint blockade of inhibitory pathways and infusion of *ex vivo* tumor-derived or T cell receptor (TCR)-transgenic or chimeric antigen receptor (CAR)-modified T cells. While the beneficial and toxic effects of T cell-based immunotherapies in the clinic are being extensively explored, the precise mechanisms of tumor elimination and irAE remain the subject of intense investigation. In the present study, we treated established tumors with melanoma-specific adoptive CD4+ T cell transfer and costimulation via OX40 or checkpoint blockade with anti-CTLA-4. We found that, in spite of co-opting the adaptive immune response to treat cancer, acute local inflammation, resembling delayed-type hypersensitivity, plays a fundamental role in tumor elimination and related toxicities in a model of irAE. While OX40 or CTLA-4 antibodies stimulated T cells are necessary for initiating a therapeutic response, activation of endogenous neutrophils constitutes an important and necessary effector mechanism of tumor destruction and irAE. Upon closer examination, we found extensive neutrophil extracellular traps (NETs) in ear pinnae of treated mice and in melanoma patients suffering from immunotherapy-induced irAE. Our results illustrate the involvement of innate immunity in promoting tumor elimination and subsequent side effects with immunotherapies that engage T cells.

T.57. IL-10 Receptor-alpha Expression in the Axillary Lymph Nodes of Patients with Breast Adenocarcinoma

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Background. Many tumor infiltrating lymphocytes (TILs) are T-regulatory cells that promote immune tolerance by via IL-10 acting on IL-10 receptor-alpha (IL-10R) on macrophages. Pro-inflammatory M1 macrophages inhibit tumor growth while anti-inflammatory M2 macrophages enhance tumor growth and metastasis, as demonstrated in pancreas, colon, liver, and lung cancer. CD206+ M2 macrophages recruit fibroblasts after tissue insult. Programmed death 1 (PD-1) protein is a cell membrane receptor expressed by T-cells, natural killer cells, and B-cells. Binding to ligands PD-L1 and PD-L2 inhibits T-cell activation and enhances regulatory function to decrease immune response.

Design. We examined IL-10R expression in axillary lymph nodes of 15 patients with breast ductal adenocarcinoma. Twelve had at least one lymph node positive for metastatic carcinoma. Tissue microarrays (TMAs) including metastatic tumor cells and lymphoid tissue were stained for IL-10R (NBP2-46046), PD-L1 (clone 28-8), CD4, CD8, and CD206 (clone15-2).

Results. IL-10R was expressed on peri-tumoral and sinusoidal macrophages also positive for CD206 and PD-L1. Macrophages in cases without metastases were also triple positive for IL-10R, CD206, and PD-L1. TILs were positive for IL-10R, the majority co-expressing CD4.

Conclusion. The presence of IL-10R and PD-L1 on the CD206+ macrophages along with IL-10R on the CD4+ T-cells in lymph nodes of patients with breast carcinoma suggests an environment that induces T-cells dysfunction and anergy. PD-1 expression was restricted on the lymphocyte within the germinal center and no PD-1 expression was noted on the TILs. These data support the use of immunotherapy to boost the immune function in breast carcinoma.

T.58. Acute Interstitial Nephritis Secondary to Programmed Death-1 (PD-1) Blockade: Role for PD-1 Ligands in Human T Cell-Renal Microvascular Endothelial Interactions

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Human renal microvascular endothelial cells (RMEC) express high levels of MHC class II proteins under non-pathologic conditions. Prior preliminary data indicated that isolated RMEC activate T cells in a class II peptide dependent manner leading to T cell proliferation and cytokine secretion. This effect was blocked by antibodies to CD40, CD58 and HLADR; and enhanced by antibodies to PDL1 (B7H1, CD274). We hypothesized that RMEC limit T cell activation to HLA class II

presented peptides by the PD-1 pathway.

Stimulatory and inhibitory second signaling proteins on RMEC (CD34+, HLADR+, CD45-) and renal T cells (CD45+, CD3+) were assessed using flow cytometry. RMEC express CD58, CD275 (ICOS L), CD274 (PDL1), CD273 (PDL2), B7H3 and B7H4; they lack CD80 and CD86. Renal T cells express CD2, CD28, CD274 (PDL1) and CD279 (PD1). Secondary signal effects on T cell activation are being evaluated with cultured RMEC and anti-CD3 stimulation of T cells.

Four cases of acute kidney injury in patients receiving PD-1 blocking monoclonal antibodies for their cancers were identified. Biopsies revealed an intense non-eosinophilic interstitial nephritis in the area of the class II expressing RMEC. This suggests a role of PD-1 ligands in limiting T cell mediated renal inflammation.

Our interpretation of RMEC's ability to activate T cells, their high levels of PD-1 ligands and now the development of interstitial inflammation in patients receiving anti-PD1 monoclonal therapy is that RMEC are poised to present peptide as an immune surveillance system, with the PD1 pathway functioning to restrain T cell activation.

W.62. PI3Kγ Signaling Enhances Microenvironmental Interaction and Survival of Chronic Lymphocytic Leukemia Cells

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CLL is the most prevalent hematologic malignancy among adults and remains incurable. CLL cells rely on BCR signaling for their survival. Interactions with stromal cells within the microenvironment are critical for CLL survival and disease progression by protecting them from chemotherapeutics. Blocking these interactions represents an important therapeutic strategy. PI3K enzymes have essential roles in signal transduction via the BCR, and Idelalisib (PI3Kδ-specific inhibitor) shows efficacy in CLL treatment. PI3Kγ has been extensively studied in T cell receptor signaling, but not in B cells. However, PI3Kγ inhibitors are now in clinical development for B cell malignancies. Here we assess whether PI3Kγ is a critical factor controlling CLL migration and retention in the microenvironment. We observed that expression of p110γ catalytic subunit and p101 adaptor subunit increased in CLL cells in response to CD40L+IL-4 stimulation but not BCR cross-linking. The adaptor subunit p84 was minimally expressed in CLL cells. PI3Kγ inhibition reduced CLL cell migration and adhesion to stromal cells. CRISPR-mediated knockdown of p110γ caused a reduction in migration of CLL cells and cell lines, while overexpression of p110γ significantly enhanced ZAP70+ CLL cell migration. PI3Kγ inhibition impaired cell migration to a similar extent as Idelalisib or the dual PI3Kδ/γ inhibitor Duvelisib. Microscopic examination of cell migration behavior within SDF1α gradients revealed that PI3Kγ has an important role in chemokine gradient sensing and impacts on cell polarization induced upon chemokine exposure. Together our findings provide evidence that PI3Kγ has unique and important functions in chemokine-dependent responses in B cells.

W.63. Single-Cell Proteomic Assessment of CD19 CAR-T Cells Reveals a Complex Landscape of Polyfunctional Antigen-Specific Response

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Single-cell multiplexed proteomics has provided an unprecedented insight into the functional heterogeneity of phenotypically similar T cells. We employ a single-cell, 17-plex cytokine assay microdevice and new data visualization methods to fully evaluate the functional profile of chimeric antigen receptor (CAR)-modified T cells targeting CD19, which have shown promising clinical efficacy in patients with B cell malignancies. The CAR-T cells were manufactured from

human peripheral blood mononuclear cells (PBMCs) transfected with lentivirus encoding the CD19-BB-z transgene. CD4+ and CD8+ CAR-T cells were stimulated with anti-CAR microbeads or control IgG at 37°C, 5% CO_2 for 24 hours. After stimulation, cells were loaded into a single-cell barcode chip (SCBC) containing ~12000 microchambers, each pre-patterned with a complete copy of a 17-plex antibody array and further incubated for 16 hours. We demonstrate the marked heterogeneity and polyfunctionality (2+ secreted cytokines) of individual CD19 CAR-T cells across 4 donors, comprising anti-tumor effector profiles mixed with a range of stimulatory, immunosuppressive, and immunotoxic/proinflammatory functions. In order to take full advantage of the high dimensionality of this data, we present new bioinformatics visualizations and dimensionality reduction techniques for effective combinatorial polyfunctionality analysis. Our analysis reveals a complex yet meaningful landscape of immune effector response in CD19 CAR-T cells to antigen-specific challenge. The ability to dissect polyfunctional subsets has the potential to guide CAR-T product pre-infusion quality assessment and result in CAR-T cells engineered with more efficacious and less immunotoxic profiles.

W.64. Radiation Combined with IL-37 Enhances Antitumor Effects in DU145 Prostate Cancer Cells

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Prostate cancer (PCa) is the one leading incidence of cancer in men in the USA, with the exception of skin cancer. Radiation therapy (RT) is a standard treatment for PCa, but there is toxicity that is caused by high radiation dosages, which causes side effects. PCa is categorized as a radio-resistant tumor. Thus, there is a need for a safe and efficient radiosensitizer that would decrease the radiation dosage. IL-37 is an IL-1 family member, which has been extensively studied in the immunological field. However, few studies have addressed the potential of IL-37 as a radiosensitizer. This study investigates whether or not IL-37 could sensitize PCa to radiation. Clonogenic survival assay, quick cell proliferation assay, immunohistochemistry (IHC), TUNEL staining, and caspase-3 activity assay kits were utilized to investigate the combined effect of IL-37 and RT (IL-37/RT) on cell proliferation, survival, and apoptosis of DU145, a PCa cell line. Further studies were performed by RT-PCR and IHC to evaluate the potential molecular mechanisms. IL-37/RT enhanced RT-induced inhibition of cell proliferation and apoptosis in DU145 cells. The expression of the anti-proliferative molecule, p27, increased and expression of the pro-proliferative molecule, cdk2, decreased which correlated with the decrease in cell survival and proliferation. The expression of pro-apoptotic molecules, Fas ligand and Bax, increased, which correlated with the increase of apoptosis.IL-37/RT enhances antitumor activity in PCa cells via modulation of anti- and pro-proliferative and pro-apoptotic molecules. These findings suggest that IL-37 has a potential as a radiosensitizer for PCa and warrants further investigation.

W.65. *In vivo* Imaging Reveals a Tumor-Associated Macrophage Mediated Resistance Pathway in anti-PD-1 Therapy

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Monoclonal antibodies targeting the immune checkpoint Programmed Death-1 (aPD-1 mAbs) have demonstrated impressive benefits for the treatment of some cancers; yet, these drugs are not always effective and we still have a limited understanding of the mechanisms that contribute to their efficacy or lack thereof. Here we employed *in vivo* imaging to uncover the fate and activity of aPD-1 mAbs in real-time and at subcellular resolution in mice. We show that aPD-1 mAbs effectively bind PD-1+ tumor-infiltrating CD8+ T cells at early time-points after administration. However, this engagement is transient, as aPD-1 mAbs are captured within minutes from the T cell surface by PD-1– tumor-associated macrophages. We further show that macrophage accrual of aPD-1 mAbs depends both on the drug's Fc domain glycan and on Fcγ-receptors (FcγRs) expressed by host myeloid cells, and extend these findings to the human setting. Finally, we demonstrate that *in vivo* blockade of FcγRs prior to aPD-1 mAb administration substantially prolongs aPD-1 mAb binding to tumor-infiltrating CD8+ T cells and enhances immunotherapy-induced tumor regression in mice. These investigations

yield new insight into aPD-1 target engagement *in vivo* and identify specific Fc : FcγR interactions that can be modulated to improve checkpoint blockade therapy.

W.66. Epigenetic Methylation Profiles of CD4 T Cell Signature Loci from Patients with Urinary Bladder Cancer

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Urinary bladder cancer (UBC) is one of the most frequent cancer diseases with 380 000 new cases diagnosed worldwide and about 150 000 deaths yearly. To dissect the role of T helper (Th) cell responses in UBC we investigate the T helper cell subpopulations; Th1, Th2, Th17 and T regulatory cells (Tregs) and their lineage commitment in draining (sentinel) and non-draining lymph nodes and blood from patients subjected to transurethral resection of the bladder (TUR-B) and/or Cystectomy. By analysing methylation in signature genes IFNG, IL13, IL17a and FOXP3 we measure the epigenetic stability of these T helper cells.
In most patients IFNG is more demethylated in sentinel nodes compared to non-sentinel nodes and blood, suggesting a Th1 activation in nodes in contact with the tumor. Aside from that, the distribution of subpopulations in all tissues investigated is highly variable in between patients. All subsets are represented, although there seem to be no, or little Th17 cells in nodes. After neoadjuvant treatment (given in between the TUR-B and cystectomy) a temporary increase in methylation of IFNG locus is seen in blood, which could suggest a translocation of activated Th cells from the blood to the tumor area, but also *de novo* synthesis of Th cells.
By analysing the intra-patient variations in distribution and relative amount of Th cell subpopulations in blood and sentinel nodes we hope to draw conclusions on differences in outcome. The long-term goal is to be able to identify which patients could respond well to immune modulatory treatments.

W.67. Immuno-reprograming of the Tumor Microenvironment using Synthetic Biotics

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The immunosuppressive milieu found within the tumor microenvironment has long been understood to be a key driver of tumor initiation and progression. More recently it has been appreciated that metabolites derived from biosynthetic pathways such as the tryptophan and adenosine pathways are major components in forming this immune privileged environment within the tumor. At Synlogic we are using synthetic biology in combination with natural probiotics to develop engineered bacteria or "Synthetic Biotics", which are programmed with exquisite precision to correct disease-causing metabolic dysregulation. Here we present results showing the development of two engineered bacterial strains that have been designed to consume either kynurenine or adenosine, two molecules known to play central roles in promoting tumor immune tolerance. In *in vitro* biochemical assays, the adenosine-consuming strain or the kynurenine-consuming strain were able to consume 180 and 80 uM adenosine or kynurenine respectively, within 2 hours. These levels of adenosine and kynurenine are ~100-fold and 20-fold higher respectively than the adenosine or kynurenine levels found in cancer patient tumors. For the kynurenine-consuming strain, this *in vitro* kynurenine consumption translated to *in vivo* activity where in sub-cutaneous CT26 tumor-bearing mice, the administration of this strain led to significant decreases in tumor kynurenine levels. Taken together these results demonstrate our ability to generate engineered bacteria that show significant *in vitro* and *in vivo* metabolic activity and support the further development of these synthetic biotics as potential immune-oncology therapies able to modulate the tumor microenvironment.

W.68. Anti-PD1 Therapy Effects on T Cell Repertoire and Functions in Patients with NSCLC Cancer: A Preliminary Study to Identify Biomarkers Of Efficacy

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Background: Immune responses protect against tumors. Conventional chemotherapy may treat cancer but its efficacy is compromised by tumor relapse. Chemotherapy "per se" have immunostimulatory effects and sustain an antitumor T cell response. Anti-PD1 antibodies are used in clinics to boost immune responses blocking of an inhibitor receptor on T cells. We evaluated the T cell repertoire and cytokines in eight NSCLC patients who underwent anti-PD1 therapy after chemotherapy.

Methods: We used PBMC to study T cell repertoire by "Spectratyping" a PCR based technique, and production of γ- IFN, IL-2, IL-4, IL-12, IL-13 and IL-17 by Quantitative PCR. Presence of cytokine message was then confirmed measuring the protein in the sera. Each patient was studied at the end of chemotherapy and after each anti-PD1 shot.

Results: We found that chemotherapy shaped a specific T cell repertoire in these patients, expanding several T cell clonotypes that were maintained by anti-PD1 administration undergoing a long-lasting expansion. Of note, a prolonged effect in term of clinical outcome was paired by a consolidated production of IL-12 and γ-IFN.

Conclusions: These data show that chemotherapy reshapes a T cell repertoire involved in antitumor response and the functional profile of these cells marked a prolonged efficient anti-tumor T cell response. Although preliminary, these results help to understand how monitor the patients undergoing therapy with anti immune-checkpoints. This is of critical importance due to the need to identify biomarkers and monitoring tools to optimize the use of these drugs, considering the high costs of these therapies.

W.69. WNT Signaling Inhibitor XAV939 Potentiates Cytotoxic Activity of **Prostate Cancer Patient'S Lymphocytes** *In Vitro*

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Inhibitors of Wnt signaling are contemporarily aimed for several clinical studies as adjuvant anti-cancer agents. As such these inhibitors might also be considered as promising molecules that could affect cytotoxic impact of prostate cancer (PCa) patient's lymphocytes on PCa cells. To test this hypothesis, we generated stably transfected LNCaP PCa cell line with a red fluorescent protein TagFP635. Lymphocytes from patients with biochemically recurrent prostate cancer were cultured for 2 days in the presence of 20 U/ml IL-2 with or without Wnt signaling inhibitor XAV939. The TagFP635-LNCaP cells were then co-cultured with lymphocytes from patients with PCa at ratio 1: 4 for 3 days. The growth of LNCaP cells was then evaluated by fluorescence microscopy using image analysis. We found that PCa patient's lymphocytes slowed down growth of TagFP635-LNCaP cells by 35% compared to untreated tumor cells control. However, when the PCa patient's lymphocytes were pre-treated with 5μM Wnt signaling inhibitor XAV939 the growth of TagFP635-LNCaP cells was abrogated. No such changes were observed when TagFP635-LNCaP cells were co-cultured with XAV939 alone. Our data indicate that blocking Wnt signaling by XAV939 represents a potential way how to improve the patient`s lymphocytes cytotoxic impact on PCa cells.

W.70. Immune Checkpoint Blockade Recovers the Functionality of Tumor-Infiltrating T cells in Hepatocellular Carcinoma

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Targeting immune checkpoint co-inhibitory pathways is a promising novel therapeutic approach for several types of cancer. Therefore, we aimed to determine which co-inhibitory pathways can be targeted to enhance the functionality of intra-tumoral T cells in hepatocellular carcinoma (HCC).

We measured the expression of co-inhibitory receptors and ligands on leukocytes isolated from paired tumors, tumor-free liver tissues (TFL), and peripheral blood of HCC patients, and studied the effects of blocking co-inhibitory pathways on functional responses of $CD4^+$ and $CD8^+$ tumor-infiltrating lymphocytes (TIL) in *ex vivo* assays.

Expression of PD-1, TIM-3, LAG-3 and CTLA-4 on $CD8^+$ T cells and/or $CD4^+Foxp3^-$ T helper cells was significantly higher in tumors than in TFL or blood. Dendritic cells, monocytes and B cells in tumors expressed their ligands. Using MHC class I multimers loaded with immunogenic Glypican-3 or MAGE-C2 peptides, we found that PD-1, TIM-3 and LAG-3 expressions were selectively increased on tumor-associated antigen-specific $CD8^+$ TIL. Compared to the TIL without co-inhibitory receptor expression, $CD8^+$ and $CD4^+$ TIL expressing those co-inhibitory receptors displayed a more activated status but similar or decreased granzyme B expression and effector cytokine production. Blocking PD-L1, TIM-3 or LAG-3 with neutralizing antibodies enhanced proliferative and cytokine responses of CD8+ and CD4+ TIL to polyclonal CD3/D28-stimulation as well as to Glypican-3 or MAGE-C2 presented by mRNA-transfected autologous antigen-presenting cells. Importantly, combining PD-L1 blockade with TIM-3, LAG-3 or CTLA-4 blockade further enhanced *ex vivo* TIL responses.

Conclusions: PD-1, TIM-3 and LAG-3 may be promising immunotherapeutic targets for the most prevalent primary liver cancer.

W.71. Typhim Vi anti-Polyssacharide Antibody For Primary Response in Hematological Malignancy

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Introduction

Infectious complications in hematological malignancy (HM) patients are the major cause of morbi-mortality. Antibody (Ab) response to protein and polysaccharide immunizations at early stages may have an important value for predicting patients prone to infections.

Objectives

To assess specific antibody response to polysaccharide and protein vaccine and the potential role of Typhim Vi in HM.

Methods

Observational study of the specific Ab response to polysaccharide and protein vaccine in a group of patients with recurrent infections and HM. All patients were immunized with PPV (Pneumo23), Salmonella Typhi (Typhim Vi) and Tetanus Toxoid (Diftavax) of Sanofi Pasteur MSD. Blood was drawn after 4 weeks post vaccination. Specific Ab concentrations in response to PPV,Typhim Vi and Diftavax were measured by ELISA (Binding Site, UK), defining 3-fold increase as normal response.

Results

Twenty-eight patients were studied (mean age 63Ã‚Â±13; 21 women).
All patients underwent clinical and immunological examination and were classified as either: Ã‚Â Advanced stages (CLL n

= 5, HL n= 1, NHL n=13) and early stages (MGUS; n=9). Twenty-one (75%) and 15 (53%) patients had protective anti-PCP and anti-TT baseline levels, respectively (12 presented both); while onlyÃ‚Â 2 (7%) had anti-Typhim baseline antibodies. Nine patients (32%) responded to Typhim, 5 on the MGUS group, with one patients responding to all three vaccines. High discrepancy was observed in the three responses, suggesting the role of primary and secondary responses.

Conclusion

Anti-Typhim responses may add value to the immunological assessment of HM patients, in whom primary responses might better predict susceptibility to recurrent bacterial infections.

W.72. Role of Circulating CD4+ CXCR5+ T Cells in Chronic Lymphocytic Leukemia

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Immune cell dysfunction is one of the key features of the B cell malignancy Chronic Lymphocytic Leukemia (CLL) relevant to poor clinical outcomes including leukemia progression, recurrent severe infections and second malignancies. CD4+ CXCR5+ T cells in peripheral blood, known as circulating follicular helper T cells (cTfh), have specialized functional properties including ability to migrate into B cell zones and promote B cell activation. However, little is known about how cTfh impact on the pathogenesis and progression of CLL. Here we examine the phenotype, activation status, clinical relevance and potential function of cTfh cell subsets in CLL patients. Preliminary flow cytometry data demonstrate that the percentage of cTfh cells is significantly increased in CLL patients compared to healthy age matched controls. The percentage of cTfh cells is significantly higher in CLL patients with poor prognostic markers compared to patients with markers of indolent disease. Furthermore, CLL patient cTfh exhibited different composition of Tfh1, Tfh2 and Th17 subsets and showed higher expression of activation markers compared to healthy controls. Our data suggest that specific cTfh subsets are activated and expanded in CLL patients with more progressive disease. In the future, we will correlate the Tfh subset phenotype to long term clinical outcomes and investigate lymphoid tissue Tfh in CLL patients. We will also investigate the function of cTfh including cytokine and chemokine profiles as well as their migration capacity. Potentially, detailed Tfh subset profiles could serve as useful biomarker to evaluate the progression of CLL.

W.74. Immunotherapy Target Evaluation for Myeloid Diseases

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Current therapies for myeloid diseases have only modest success and the vast majority of patients will ultimately die. Clearly, novel therapies are warranted and adoptive immunotherapies represent an exciting and promising cancer therapy. The success of antibody-mediated therapy targeting immune checkpoint underscores the therapeutic potential of counteracting immune inhibition and T cell exhaustion. Other alternative immunotherapies consist of bispecific T cell engagers (BiTEs), which have had some success for leukemia. A major advance for adoptive T cell therapy is the chimeric antigen receptor (CAR), which is a single chain variable fragment (scFv) fused to the signal domains of a T cell receptor. CAR-T cell therapy has significant potential as a cancer therapy because T cells can expand in numbers to eradicate large volume disease, traffic throughout the body, and provide patients with long-lived tumor immunity. In particular, clinical success with CART19 has generated high complete response rates in patients with B-cell acute lymphoblastic leukemia (B-ALL).

Currently, CAR T cells are being developed against CD33, CD123, CD44v6, as well as other antigens. Early pre-clinical work suggests that CAR-T cell directed towards CD33 and CD123 are effective, but unfortunately also kill normal myeloid and/or hematopoietic stem cells. Considering the extensive B cell aplasia reported with CD19-targeted CAR T cells, a similar off-tumor, on-target toxicity for myeloid malignancies could result in bone marrow failure and/or death. Therefore,

we have decided to compare the co-expression of various tumor markers on AML to identify candidates for multi-antigen targeting. CAR-T cells with a combination of either of these markers would prove effective targeting against leukemic stem cells leaving normal stem cells unharmed.

W.75. Alloresponse to anti-leukemia (CLL): reassessing the role of CD4+ T cells

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Tumor-specific mutations are ideal targets for cancer immunotherapy as there is a lack of expression in healthy tissue; potentially they can be recognized as neo-antigens by the mature T-cell repertoire which can reject the tumor.
Our aim was to characterize neopeptides of CLL-patient's tumors from the protein-encoding sequences of 500 genomes (exomes) that can be recognized by T cells through their binding affinities to HLA molecules.
Neopeptide missense mutations was used to epitopic prediction: concatamers (overlapping sequences) of these neopeptides was analyzed for epitopic predictive binding in all HLA molecules.

At last, by co-relating these peptides with the specific HLA typing from each patient we define most reactive neopeptides to test *in vitro*.

W.76. Functional Dynamics of Regulatory T Cells in Oral Squamous Cell Carcinoma

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Oral squamous cell carcinoma (OSCC) is one of the major cancers affecting in Asian countries. The main causative factor has been tobacco habit. It has been reported that immune dysfunction in these patients is one of the major factors for tumor growth and dissemination that affects disease-free survival of the patients. We assessed the phenotypic and functional characteristics of Regulatory T (T_{reg}) $CD4^+CD25^+FoxP3^+$subsets in patients with OSCC by multicoloured flow cytometry. The effects of garcinol mediated, MACS-purified Tregs inhibition on growth of cell lines was also studied. An increased frequency of $CD4^+CD25^+$, $CD4^+FoxP3^+$, $CD8^+FoxP3^+$ and $CD4^+CD25^+FoxP3^+$ was observed in the peripheral circulation of OSCC patients that positively correlated with clinicopathological features. The increased frequency of $CD4^+CD8^+CD25^+FoxP3^+$, CTLA-4^+, $GITR^+$, $NrP1^+$, HLA-DR^+, $CD127^+$, $Tbet^+$ and granzyme B^+ (GzmB) T_{regs} alsoshowed a significantly higher prevalence in OSCC patients. Functionally $CD4^+FoxP3^+$ T_{regs} showed skewed expression of IL-2, IL-10 and IL-35 in patients as compared with the normal controls. Further, enhanced expression of CCR5 and CCR7 on T_{regs} with up regulation of their ligands (CCL5, CCL19 and CCL21) in tumor cells indicates efficient recruitment and trafficking of T_{regs} to the tumor site. Additionally, garcinol treatment significantly ($P < 0.001$) inhibited the growth of OSCC cells with a concomitant induction of apoptosis, cell cycle arrest and anti-angiogenesis. It appears that garcinol concurrently prevents many tumour-promoting effects Treg. Hence, modulation of functional dynamics of selective T_{reg} subsets may be useful in enhancing anti tumor immunity and developing immunotherapeutic strategies for patients with oral squamous cell carcinoma.

Inflammatory bowel diseases

W.17. Microbial Metabolites Regulate Human T Cell Responses

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Microbial metabolites have strong potential to influence host immune responses. To advance the understanding of microbial functions on T cell responses, we screened a list of predicted differential abundant microbial metabolites in CD. Among 139 screened metabolites, we found 15 bioactive metabolites regulating CD4+ T cells through different actions, reflecting both proven and novel effects on human CD4+ T cells. We discovered twelve novel metabolites regulating T cell cytokine profiles with categorized actions, including pan-inhibition, pan-enhancement, and selective effects on cytokine productions. One novel microbe-derived molecule, ascorbate, selectively induced the apoptosis of activated human CD4+ T cells via modulating energy metabolism. Our findings demonstrate that microbial products can function as a metabolic modulator to influence host CD4+ T cell responses and this can be one novel microbial factors to influence CD progression.

W.18. Therapeutic Potential of Recombinant Wuchereria bancrofti L2 (rWbL2) in the Treatment of DSS-induced Chronic Ulcerative Colitis

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Current research advances for therapeutic interventions against inflammatory diseases including ulcerative colitis shows the use of helminths and their molecules. Hence, in present study, we investigated the anti-inflammatory and therapeutic effect of *Wuchereria bancrofti* L2 against dextran sulfate sodium (DSS)-induced chronic colitis in BALB/c mice.

Mice were administered with four cycles of DSS (4%) with one week interval after each cycle for the induction of chronic colitis. Mice were treated intraperitoneally with four consecutive doses of rWbL2 (25 µg in 200 µl PBS) starting from the 21st day of colitis induction. The body weight and disease activity index of mice were monitored daily. At the end of the experiment, colons were harvested for macroscopic and microscopic examination and also for the assessment of myeloperoxidase activity. The relative mRNA expression of the cytokines in the splenocytes was analyzed by real-time polymerase chain reaction.

The mice treated with rWbL2 ameliorated the disease severity measured in terms of disease parameters *viz.*, disease activity index ($p \leq 0.05$), colon length ($p \leq 0.005$) and degree of mucosal edema ($p \leq 0.005$). Histopathological analysis revealed significantly improved macroscopic and microscopic and decreased myeloperoxidase activity, which correlated with the significant (p

In conclusion, above findings provide valid proof for exploring rWbL2 as a therapeutic candidate against ulcerative colitis.

W.19. IL-7 Pathway Controls Human T Cell Homing to the Gut and Culminates in Inflammatory Bowel Disease Mucosa

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Signaling networks perpetuating chronic inflammatory bowel diseases remain unclear. Here we report that key players of the IL-7 pathway as well as α4/β7 gut-homing integrins accumulate in diseased colon biopsies. While in mice IL-7 is known to play a role in systemic inflammation, we found that IL-7, specifically in humans, also controls α4/β7 integrin expression and imprints gut-homing specificity on T cells. IL-7 receptor blockade reduced human T cell homing to the gut and colon inflammation in different humanized mouse models of colitis and reduced *ex vivo* production of IFNγ by tissues punched from ulcerative colitis patients. In addition, transcriptional analysis of diseased colon mucosa indicated that IL-7 receptor signaling pathway discriminated response to conventional therapies. Our findings suggest that resistance to treatment in inflammatory bowel diseases may occur at least in part by dysregulated IL-7 receptor signaling pathway and point IL-7 as a fuel for gut chronic inflammation.

W.20. A Novel CD177+ Population of FOXP3+ Regulatory T Cells in the Intestinal Mucosa is Decreased in Inflammatory Bowel Disease

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Background: CD177, the receptor for platelet endothelial cell adhesion molecule (PECAM-1, CD31), is expressed on neutrophils, but is not known to be on lymphocytes. We found CD177 mRNA selectively expressed by CD25+, CD127- regulatory T cells (Tregs) from human colon lamina propria lymphocytes (LPL).

Methods: LPL from colonoscopic biopsies of 24 patient with or 9 without inflammatory bowel disease (IBD) were evaluated by flow cytometry. A pilot comparison of the whole genome mRNA expression of CD177+ and CD177- Tregs from the surgically resected colon of a single ulcerative colitis (UC) patient was performed.

Results: High levels of surface CD177 was expressed by up to 60% (mean 18%) of FOXP3+, Helios+, but not Helios-, LPL Tregs. The fraction of FOXP3+, Helios+ LPL Tregs expressing CD177 was smaller in LPL from patients with than without IBD.Â However, because there were more Tregs overall in IBD LPL, the total number of CD177+ Tregs was no lower in IBD biopsies. CD177+ Tregs had a gene expression profile similar to that of CD177- Tregs from the colon of a UC patient, except for a subset of genes up-regulated in the CD177+ population, including chemokines (specifically CXCR2 ligands), cytokines, cell surface receptors, and matrix proteins, suggesting a unique function for this population in mucosal immunity.

Conclusions: CD177+ Tregs represent a novel subpopulation of FOXP3+, Helios+ Tregs in the intestinal mucosa that is relatively under-represented in IBD, and may have a novel gene expression profile.

W.21. Induction of Human Regulatory T Cells by a Parasite-Derived TGF-**β** Mimic

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Immune homeostasis in the intestine is tightly controlled by FOXP3+ regulatory T cells (Tregs); loss of Treg-mediated control is linked to inflammatory bowel disease (IBD). As a mechanism of immune evasion, some intestinal parasites can strengthen Treg activity, indicating that parasite-derived products could be harnessed and used as immunotherapy for IBD. It has been previously demonstrated that *Heligmosomoides polygyrus*, a natural murine parasite, secretes a molecule which mimics the ability of mammalian TGF-β to induce FOXP3 expression in CD4+ T-cells. To determine whether this molecule (TGM), promotes the development of human FOXP3+ Tregs, naïve CD45RA+CD4+CD25– T cells were stimulated with anti-CD3/CD28 mAbs in the presence of TGM or TGF-β. We found TGM efficiently induced FOXP3

expression (>50%) in naïve CD4+ T-cells, and caused loss of methylation at the Treg-specific demethylated region (TSDR) of *FOXP3* to a greater extent than TGF-β, indicative of stable lineage differentiation. We performed the same experiments with sorted *ex vivo* human Th1, Th2 and Th17 cells and, surprisingly, found that TGM (and to a lesser extent TGF-β) promoted the conversion of Th1 and Th17, but not Th2 cells, into FOXP3+ cells which acquired regulatory function and lost methylation at the TSDR. TGM also induced IL-10 production and stabilised the phenotype of CD25+CD127– Tregs *in vitro*. These data indicate that TGM could potentially be used to generate Tregs to treat IBD, and via its ability to convert Th1 and Th17 cells into regulatory cells, may have a unique capacity to reverse harmful pro-inflammatory effects of these cells.

W.22. IRGM1 Expression in Neutrophils Protects Mice from DSS-induced colitis

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Autophagy related gene IRGM1 has been linked to Crohn's disease by genome-wide association studies. Deletion polymorphism upstream of IRGM1 was associated with the down-regulation of IRGM1 expression in patients with Crohn's disease. However, it is still largely unknown how IRGM1 may contribute to Crohn's disease progression. Growing evidence have suggested that neutrophils are actively involved in Crohn's disease activities. Autophagy is important for the function of neutrophil. Here, we investigated the potential role of IRGM1 mediated neutrophil autophagy in inflammatory bowel disease using DSS-induced colitis mice model. We showed that IRGM1 was strongly upregulated in the tissue infiltrated neutrophils after DSS administration. In addition, IRGM1 knock-out (KO) mice developed more severe disease phenotype as indicated by both greater body weight loss and tissue damage. More importantly, the injection of IRGM1 KO neutrophils to the neutrophil deficient mice copied the phenotype of IRGM1 pan KO mice indicating the important role of neutrophil IRGM1 during disease progression. Furthermore, we demonstrated both in *vitro* and in *vivo* that IRGM1 deficient neutrophils showed a decrease of autophagy activity. These data together suggested that IRGM1 mediated neutrophil autophagy maybe important for the pathogenesis of Crohn's diseases and provide a potential novel therapeutic target.

W.23. Involvement of MAIT cells in Crohn's disease

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Crohn's disease (CD) is a chronic inflammatory disease characterized as intestinal disorder in consequence of a dysregulated response in mucosal immune system. Mucosal associated invariant T cells (MAIT), a population of T cells expressing Va7.2, a semi-invariant T cell antigen receptor, abundant in gut mucosa, have antimicrobial activity and ability to produce cytokines. Our study focus in the involvement of MAIT cells in CD. We analyzed mucosal tissue and peripheral blood from healthy controls and CD patients in treatment with anti-TNF (biological therapy) or corticosteroids. We observed an increase number of MAIT cells in mucosal tissue from CD patients, and increased numbers of MAIT cells Va7.2+CD161+CD8+TBET+CD45RO+ in both CD groups compared to healthy controls and between biological and corticosteroid groups. MAIT cells Va7.2+CD161+CD8+TBET+CD45RO- are increased in biological group compared with corticoid or control group. Furthermore, we observed that MAIT cells has higher TNFalpha expression in biological and corticosteroid groups when compared to healthy controls. Moreover, our data demonstrate that patients from biological group who are in clinical activity have an increase numbers of MAITCD8+TBET+CD45RO+ cells compared to patients in the same group but in clinical remission. Aditionally, our data indicate an increase number of MAIT cells with memory phenotype, more responsive than those with CD45RO- phenotype. Furthermore, these cells are producing large amounts of TNFalpha even in patients treated with anti-TNF inhibitors. So, we suggest that accumulation of MAIT cells in peripheral blood and mucosal tissue of CD patients, associated with increased TNFalpha production, contributes to progression of CD disease.

W.24. E. coli OmpC-**specific T cells clones from Crohn's patients express diminished IL**-10 and IL-2

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Background: Crohn's disease (CD) may be an inappropriate immune reaction to normally well-tolerated intestinal bacterial antigens. Indeed, many CD patients possess abnormally high titers of antibodies specific for intestinal bacterial proteins, such as the outer membrane porin C (OmpC) of E. coli.

W.25. Human Resident Intestinal Lamina Propria Cells Rapidly Upregulate Protein Translation and mTOR Pathway Activity During the Induction of an Inflammatory Response

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Under steady-state conditions, human resident intestinal lamina propria (LP) leukocytes (LPL) exhibit low reactivity to bacterial/nutritional antigens as present in the gut lumen and thereby contribute to local immune homeostasis. Under adverse conditions, however, LPL rapidly acquire an activated phenotype characterized by the expression of inflammation-associated genes, and hence actively participate in the regulation of intestinal inflammation. Molecular mechanisms driving the switch from a hyporesponsive to an inflammatory state in LPL are currently unkown.

Here, we addressed the regulation of protein translation during the induction of an inflammatory response in LP cells. For this purpose, we employed a human intestinal organ culture model, in which an inflammatory response is elicited in LP cells upon loss of the epithelial layer (LEL model). Using OPP Click-It technology, we observed that –in comparison to epithelial cells or peripheral blood immune cells- global protein synthesis was low in LP cells under homeostatic conditions and was rapidly upregulated during onset of inflammation, particularly in IgA^+plasma cells and $CD68^+$macrophages. Upregulation of protein translation in macrophages but not plasma cells was partially dependent on mTOR activation. In line with this observation, mTOR activation contributed to the inflammation-associated secretion of the chemokines CCL2/CCL19/CCL22/CXCL1, which are known to be produced by macrophages.

In summary, upregulation of global protein translation and mTOR pathway activity represent early events during the initiation of an inflammatory response in human resident LPL. By mediating chemokine secretion in LP cells they may contribute to the recruitment of immune cells to inflamed mucosal sites.

W.26. Gut-localized PD-L1 Expression Using a Novel Dually-Derivitized Chitosan DNA carrier for Treatment of Murine Chronic Colitis

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Inflammatory bowel disease results from a loss of tolerance to commensal microbiota within the mucosal environment. Immunoregulatory T cell costimulation by programmed death 1 (PD- 1) receptor and PD ligand 1 (PD-L1) can provide a negative signal to inhibit T cell proliferation, mediate tolerance and prevent auto-inflammation. Therefore, we hypothesize that modulation of PD-L1 levels in the gastrointestinal tract (GIT) provides a novel mechanism to control intestinal tolerance. We have developed a novel dually-derivitized (DD) chitosan to package plasmid DNA into nanoparticles for delivery to the GIT for treatment of mucosal disease, such as IBD. Codon optimized gene sequences of human full-length PD-L1 or PD-L1-Fc were sub-cloned into an expression plasmid and formulated in DD-chitosan. *In vitro* potency of the PD-L1 constructs was assessed by inhibition of T cell activation and a receptor binding assay. *In vivo* expression of PD-L1 mRNA was confirmed and disease outcome was evaluated in the T cell transfer model of colitis. Following transfer of

$CD4^+CD25^-CD45RB^{hi}$ naïve T cells, significant effect on weight loss, survival and clinical signs was observed with mice treated with PD-L1-PPs relative to controls. A significant increase in FOXP3 expression was observed in $CD4^+CD25^+FOXP3^+$ cells in PD-L1-PP treated mouse lymphoid tissues relative to controls. Our data suggest that local expression of PD-L1 improves clinical manifestations of murine IBD. Our findings provide a strong foundation for exploring the therapeutic effect of local delivery of T-cell inhibitory checkpoint proteins to treat IBD using the DD-chitosan-based gene vector platform optimized for gut delivery of DNA.

W.27. Identification and Characterization of a Novel Association Between Dietary Potassium and Risk of Crohn's Disease and Ulcerative Colitis

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Crohn's disease (CD) and Ulcerative Colitis (UC) that constitute IBD are caused by imbalance between Th1 and Th17 immune cells, and Foxp3+ T-regulatory immune cells. However, in response to environmental and gut microbial triggers, T cell immune balance is disrupted which lead to inflammation in IBD.

It is shown that salt (sodium) can alter the balance between pathogenic Th17 and Treg cells. We identified that extracellular potassium induces the generation of Foxp3+ Tregs in human by enhancing TGF-β1-signaling. We found that extracellular potassium activates Smad 2 and 3 while suppresses Smad7 and SGK1 in human T cells. We found that extracellular potassium not only induces Foxp3 expression in human T cells but also inhibited generation of human Th1 and Th17 cells.

We translated these findings in a prospective cohort of nearly 170,000 US women who had been followed up for over 20 years. Among a total of 194,711 women over a follow-up of 3,220,247 person-years, we documented 273 cases of CD and 335 cases of UC. Dietary intake of potassium (*P*trend = 0.005) but not sodium (*P*trend = 0.44) was inversely associated with risk of CD. Although, both dietary potassium and sodium were not significantly associated with risk of UC, there was a suggestion of an inverse association with dietary potassium (*P*trend = 0.08). The association of potassium with risk of CD and UC appeared to be modified by loci involved in the TH17 pathway that have previously been associated with susceptibility to CD, particularly SNP rs7657746 (*IL21*). Dietary potassium is inversely associated with risk of CD potentially by regulating immune tolerance through its effect on Tregs and Th17 pathway.

W.28. Evaluation of Novel Antisense Oligonucleotides Targeting RORγt for Treatment of Chronic Inflammatory Diseases

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T helper cells are comprised of several closely related T cell populations that play an important role in every immune response. Our work focuses on T helper cell lineage, Th17 that in characterized by production of a key cytokine, IL17 and is highly involved in chronic inflammation. The differentiation, survival and function of this population is dependent on a RORγt, a member of retinoid-related orphan receptor (ROR) family. Therefore, RORγt is an attractive target for Th17-mediated autoimmune diseases.

The difficulty in targeting RORγt relies in its high similarity to another ROR member, RORγ. RORγ and RORγt transcripts are identical except for the initial 150 bases of the 5' region. Beside this difference the residual sequences, including DNA-

and ligand-binding domains are identical. The expression of RORγt is restricted to the cells of the immune system while RORγ shows highest expression in the liver and kidney. Additionally, these factors regulate expression of different target genes. RORγt drives Th17 lineage commitment and function while RORγ regulates blood sugar and triglyceride metabolism in the liver.

We designed and selected effective RORγt-specific LNA^{TM}-Gapmer antisense oligonucleotides. Our targeting approach is highly RORγt specific and advantageous to small molecule inhibitors that target ligand binding domains of both RORγ and RORγt. *In vitro* data in primary human Th17 cells as well as *in vivo* data in different mouse IBD models show efficacy of antisense oligonucleotides after unassisted delivery. Our data provide first evidence for targeting of RORgt without safety issues of co-targeting of other ROR family members.

W.29. Increased T-Cell Activation and Differentiation in Patients with Alcohol Use Disorder

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Chronic alcohol consumption promotes the activation and differentiation of T-cells. These effects have been associated with increased apoptosis, reduced ability to recognize new antigens, and risk of autoimmunity. We aimed to analyze the profiles of CD4 and CD8 T-cell activation and differentiation in patients with alcohol use disorder (AUD).

Patients and Methods: cross-sectional study in patients seeking detoxification. Blood samples for immunophenotyping (FACSCalibur, BD Biosciences) were obtained the first day of admission. CD4 and CD8 T-cells subpopulations were studied using combinations of monoclonal antibodies for activation (anti-HLADR V500 and anti-CD38 APC) and differentiation (anti-CD45RA PECy7 and anti-CCR7 PE). Patients under immunosuppressive therapy and/or HIV infection were excluded. We analyzed 50 healthy blood donors as controls.

Results: 79 patients were included (81% M); median age was 50 years [IQR:45-56 yrs] and, alcohol consumption was 150g/d [IQR:100-200]. With respect to controls, patients had higher expression of CD4+CD38-HLADR+ and CD4+CD38+HLADR+ activation phenotypes *($p < 0.05$)* and higher expression of all CD8+ activation profiles *($p < 0.001$)*. Regarding T-cell differentiation, patients showed lower proportions of naïve CD4+ T-cells (CCR7+CD45RA+) (32±25% vs. 41± 14%, p) and naïve CD8+ T-cells (22±15% vs. 39±17%, $p < 0.001$). The percentage of Central Memory (CCR7+CD45RA-) and Effector Memory (CCR7-CD45RA-) of CD4+ and CD8+ T-cells were increased in patients than in controls *($p < 0.05$)*.

Conclusions: an increased expression of T-cell activation and differentiation is observed in patients with alcohol use disorder. These findings could help to identify mechanisms of both immunodeficiency and/or inflammation in this population.

Innate immunity

T.59. The Role of CD160 Receptor on NKT Cells in Acute Hepatitis

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Natural killer T (NKT) cells play an important role in both innate and adaptive immunity. CD160, a glycosylphosphatidylinositol-anchored Immunoglobulin (Ig) domain protein that binds to HVEM (herpes virus entry mediator), is expressed on T cells and NKT cells. Although the inhibitory role of CD160 in antigen-specific CD8+ T cells is

well documented in conditions of chronic antigen exposure, the role of CD160 in NKT cells have not been clearly understood. Therefore, we investigated the role of CD160 in NKT cells isolated from WT and CD160-deficient mice. Although CD160-/- mice showed no apparent developmental defects, they were more susceptible to Concanavalin A (Con A)-induced hepatitis than WT mice *in vivo*. While WT mice recovered from acute hepatitis, all CD160-/- mice died within 24 hours following Con A challenges. Serum AST and ALT levels were increased along with increased IL-4, IL-6, IFN-γ and TNF-α. Furthermore, NKT cells harvested from the liver of CD160-/- mice showed significantly higher levels of IFN-γ, TNF-α, and IL-4 upon *in vitro* challenges of α-Galactosylceramide (α-GalCer) or Concanavalin A (Con A) than those from WT mice. These results demonstrate that CD160 plays a critical role in the resolution of acute hepatitis and liver damage, and dysregulation of CD160 receptor signaling in NKT cells resulted in liver injury and death associated with uncontrolled cytokine responses in NKT cells. Therefore, we conclude that CD160 is a negative regulator of NKT cells.

T.60. Autoimmune-associated CLEC16A modulates inflammasome activity in human macrophages

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C-type lectin domain family 16 member A (CLEC16A) is genetically-associated with a spectrum of autoimmune diseases including type I diabetes, multiple sclerosis and systemic lupus erythematosus (SLE). Functional characterization studies of Drosophila and mammalian CLEC16A homologues revealed their regulatory roles in different aspects of autophagy. Recent research advances in autophagy reveal its cross-regulatory relationship with inflammasome and have prompted us to evaluate the role of CLEC16A in inflammasome induction. The functional role of CLEC16A in inflammasome pathway was investigated using human monocyte-derived macrophages. Specific siRNAs targeting CLEC16A in macrophages resulted in a reduction in IL-1β secretion upon lipopolysaccharides stimulation with nigericin or poly(dA-dT), indicating that CLEC16 could modulate NLRP3 and AIM2 inflammasomes activity. Expression analyses showed that the inhibition of CLEC16A had minimal impact on mRNA levels of NLRP3, adaptor protein apoptosis-associated speck-like protein containing a caspase activation and recruitment domain (ASC), interleukin-1 converting enzyme caspase-1 and the precursor pro-IL-1β, suggesting CLEC16A may act indirectly on the NLRP3 inflammasome pathway. Macrophages derived from SLE patients exhibited higher CLELC16A mRNA expression when compared with healthy controls. Interestingly, SLE macrophages produced more IL-1β upon NLRP3 as well as AIM2 inflammasomes activation. Taken together, CLEC16A may indirectly modulate inflammasome activity and affect IL-1β production in SLE macrophages. The mechanism involved is currently under further investigation.

T.61. Hematoma-Infiltrating Macrophages Transition from Inflammatory to Reparative Programs in Intracerebral Hemorrhage Patients

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Intracerebral hemorrhage (ICH) is a devastating form of acute stroke accounting for greater than half of stroke-related deaths. Circulating leukocytes are rapidly recruited to the hemorrhage; however, their role in disease progression and tissue repair in the brain are poorly understood. Findings from animal models have failed to translate into effective therapies for ICH, emphasizing the importance of studying the disease in the patient population. To gain insight into the inflammatory response in patient hematomas, we are utilizing mass cytometry, flow cytometry, and RNA-seq to characterize hematoma-infiltrating leukocytes isolated from ICH patients over a five-day period, in conjunction with an ongoing trial for surgical evacuation of ICH. We have found that the hematoma immune infiltrate is predominantly composed of neutrophils and monocyte-derived macrophages, rather than CNS-resident microglia. We have observed that hematoma macrophages acquire a distinct phenotype from peripheral blood monocytes, suggesting that their gene expression is strongly influenced by local signals in the hematoma. Preliminary transcriptional analysis of hematoma

macrophages 24-50 hours post-ICH has revealed an inflammatory profile characterized by increased expression of antigen presentation, TLR signaling, glycolytic metabolism, and prostaglandin production pathways. Intriguingly, by 100 hours post-ICH, macrophages downregulated these pathways and engaged a wound healing program characterized by TGF-beta signaling, fatty acid metabolism, and collagen deposition. These findings suggest that recruited macrophages may contribute not only to initial inflammatory damage after ICH, but also to clearance of the hematoma and resolution of inflammation, making them a potentially ideal target for therapeutic intervention.

T.62. Modulation of Monocyte Phenotype and Function by Regulatory T Cells

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CD4+CD25+FOXP3+ regulatory T cells are effective suppressors of the adaptive immune system. However, the nature of their interactions with innate immune cells, such as monocytes, is less well characterised. Previous data from our lab showed that Tregs can modulate monocyte function, including a down-regulation of LPS-induced IL-6 release that can persist for 48 hours after Tregs have been removed from co-culture, suggesting a long-lasting mechanism of modulation. The current work aims to further characterise and examine the molecular basis for Treg-mediated monocyte modulation.

Human CD14+ monocytes were isolated by MACS separation. $CD4^+CD25^+CD127^{low}$ regulatory T cells (Tregs) $CD4^+CD25^-CD127^{high}$ and effector T cells (Teffs) were purified using FACS sorting. CD14+ monocytes were cultured alone or at a 2:1 ratio with autologous Tregs or Teffs with anti-CD3 mAb for 16/40 hours, followed by LPS addition.

LPS-induced pro-inflammatory cytokine production was decreased in monocytes upon co-culture with Tregs and increased upon co-culture with Teffs, when measured by ELISA or MSD (TNFα, IL-6, IL-8, n=11) and intracellular staining (IL-6, TNFα, n=6). Monocytes co-cultured with Teffs displayed increased expression of CD80, CD86 and HLA-DR, whereas monocytes co-cultured with Tregs displayed increased expression of CD163, measured by flow cytometry (n=8). These results indicate that Tregs modulate monocyte function and phenotype. We are currently analysing the gene expression profiles of CD14+ monocytes following co-culture without/with Tregs vs. Teffs, using RNA-sequencing. Our aim is to identify differentially expressed genes of interest and characterise the molecular mechanisms underlying modulation of monocytes by Teffs/Tregs.

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T.63. Role of HSP70 in the Activation of the NLRP3 Inflammasome

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The inflammation is a key process in the general physiology of an organism, and one of the first to be involved in the immune response. Its activity is mediated by molecular platforms called inflammasomes. The NLRP3 inflammasome is the most studied and is involved in the maturation of caspase-1, a protease responsible for the secretion of Il-1β and Il-18. Its regulation is tightly controlled via a wide variety of phenomenon and is a vital process to maintain the correct physiology of an organism.

The goal of our study is to determine the effects of the protein HSP70 on the NLRP3 inflammasome. We have shown in human Thp1 cells and in mice macrophages that a heat shock of 42°C during 1 hour leads to an increase in the HSP70 expression 1 hour after the treatment and decreases the activity of the NLRP3 inflammasome. An overexpression of

HSP70 was shown to mimic the effect of the heat shock, whereas an inhibition of the expression of HSP70 (via siRNA or knock-out mice) led to an increased activation of NLRP3.

A promising way to explain the activity of HSP70 is the ubiquitination state of the adaptator protein ASC.

W.77. Cellular Metabolic and Translational Constraints in Preterm Monocytes

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Human innate immune responses are broadly attenuated below 32 weeks of gestation, resulting in increased infections in infants born prematurely. However, despite decades of functional characterization of these cells, the underlying mechanism(s) remain unclear. A switch in energy metabolism, from oxidative phosphorylation to aerobic glycolysis, is required to increase protein synthesis during immune activation. Here, we performed a genome-wide transcriptomic analysis (Illumina HT-12 Human Bead Array) of LPS-stimulated (5h) genes comparing preterm, term (cord) and adult (peripheral) blood monocytes. We found comparable cytokine/chemokine gene responses across all three age groups, despite a lack of corresponding protein expression. In contrast, genes encoding ribosomal subunit were down-regulated in preterm monocytes. On that basis, we hypothesized that the lack of strong immune responses in preterm neonatal monocytes is due to a decreased glycolytic capacity. Using radioactive Met/Cys pulse-labeling, we confirm that blocking glycolysis impaired the increase in translation in primary human monocytes, after LPS stimulation. Accordingly, the glycolytic activity of preterm mononuclear cells was impaired relative to their adult and term counterparts, as shown by reduced lactate production (post-LPS) and reduced rate of extracellular acidification upon adding glucose (Agilent Seahorse Analyzer). Our preliminary data suggests that translation is substantially reduced in preterm monocytes, and that this mechanism is mTOR-related as evidence by lack of mTOR phosphorylation in preterm monocytes. Polysome profiling/qPCR experiments are underway to determine how selective this impairment in translation is. Altogether, our data provide a novel mechanism through which immune activation is constrained during fetal life.

W.78. Expansion of Human Invariant NKT Cells *Ex Vivo* Augments Th2 Polarization and Anti-Tumor Cytotoxicity

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Invariant natural killer T (iNKT) cells are potent innate immune mediators of immune regulation and tumor immunosurveillance. Key obstacles to the translation of human iNKT immunotherapy include the lack of dependable protocols for robust expansion of human iNKT cells and the requisite data on post-expanded iNKT cell phenotypes necessary for clinical application. We have developed a robust human peripheral blood $CD3^+Va24^+Vb11^+$ iNKT cell expansion platform and defined a method to simultaneously augment both Th2 polarization and anti-tumor cytotoxicity of expanded human iNKT cells. Expanding from 2 x 10^8 starting PBMC, the cumulative 21-day mean increase in absolute number iNKT cells was 1750-fold (N = 41 random donor expansions). The $CD4^+$ iNKT subset was preferentially expanded and was $CD56^{neg}CD161^+$ and $CD45RO^+CD45RA^{neg}$, reflecting maturation and memory phenotypes. Cytokine gene and protein profiling showed an augmented Th2 cytokine profile (IL-4, IL-5, IL-13) in expanded iNKT cells following stimulation with anti-CD2/CD3/CD28 antibodies. Anti-CD2/CD3/CD28 stimulation enhanced expression of cytotoxic effector molecules including granzyme B in expanded iNKT cells. Direct cytotoxicity assays using ^{51}Cr-loaded targets and unstimulated expanded iNKT cell effectors revealed a-galactosylceramide (a-GalCer)-dependent killing of the T-ALL line Jurkat. Anti-CD2/CD3/CD28-stimulated iNKT cell effectors killed Jurkat cells in an a-GalCer-independent manner. These data demonstrate a robust protocol to expand and novel pathways to augment cytokine secretion and cytotoxicity in iNKT cells from human peripheral blood. The functional studies suggest that expanded iNKT cells have the potential to impact not

only cancer immunotherapy but also could impact the field of auto-immunity, vaccine augmentation, and anti-infective therapeutics.

W.79. Antiviral Activity of Double-stranded RNA-binding Protein PACT Against Influenza a Virus Mediated Through Suppression of Viral RNA Polymerase

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Interplay between host innate immune responses and viral antagonism determines the effectiveness of viral replication and pathogenicity. Influenza A virus (IAV) confronts host antiviral responses including type I interferon (IFN) production using multiple viral proteins. PACT is a double-stranded RNA-binding protein implicated in host-IAV interaction. On one hand, its activation of RIG-I-dependent type I IFN production is inhibited by viral non-structural protein NS1. On the other hand, PACT is one of cellular proteins identified to interact with IAV RNA polymerase subunit PA. Exactly how PACT exerts its antiviral activity during IAV infection remains to be elucidated. In this study, we demonstrated mutual antagonism of PACT and IAV polymerase. PACT activated RIG-I in the induction of IFN-β by IAV ribonucleoprotein complex. PACT-dependent activation of IFN-β production was suppressed by IAV polymerase subunits PA, PB1 and PB2. PACT associated with PA, PB1 and PB2. Compromising PACT in IAV-infected A549 cells resulted in the augmentation of viral RNA transcription and replication as well as IFN-β production. Furthermore, viral RNA replication was boosted by knockdown of PACT in both A549 cells and IFN-deficient Vero cells. Thus, the antiviral activity of PACT against IAV is mediated primarily through its interaction with and inhibition of viral polymerase. Taken together, our findings reveal a new facet of host-IAV interaction in which PACT and viral RNA polymerase antagonize each other to regulate viral replication and host antiviral response.

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W.80. ILC2-**expressed β2 Integrins Mediate Allergen Induced ILC2 Migration to the Lungs**

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Group 2 innate lymphoid cells (ILC2s) have been shown to expand in the airways during type 2 inflammation. However whether the increase in ILC2 numbers is due to local proliferation or migration from the circulation remains controversial. In this study, we demonstrate that following challenge with the fungal allergen *Alternaria alternata*, ILC2s in the lungs of bone marrow transplanted mice are of donor origin and thus demonstrating that ILC2s can migrate to the lungs. In addition, we show that irradiation of IL-7R$^{-/-}$ mice (which lack ILC2s) and subsequent transplantation of wildtype bone marrow results in significant levels of ILC2s in the lungs, compared to naïve IL-7R$^{-/-}$ mice. We investigated the role of integrins in ILC2 migration as leukocytes primarily traffic from the circulation into the tissues utilizing β_1 and β_2 integrins. Our data suggests that while ILC2s express both β_1 and β_2 integrins, the major contributors to ILC2 migration to the airways are β_2 integrins and more specifically $\alpha_L\beta_2$. *In vivo* administration of a β_2 integrin blocking antibody significantly diminished ILC2 levels in the lungs following *Alternaria* challenge. This was likely a direct effect of blocking the interaction between β_2 integrins on ILC2s and intercellular adhesion molecule-1 (ICAM-1) in the vasculature, as blocking of β_2 integrins on sorted ILC2s diminished adhesion to ICAM-1 *in vitro*. These findings suggest that during allergic inflammation, ILC2s traffic from the circulation into the tissues using firm adhesions between ILC2 β_2 integrins and vascular ICAM-1.

Organ transplantation

T.86. Identification of the Donor-specific Regulatory T Cell Repertoire for Tracking in Human Transplant Recipients

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We aimed to optimize the ability to identify and track donor alloantigen-specific Tregs in transplant recipients using a high throughput TCR sequencing-based approach. On three healthy control samples, we looked for TCRb CDR3 sequences identified in unstimulated FACS-sorted Tregs ($CD4^+CD25^+CD127^-$) and CD4 "nonTregs" ($CD4^+CD25^-$)in several activated T cell populations: 1) CFSElow CD4 cells from bulk CFSE-MLR; 2) FACS-sorted Tregs ($CD4^+CD25^+CD127^-$) expanded by culture with irradiated activated donor B cells; 3) FACS-sorted Tregs expanded by primary MLR in the presence of CTLA-4Ig (Belatacept), and following donor restimulation in secondary MLR with Belatacept. Overlap between unstimulated Tregs and CFSElow-MLR CD4 cells included 20 to 318 unique sequences; Tregs expanded with donor B cells included 1836 to 5139 unique sequences; primary Belatacept MLRs included 42,189 to 92,312 unique sequences; and secondary Belatacept MLRs 18,513 to 25,357 unique sequences. The primary MLRs with Belatacept had similar TCRb repertoires to the unstimulated Treg repertoire (top 100 sequences Jensen-Shannon Divergence [JSD] values: 0.05-0.13), suggesting minimal enrichment for donor-specificity. JSD values comparing the other methods to each other and to the unstimulated Treg repertoires showed greater divergence. The cumulative frequency of nonTregs was higher than that of Tregs in the secondary Belatacept MLR sequences, suggesting the prominent induction of Tregs from nonTregs, whereas the reverse was true in the Treg culture with donor B cells. These data indicate that activated donor B cells preferentially expand natural donor-specific Tregs and that additional natural Treg sequences are identified by each of the methods evaluated.

T.87. Transient Antibody Targeting of CD45RC Induces Transplantion Tolerance and Potent Donor Antigen-Specific Regulatory T Cells

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Rat $CD4^+$ and $CD8^+$ $CD45RC^{low/-}$ have been described as Tregs whereas $CD45RC^+$ are Th1 cells. We reasoned that depletion of $CD45RC^+$ cells with a MAb would eliminate T cell involved in graft rejection and spare Tregs. Transient administration (10 or 20d) of an anti-rat CD45RC MAb in a rat cardiac allotransplantation model (2 different strain combinations in both cases complete MHC mismatch) induced transplant tolerance (>83 % >120 d survival, n=9) whereas isotype-treated controls rejected in + T cells *in vitro* and *in vivo* through intrinsic $CD45RC^+$ signaling. *In vivo* anti-rat CD45RC treatment increased the number of $CD4^+$ and $CD8^+CD45RC^{low/-}$ Tregs, potentiated their *in vitro* suppression, modified their transcriptional signature and potentiated adoptive transfer of donor-specific tolerance to grafted recipients vs. Tregs from controls. We also demonstrate that human CD45 isoforms are expressed differentially by Tregs. Human $CD4^+$ and $CD8^+Foxp3^+$ Tregs were both largely $CD45RC^{low/-}$ and $CD45RA^+RB^+RO^+$. Anti-human CD45RC treatment inhibited GVHD in immune-humanized NSG mice both, when $CD45RC^+$ cells were depleted before and after *in vivo* PBMC administration (n=4 in each group). Anti-human CD45RC MAb induced apoptosis of only human $CD45RC^+$ T cells. In conclusion, short-term anti-CD45RC MAb administration eliminates cells implicated in organ rejection and GVHD while preserving $CD4^+$ and $CD8^+$ $CD45RC^{low/-}$ Tregs that become primed to donor alloantigens and is thus a potent novel therapeutic candidate to favor transplantation tolerance in humans.

T.88. Ethnic and Gender Disparities in Organ Transplant Clinical Trials and Electronic Health Records

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Understanding ethnic and gender disparities in solid organ transplantation has been previously shown to have social and clinical implications. While general disparities at the national level have been mostly studied, it is not clear how generalizable these broad observations are across other levels of the U.S. organ transplant community, particularly in clinical trials, individual centers, and across states. Here, we investigate disparities by comparing gender and ethnic trends from clinical trials and studies in the ImmPort database (https://immport.niaid.nih.gov/), with UCSF electronic health records (EHR), and the U.S. national registry, hosted by the United Network for Organ Sharing (UNOS), for kidney living donors (LDs) and recipients (RPs). We found that 70% of the UNOS kidney transplant LDs are of European ancestry, compared to 43.4% in the UCSF EHR, and 87% LDs in ImmPort. We also observe discrepancies in gender and ethnic trends across clinical-trial, national and center-specific data within the LD and RP populations, especially when we stratify by the age of donation. Our analyses suggest that, while general trends at the national level are useful in painting a broad scenario, they are not necessarily reflective of the kidney transplant populations in clinical trials and various centers. The implications are two-fold: (1) inferences from the results of clinical trials might not be immediately applicable to all transplant populations, which can vary in ethnic and gender composition; (2) strategies in LD and clinical trial recruitment should incorporate and reflect local gender and ethnic shortfalls and disparities.

Other

F.93. True-**Stain Monocyte Blocker™, An Effective Blocking Buffer to Eliminate Nonspecifi**c Cyaninelike Dyemediated Monocyte Binding

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Cyanine dyes and their analogs are commonly used as acceptors in commercially available antibodytandem fluorophore conjugates. However, it has been confirmed that the dyes have a tendency to bind monocytes or macrophages nonspecifically, which limits the ability to do multicolor flow cytometric analysis for lower density antigens. We are introducing TrueStain Monocyte Blocker™ buffer which eliminates this nonspecific binding. This reagent blocks nonspecific binding of Cyanine dyes (PE/Cy7, PE/Cy5, PercpCy5.5, APC/Cy7, APC/Fire™ 750, PE/Dazzle™ 594) to monocytes and macrophages. It does not affect binding of e.g., CD64, CD14 and other tested antibodies. Multiple Cyanine dyes can be blocked at once and stability data shows no decrease in signal intensity compared to the conjugate alone. In addition the reagent has no impact on cell viability. Mock sorting experiments were performed with PBMC to determine if the TrueStain Monocyte Blocker™buffer shows inhibitory or enhanced function on inflammatory cytokine/chemokine production. Our data indicates minimal to no effect above conjugated antibody for IL6, IL8, IL10 and MCP1 production. We also tested TrueStain Monocyte Blocker™ buffer for its ability to affect PBMC proliferation in response to antiCD3/ antiCD28 stimulation. We observed minimal to no effect on proliferation by measuring BrdU incorporation.

W.53. The Immunomodulatory Role of Exosomes Derived from Naïve and Primed Mesenchymal Stem Cells

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Mesenchymal stromal cells (MSCs) are under investigation for treating a wide range of immune disorders due to their immunomodulatory capacity. Although intracellular factors like indoleamine-2,3-dioxygenase have been implicated as important, secreted factors, including exosomes, may also play a major role. Curiously, while MSCs are not very immunosuppressive at baseline, as they have not been “primed” towards a suppressive phenotype, exosomes from the

supernatant of these naïve MSCs still show therapeutic efficacy. Continuing our previous work on how MSC priming influences their therapeutic efficacy, we sought to extend this exploration to their exosomes. We used a polymer method to isolate exosomes from the supernatant of unprimed MSCs or those primed with 48-hours of combined hypoxia/IFN-γ stimulation. There were consistently 2-4x more exosomes secreted from primed MSCs. We labeled these exosomes and dosed them into mixed lymphocyte reactions (MLRs) at concentrations of 10, 50, 125 and 250 µg/mL. This showed dose-dependent uptake of the exosomes into mononuclear cells (PBMCs), but the uptake was less profound when exosomes came from primed MSCs. While no division occurred when the exosomes were added to only responder PBMCs, in a full MLR, the exosomes attenuated T-cell division in a dose-dependent manner. Doses > 50 µg/mL exacerbated T-cell division from both exosome sources, but exosomes from primed MSCs were able to inhibit T-cell division once the concentration was lowered to 50 µg/mL. We are still investigating the mechanisms of this dose and source-dependent attenuation, but it is clear that monocytes (not T-cells) predominantly take up the exosomes.

W.54. Loss of Murine, T Cell Expressed CD70 Does Not Reduce T Cell Expansion

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It is understood that naïve CD4 and CD8 T cells express the TNF receptor superfamily member CD27, while dendritic cells or activated T cells express the CD27 ligand, CD70. The co-stimulatory interaction of CD27 with its ligand, CD70, enhances the activation and proliferation of naïve T cells. Following such activation in humans, there is an upregulation of CD70 on T cells, which can interact with CD27 on other T cells, leading to further enhancement of proliferation. Upon blocking of human CD70, there is a reduction in the proliferation of CD8 T cells, and thus, suggests that CD70 on T cells may serve a role in T cell expansion. This contribution of T cell expressed CD70 has not been well described in mice. As CD70 blocking reduced the proliferation of human T cells, we expected a similar response when stimulating murine T cells. However, we find no reduction in murine lymphocyte expansion when stimulating cells *in vitro* with anti-CD3 and anti-CD28 while blocking CD70 with FR70 antibody. Furthermore, we see no effect of CD70 blocking when enriching for and stimulating CD4 T cells alone. *In vivo,* there is no reduction in activation and expansion of CD8 T cells when adoptively transferring CD70 knockout OTI cells to B6 mice. Together, these data lead us to conclude that murine T cells do not exploit CD70 for expansion. In brief, human and mouse T cells differ in their utilization of T cell expressed CD70.

W.81. Data Cleaning with flowAI Ameliorates Agreement Between Flow Cytometry Analysis and Gene Expression Deconvolution

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The cell composition of the hematopoietic tissue is highly heterogeneous and the characterization of phenotype and functionality of all the immune cell types has high impact on the treatment of diseases and increasing of life expectancy. The instrument of choice for both research and clinical environments that focuses on immunology is flow cytometry. The latest technology analyses up to 30 fluorescence signals and in general researchers struggle to obtain reproducible and robust results. We present flowAI, a software for cleaning the flow cytometry data from unwanted events. flowAI performs its task in three steps by detecting and removing: 1) surges in the flow rate, 2) shifts in the signal acquisition, and 3) margin events in the dynamic range. Our testing revealed that flowAI could be particularly useful to discern anomalies from rare cells, reveal outlier samples, and detect technical anomalies from less reliable instruments. Moreover, we are optimizing flowAI to implement it in automatic pipelines of analysis and to favor the switch from laborious and subjective manual analysis to more effective and reproducible ones. Here, we validated flowAI on a small flow cytometry dataset by evaluating the improvement of the calculation agreement of the cell types' proportions by flow cytometry and the deconvolution algorithm CIBERSORT. flowAI is available from Bioconductor (http://bioconductor.org/packages/flowAI/) and ImmPort Galaxy (https://immportgalaxy.org/).

W.82. Integrated Molecular and Clinical Analysis for Understanding Human Disease Relationships

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By performing gene expression meta-analysis of 36,000 patient samples from 103 diseases, we find that only 20% of published gene associations exhibit significant differential expression. We contextualize the gene expression analysis with phenotypic disease similarity learned from electronic health records of two million patients. We compare disease similarity profiles based on molecular and clinical manifestations. We observe that many autoimmune diseases cluster with infectious diseases that are hypothesized to trigger the disease onset. For example, the only non infectious disease in a cluster of viral infections was systemic lupus erythematosus, a complex autoimmune disease whose pathogenesis is associated with viral infection. Similarly, Wegener's granulomatosis and sarcoidosis are clustered with bacterial infections, which are hypothesized to trigger the onset of those diseases.

To uncover new biological relationships between diseases, we identify surprising disease pairs with significant similarity. One surprising pair of diseases that was positively correlated in both the clinical and molecular data is the autoimmune disease rheumatoid arthritis (RA) and the muscular disorder inclusion body myositis (IBM). We validate clinical utility of our analysis by showing that positively correlated diseases tend to share drug indications. From this therapeutic perspective, the example of RA and IBM is particularly promising for improving treatment of IBM because RA has many approved therapies and IBM has none.

By integrating molecular and clinical data, our analysis identifies diseases with under-appreciated relationships and enables drug repositioning by connecting disparate research communities.

Reproductive immunology

F.81. Permissive Epigenetic Marks at Regulatory T Cell Signature Genes in Fetal Naïve CD4+ T Cells Prime their Differentiation into Regulatory T Cells

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The developing human fetal immune system achieves immune tolerance through active generation of a prevalent population of $CD25^{+}FoxP3^{+}$ regulatory T (Treg) cells present in secondary lymphoid organs. Crucially, fetal $CD4^{+}$ naïve T cells preferentially differentiate into Treg cells upon T cell receptor (TCR) stimulation *ex vivo*. In addition to the expression of the lineage-determining factor *FOXP3*, commitment to the Treg cell fate is preceded by the acquisition of permissive epigenetic modification at Treg cell-specific super-enhancers (Treg-SE) associated with the transcriptional control of Treg signature genes. Through ATACseq (Assay for Transposase-Accessible Chromatin Sequencing) and H3K27ac ChIPseq (Chromatin Immunoprecipitation Sequencing), we show that fetal naïve $CD4^{+}$ T cells share large regions of chromatin accessibility with adult Treg cells that are absent in adult naïve $CD4^{+}$ T cells. A significant proportion of these regions maps within Treg-SE and are associated with Treg signature genes such as *IKZF2* (Helios). We show that Helios is highly expressed in fetal and not in adult naïve $CD4^{+}$ T cells. Cas9-mediated Helios knockdown in fetal naïve $CD4^{+}$ T cells results in the reduced induction of $CD25^{+}FOXP3^{+}$ Treg cells, indicating a potent contribution of Helios expression in enhancing fetal Treg cell differentiation. The establishment of permissive epigenetic marks at Treg-SE within fetal naïve T cells might thus underlie the predisposition for fetal Treg cell differentiation. Further work in this project will correlate these permissive chromatin regions with active gene transcription through RNA sequencing, and validate their key role in driving fetal Treg cell differentiation.

F.82. Osteoporosis in Menopausal Women- a Need for Finding Biochemical Marker

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Mean serum E2 is significantly low with significant increase of Serum OST and Calcium in Cases than Control (E2; 43.40pg/ml vs 162.82pg/ml, OST; 15.87ng/ml, Calcium; 14.57mg/dl vs 8.37mg/dl). Significant negative correlation exists between Serum E2 and OST in both cases and control (cases ; r value =-0.628, p value =0.001, control; r=-0.314, p= 0.026).Serum E2 is significantly more low with significant more increase of Serum OST and Calcium in Cases with positive history of joint pain (N=26) than with negative history (N=24) (E2; Mean ± SD: 38.80±19.00 vs 48.37±22.03,OST; Mean ± SD: 19.22±6.76vs 12.25±7.00,Calcium; Mean ±SD:15.90±3.49 vs 13.12±3.23). Cases with positive history of fracture (N=6) also have more reduction of serum E2, with more increase of Serum OST and Calcium than with negative history (N=44) (E2; Mean±SD:22.50±6.12 vs 46.25±20.56, OST; Mean±SD:25.26±4.34 vs 14.59±7.12, Calcium; Mean±SD:17.14±3.98 vs 14.22±3.47). Cases with positive history of Calcium intake (N=13) have significant increase of Serum OST and Calcium than with negative history (N=37) (OST; Mean±SD:23.24±5.27 vs 13.29±6.65, Calcium: Mean ±SD: 16.43±3.76vs13.91±3.38).

In menopausal women, serum 17β-Estradiol (E2) is lowered as a result failing ovarian function. This decline is associated with increased Osteocalcin level, suggesting clear correlation between serum 17β-Estradiol (E2) and serum Osteocalcin. So they can be considered as biochemical markers osteoporosis in menopausal women. However, larger study is required to arrive at concrete conclusion.

Therapeutics/pharmacology

F.83. Cellular Biomeasures in Pre-Clinical Development of a Monoclonal Antibody Against a Cytokine Receptor Implicated in Autoimmune Disorders

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Biomeasures form an important component of the pre-clinical pharmacodynamic markers to assess the functional competence of a biologic and for enabling theoretical modeling for dose projections. Here, we share our results with flow and mass cytometry based biomeasures and pharmacodynamic markers employed for human and cynomolgus monkey studies for the development of Antibodies A and B designed for treatment of autoimmune diseases. Antibody A and Antibody B are monoclonal antibodies against a cytokine receptor that is present on several different types of immune cells. In order to characterize the *in vivo* pharmacology and safety of our antibodies and to translate the results into a human setting, we first developed multi-parameter flow and marker mass cytometry panels for deep phenotyping human and cynomolgus immune cells and quantified the expression of the target cytokine receptor on single cells from the immune subsets identified above using Quantibrite™ PE based flow cytometry and mass cytometry. The highest expression was seen on pDC, memory fractions of CD4 and CD8 T cells and gamma delta T cells. Finally we show results from two *in vivo* cynomolgus monkey studies treated with either Antibody A or Antibody B where the antibodies efficiently depleted the cells expressing target cytokine receptor consistent with the level of expression of the receptor. In both studies, no in-life adverse events or significant safety concerns were observed. The results from these studies were used in the development of a theoretical model to predict the kinetics of cell depletion in cynomolgus monkeys.

F.84. Enhanced Immunostimulation by TLR9 Agonist Spherical Nucleic Acids *in vitro* and in Mice and Non-Human Primates

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Spherical nucleic acids (SNAs) are a novel class of oligonucleotide agents based on densely packed oligonucleotides radially arranged on a spherical nanoparticle. As a result of their structure, SNAs exhibit increased cellular uptake and avidity for targets compared with the same oligonucleotide that is not in SNA format (linear oligo). Oligonucleotides containing CpG motifs induce TLR9-mediated immune responses. Here we designed, synthesized, and assessed immunostimulatory characteristics of TLR9 agonist SNAs using cell-based assays, and *in vivo* in mice and monkeys. TLR9 agonist SNAs displayed increased uptake in primary cell cultures and increased TLR9 activation in reporter cells compared with the linear oligo. In primary cell cultures TLR9 agonist SNAs induced dose-dependent T_H1-type cytokine secretion and the levels of cytokines induced are relatively higher compared with the linear oligo. Subcutaneous (SC) administration of TLR9 agonist SNAs to wild-type and $TLR7^{-/-}$ mice induced higher levels of cytokine secretion compared with a linear oligo and no cytokine induction was observed in $TLR9^{-/-}$ mice, suggesting that TLR9 agonist SNAs mediate immune responses via TLR9 engagement. A single dose SC administration of TLR9 agonist SNAs to monkeys induced time- and dose-dependent T_H1-type cytokines and immune cell activation. Taken together, these studies demonstrate that SNAs targeting TLR9 protein induce potent immune responses *in vitro* and *in vivo* compared with linear oligos. TLR9 agonist SNAs are a promising approach to cancer immunotherapy, asthma treatment, and vaccine adjuvant applications. One such TLR9 agonist SNA, AST-008, is being developed for immuno-oncology in combination with a selected checkpoint inhibitor.

F.85. Designing the Next Generation of Chimeric Antigen Receptors for Regulatory T Cell Therapy

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Manipulating human regulatory T cells (Tregs) offers the opportunity to induce tolerance in a clinical setting. However, low numbers of antigen-specific Tregs and Treg instability upon prolonged expansion have hampered the implementation of Treg-based therapies. Chimeric antigen receptor (CAR) technology has greatly expedited the generation of tumor antigen-specific effector T (Teff) cells. CARs are synthetic receptors comprising an extracellular antigen-binding domain and an intracellular signaling domain. The latter is commonly a fusion of CD28 and CD3z, allowing for potent T cell activation directly downstream of antigen recognition. Adoption of the CAR platform for Treg engineering represents a promising strategy to generate custom-made antigen-specific Tregs for therapy. Yet, there are marked differences in function and signaling between Tregs and Teff cells. Here, we interrogated CAR-mediated signaling in human Tregs and Teff cells by systematically modifying the CAR cytoplasmic domain. We constructed CARs with three different CD28 mutants, abrogating binding to PI3K, ITK, and LCK. Interestingly, disrupting CAR-mediated PI3K signaling did not significantly alter activation or proliferation of either Treg or Teff cells. However, preliminary data show that loss of PI3K signaling impairs CAR-Treg suppressive capacity. In addition, CARs with three different CD3 chains downstream of CD28, namely CD3z, CD3d and CD3e were examined. Replacing CD3z with other subunits invariably decreased CAR-mediated activation. Altogether, our results demonstrate that PI3K signaling is crucial for CAR-Treg function and that CD3z is essential for optimal CAR-Treg signaling, suggesting that novel CAR signaling modules may maximize CAR-Treg therapies.

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T.67. An Engineered, Particulate-based System for Immunotherapy of Rheumatoid Arthritis

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Current paradigms for the treatment of Rheumatoid Arthritis (RA) are inadequate. They do not address the root cause of the disease - aberrant immune reactions that are intiated by dendritic cell (DC) presenation of modified self antigens. To

address this, we are developing a multi-component, microparticle-based 'anti-vaccine' using biodegradable materials with encapsulated factors. In combination, this MP formulation provides antigen delivery to DCs, and controlled-release of DC recruitment and tolerance-promoting factors to create an immune-modulating microenvironment localized subcutaneously, in which antigen-specific, tolergenic DCs can be generated. Specifically, we have developed poly (lactide-co-glycolide) (PLGA) microparticles (MPs) encapsulating immunomodulatory agents (vitamin D3, transforming growth factor-beta 1, and granulocyte macrophage colony-stimulating factor) and denatured auto-antigen, collagen (for collagen-induced arthritis [CIA] model). We have demonstrated, *in vitro,* the ability of our MP formulation to induce suppressive DCs, which subsequently inhibit allogenic T-cell proliferation and induce regulatory, FoxP3-expressing T-cells in a MLR. Administration of this MP system to CIA mice also proved to be therapeutically beneficial based a marked decrease in clinical scores of MP-treated mice in comparison to an untreated cohort. Additional metrics that support the therapeutic efficacy of this particulate system include: a) histological analysis - which showed decreased cellular infiltration of the synovium of joints taken from MP-treated mice; b) fFDG PET analysis - demonstrated decrease in PET intensity in joints (relative measure of inflammation) of MP-treated groups compared to control groups; and c) RT-PCR analysis of the cytokines which showed increased expression of anti-inflammatory cytokines (IL-10/ TGF-β1) compared to controls.

T.68. Initial Data from a Phase 2 Multiple Ascending Dose Clinical Trial of SEL-212 Indicates That the Addition of Tolerogenic Nanoparticles to Pegylated Uricase Enables Sustained Control of Serum Uric Acid in Symptomatic Gout Patients

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The currently pegylated uricase treatment for refractory gout is compromised by the development of anti-drug antibodies (ADAs) for most patients, adversely affecting efficacy and safety. We have developed synthetic nanoparticles encapsulating rapamycin (SVP-R) that are capable of inducing antigen-specific immunological tolerance to biologic drugs (Kishimoto et al., Nature Nanotech 2016). We report on initial data from a Phase 2 open label multidose clinical study of SEL-212, the combination of SVP-R and the pegylated uricase enzyme pegsitacase, in symptomatic gout patients with hyperuricemia. The primary and secondary endpoints for this trial include the safety, tolerability and pharmacokinetics of repeated monthly doses of SEL-212; sustained reduction of serum uric acid levels (sUA); and prevention of ADAs. As of March 23, 2017, a total of 38 patients had been dosed at 10 U.S. clinical sites. Five of six control patients administered 0.2 mg/kg or 0.4 mg/kg of pegsitacase alone were unable to maintain control of sUA for more than 14-21 days, as expected. In contrast, 11 of 13 patients administered 0.08 mg/kg of SVP-R + 0.2 mg/kg or 0.4 mg/kg of pegsitacase had thus far maintained control of sUA levels for up to 56 days and continue to be dosed. One patient in these cohorts was lost to a protocol deviation and the other reached the trial's stopping rules for failure to control sUA levels. These data suggest that SVP-R can significantly enhance the activity of pegsitacase. Additional data will be updated at the time of presentation.

T.69. Human anti-MUC1 Antibodies Elicited by a Prophylactic Cancer Vaccine for CAR T Cell and mAb Immunotherapies

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Hypoglycosylated MUC1 is a tumor-associated protein that is expressed on over 80% of all human cancers. In our recent clinical trial, individuals at-risk for colonic adenocarcinoma received a prophylactic MUC1 cancer vaccine. Many individuals responded producing high titers of anti-MUC1 IgG antibodies with no detectable toxicity. TCR repertoire sequencing also

revealed several differentially abundant T cell clones induced by the vaccine. This trial provided a rare source of human antibodies raised and affinity-matured in a healthy human host to abnormal MUC1. We isolated and identified 13 anti-MUC1 monoclonal antibodies that could bind to several different epitopes on the MUC1 vaccine peptide with a range of affinities. They also stain MUC1 on human cancer cell lines and colon, breast, lung, and pancreas adenocarcinoma tissue sections while showing no reactivity against a large panel of normal tissues. We found that two of the antibodies are capable of performing MUC1-depdendent complement-mediated cytotoxicity (CDC). Next, we constructed a series of lentiviral vectors encoding chimeric antigen receptors (CARs) using scFv's of the antibodies as antigen binding regions. Several CARs were able to retarget human primary T cells to become activated and produce cytokines in a MUC1-dependent manner, and were able to mediate lysis of MUC1+ human tumor cell lines. Preclinical testing in MUC1.tg mouse tumor graft model is underway. Being of fully human origin and showing a high-degree of tumor specificity and efficacy in preclinical experiments this research has the potential to enable clinical trials needed for approval of these reagents for cancer therapy.

W.83. Novel Immunomodulatory Proteins Generated Via Directed Evolution of Variant IgSF Domains

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The immunoglobulin superfamily (IgSF) is a large, diverse family of proteins expressed on immune cells extensively targeted for treatment of cancers and autoimmune diseases. Most of the therapeutic strategies targeting this family have focused on high affinity antibodies binding a single receptor. Moreover, wild-type IgSF receptors typically exhibit low affinities for their counter-structures, limiting their utility in therapeutic modulation of immune responses. We have developed a novel variant Ig domain (vIgD™) platform using directed evolution and yeast display to affinity mature human IgSF extracellular domains. In this platform, libraries of mutagenized IgSF domains are selected for enhanced or altered affinity to specific recombinant proteins. Fc fusion proteins incorporating these evolved immunomodulatory IgSF domains are then tested *in vitro* for their ability to either agonize or antagonize T cell responses. Multiple novel vIgD™ fusion proteins have been generated which significantly attenuate or accentuate T cell activation *in vitro* as assessed by proliferation and cytokine production. Lead molecules also exhibited *in vivo* efficacy in the human PBMC-NSG™ GVHD mouse model. Efficacy *in vitro* and *in vivo* was superior to wild-type IgSF domains due to the induced alterations in affinity for cognate ligand and through specifically directed changes in their ability to bind additional counter-structures. Our results demonstrate that vIgDs™ evolved to acquire unique biochemical properties significantly enhance therapeutic utility as immunomodulatory agents. This vIgD™ therapeutic platform has broad potential to enhance the activity of biologics in treatment of autoimmune diseases, cancer, and other disorders.

W.84. Generation of Human Alloantigen-Specific Regulatory T Cells by IL-10 Gene Transfer

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Regulatory T cells (Tregs) play a key role in modulating T cell responses. Clinical trials showed that Tregs modulate Graft-versus Host Disease (GvHD) after allogeneic hematopoietic stem cell transplantation (allo-HSCT). In a clinical trial aimed at promoting immune-reconstitution in the absence of GvHD, we demonstrated the safety and feasibility of Type 1 regulatory T (Tr1) cell-infusion after allo-HSCT. Tr1 cells are generated *in vitro* in an antigen-specific manner with recombinant IL-10 or DC-10; however, this cell product still contains effector T cells that may limit the efficacy of Tr1 cells. We developed a method to generate a homogeneous population of Tr1-like ($CD4^{IL\text{-}10}$) cells by lentiviral vector-mediated human IL-10 (LV-IL-10) gene transfer. Here, we show the generation of human alloantigen-specific (allo)-$CD4^{IL\text{-}10}$ cells by LV-10 gene transfer. Allo-$CD4^{IL\text{-}10}$ cells were generated by transducing naïve or total $CD4^+$ T cells with LV-IL-10 following

a second stimulation with allogeneic mature DC. Allo-CD4$^{IL\text{-}10}$ cells secreted high levels of IL-10 upon allo-specific stimulation, displayed an anergic and suppressive phenotype, released Granzyme-B, and selectively eliminated myeloid leukemic cell lines *in vitro*. Allo-CD4$^{IL\text{-}10}$ cells can be differentiated starting from naïve or total CD4^{+} T cells, this latter leading to a sufficient number of allo-CD4$^{IL\text{-}10}$ cells suitable for cell-based therapy. *In vivo* testing of this allo-specific CD4$^{IL\text{-}10}$ cells in humanized model of GvHD are ongoing. These results represent the first step towards the development of allo-specific Tr1-like cells by IL-10 gene transfer and will contribute to broaden the use of Tr1-based immunotherapy to induce tolerance after transplantation.

W.85. Nanodiamonds as Novel Therapeutics to Treat Intracellular Bacterial Infections in Human Bladder Cells

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Recurrent urinary tract infections (UTIs) causing *Escherichia coli* (*E. coli*) pose a significant health threat by evading the immune system via internalization into host cells. In absence of vaccines, antibiotics are the main line of treatment for UTIs, but their overuse contributes towards bacterial resistance, resulting in worldwide public health concern. Thus, it is critical to develop novel therapeutics for UTIs. Nanodiamonds (NDs) are advantageous over conventional nanomaterials as they can be functionalized to adsorb therapeutics and develop novel, slow drug-releasing, targeted delivery systems. Safety studies demonstrate that NDs display minimal toxicity in variety of cells compared to carbon nanotubes. The objective of the current study was to investigate the ability of internalized NDs to kill *E. coli* that invade bladder cells.

We utilized an established *in vitro* bacterial invasion model, comprising of human bladder T24 epithelial cells infected with invasive *E. coli*. Acid-treated 6 and 25 nm NDs were tested for their capacity to kill bacteria by treating infected bladder cells with NDs for 24 hours. We found that 6 nm NDs were able to kill *E. coli* internalized in T24 cells more effectively than 25 nm NDs in a dose dependent manner (46.1% vs 81.1% at 100 µg/mL, P). We further confirmed actin-dependent internalization of 6 nm NDs by using cytochalasin D and determined that we could prevent the efficient killing of intracellular bacteria by inhibiting this internalization of NDs.These findings provide a foundation for establishing NDs as novel therapeutics to kill internalized bacteria causing recurrent UTIs.

W.86. Mitigation of Anti-drug Antibodies against a Peglylated Uricase in Patients with Hyperuricemia Results in Enhanced Control of Serum Uric Acid

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The development of ADAs is a common cause of treatment failure and hypersensitivity reactions. We have demonstrated that synthetic vaccine particles encapsulating rapamycin (SVP-R), are capable of inducing durable immunological tolerance to biologics, resulting in improved efficacy in disease relevant animal models (Kishimoto et al., Nature Nanotech 2016). Here, we report on our translation of these findings to humans by demonstrating the addition of SVP-R to pegsiticase, a pegylated uricase enzyme, mitigated the formation of ADAs, enabling sustained control of serum uric acid levels for at least 30 days after a single dose in patients with hyperuricemia in a Phase 1 open-label multicenter clinical trial. Patients with sUA >6 mg/dl dosed with pegsiticase alone showed an immediate drop in sUA, which returned to baseline levels by 14-21 days in 4 of 5 subjects, correlating with the induction of anti-uricase antibody titers >1000. Patients treated with SVP-R alone showed no meaningful change in sUA. Those treated with SEL-212, the combination of SVP-R and pegisiticase, showed a dose-dependent inhibition of anti-uricase ADAs and corresponding decrease in sUA levels through at least day 30 after a single injection. There was a strong correlation between maintenance of low uric acid levels and low or no ADA titers. These results support monthly dosing in an ongoing Phase 2 multi-dose study in symptomatic gout patients and the potential use of SVP-R to mitigate ADAs for other immunogenic biologics.

W.87. Prophylaxis with Recombinant Human C1 Inhibitor (rHC1INH) Is Efficacious and Well Tolerated for Prevention of Hereditary Angioedema (HAE) Attacks

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Background: rhC1INH is indicated for the treatment of acute HAE attacks. However, prophylactic replacement therapy to provide functional C1INH levels may deter HAE attacks.

Methods: A phase 2, randomized, double-blind, 3-period, crossover study was conducted in patients aged Ã¢â€°Â¥13 years with functional C1INH levels < 50% of normal and Ã¢â€°Â¥4 HAE attacks during previous 3 months.Ã‚Â Patients received rhC1INH 50 IU/kg (max, 4200 IU) once or twice weekly or placebo in three 4-week periods; each treatment was separated by 1 week and symptoms monitored by a daily diary. Primary endpoint (intent-to-treat [ITT] population) was number of HAE attacks per 4-week period. Percentage reduction in number of attacks with rhC1INH from placebo treatment was examined in 10% increments (per-protocol [PP] population).

Results: Thirty-two patients (mean age, 45.9 years [range, 16.9-73.5]) were included in the ITT population (modified ITT, n=31; PP, n=23). Mean number of HAE attacks was reduced from 7.2 (placebo) to 4.4 (rhC1INH once weekly; *P*=0.0004) and 2.7 (rhC1INH twice weekly; *P* < 0.0001).Ã‚Â The majority who received rhC1INH twice weekly (modified ITT: 74.2% [95% confidence interval (CI), 56.8Ã¢Ë†'86.3] and PP: 95.7% [95% CI, 79.0Ã¢Ë†'99.2]) had Ã¢â€°Â¥50% reduction in number of attacks that occurred during active treatment versus during placebo. A greater percentage of patients had 90%-100% reduction in HAE attacks with twice weekly rhC1INH (26.1%) than with once weekly rhC1INH (8.7%). The most common adverse events with rhC1INH were headache and nasopharyngitis.

Conclusions: rhC1INH as prophylactic replacement therapy was efficacious for the prevention of HAE attacks and results support further research.

W.88. Thymol Derived from *Trachyspermum Ammi* a Novel Therapeutic Drug for Filarial Induced Secondary Lymphedema

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Lymphatic filariasis is a major neglected disease of the tropics, caused by *Brugia malayi* and *Wuchereria bancrofti* parasites. These parasites are inhabitant of lymphatic vessels and ultimately choke the lymphatic circulation eventually leading to chronic lymphedema. Stagnation of lymph fluid nourishes the growth of bacteria and fungi predominantly in the dermal cells of lower limbs. Our present study aimed at evaluating the bacterial killing, free radical scavenging and cytokine inhibiting potential of thymol derived from *Trachyspermum ammi*. Thymol shows a clear zone of inhibition in paper disc and agar diffusion methods, indicating significant anti-bacterial activity against *Bacillus cereus* and *Staphylococcus epidermis* associated with filarial lymphedema. Further, thymol scavenges the DPPH (diphenyl-picryl-hydrazyl-hydrate) and Hydrogen peroxide (H_2O_2) free radicals at a dose dependent manner, indicating its inherent anti-oxidant property. *In-vitro* lymphangiogenic activity of thymol was evaluated by 2D matrigel and Nitric Oxide (NO) production in human endothelial cells in response to TNF-α (10ng/ml). These studies showed that thymol favours the formation of the tubular

network and interestingly the NO production is elevated with increasing concentrations of thymol. Bacterial Infection Assay and Viable cell count analysis were carried out to evaluate the internalization of bacteria into the host cells and the viability of host cells after infection respectively. Real Time-PCR analysis and Confocal Imaging studies are under progress. Taken together, thymol efficiently kills bacteria, scavenges free radicals and induces lymphangiogenesis. Thus thymol derived from *Trachyspermum ammi* can be a novel therapeutic agent in the lymphatic filarial treatment.

W.89. Dedifferentiation of Human Terminally Differentiated Dendritic Cells Shuts Down Their Innate and Adaptive Immune Mechanisms

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Induced pluripotent stem cells (iPSC) can be used to generate donor-specific desired cell types, including naïve immune effectors. Although donor-derived iPSC lines are believed to be genetically and immunologically matched, studies in mouse models have claimed that the syngenic mouse iPSC lines can be immunogenic. Therefore, a greater understanding of the inherent immunogenicity of human iPSC and their cellular derivatives is needed for the development of safe and effective cell replacement therapies (CRT). We here report characterization of the innate and adaptive-immune mechanisms in human DC-derived iPSC lines. We show that these iPSC lines express mRNAs of Toll Like Receptor (TLR) molecules and the antigen-presentation pathway intermediates, however, these mRNAs are not translated into functional proteins, and these iPSC lines do not induce TLR-mediated inflammatory cytokine response or inflammasome activation. We also show that these iPSC lines do not activate T cells in an allogenic mixed lymphocyte reaction (MLR), however, they do express low-levels of MHC class I molecules that can efficiently acquire antigenic peptides from their microenvironment and present them to antigen-specific T cells. In addition, we show that these iPSC lines can be efficiently differentiated into hematopoietic stem cell (HSC) precursors as well as antigen presenting cells. Taken together, our data show that de-differentiation of human DC effectively shuts down their immunogenic pathways and implicate transcriptional as well as post-transcriptional mechanisms in this process. Our findings demonstrate that human DC-derived iPSC lines could be useful to understand the development of innate and adaptive immune mechanisms in human DC.

Transplantation

F.90. Il-2 Therapy Restores Regulatory T Cell Dysfunction Induced by Calcineurin Inhibitors

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CD4+CD25+FOXP3+ regulatory T cells (Tregs) constitute a heterogeneous lymphocyte subpopulation essential for curtailing effector T cells and establishing peripheral tolerance. Hence, Tregs have become a key target for immunotherapies in autoimmunity and transplantation. Calcineurin inhibitors (CNIs) are among the most effective agents in controlling effector T cell responses in humans, but they also reduce the size of the Treg pool. The mechanisms responsible for this negative effect and the functional consequences remain to be elucidated. Here we characterize the Tregs subsets from a cohort of transplanted patients under CNIs therapy and we investigate the effects of low-dose IL-2 therapy on Treg homeostasis and function in the presence of CNIs. Our data indicate that CNIs compromise the regulatory capacity of Tregs to a greater extent than previously reported by selectively promoting the apoptosis of the resting and activated Treg subsets, known to display the most powerful suppressive function. These effects are mainly caused by reduced access to IL-2, since Tregs remain capable to translocate nuclear NFAT even in the presence of high CNI levels. Exogenous IL-2 restores the phenotypic changes and overall gene expression effects exerted by CNIs and promotes Treg expansion by enhancing anti-apoptotic Bcl-2 expression. In a CNI-dependent transplant model, the addition of IL-2

resulted in the intra-graft accumulation of Tregs, reduced migration of effector T cells and prolonged allograft survival. Hence, combination of IL-2 and CNIs constitutes an optimal immunomodulatory regimen that enhances the pool of suppressive Treg subsets while effectively controlling cytopathic T cells.

F.91. Distinct Alloimmune Stimulatory Profile of Monocyte-Derived Dendritic Cells and CD40L-stimulated B cells

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Donor-derived allogeneic B cells have been used to measure alloimmune responses in transplant patients, and to expand alloantigen-reactive regulatory T cells (Tregs) that are being tested clinically to induce tolerance and control rejection in liver and kidney transplant patients. However, dendritic cells (DCs) are potent antigen-presenting cells (APCs) that also contribute to alloimmune responses. Therefore, we compared the T cell-stimulating capacity of human mature monocyte-derived DCs (mDCs), differentiated from CD14+ blood monocytes, to CD40L-stimulated B cells (sBcs). In mixed PBMC and purified T cell cultures, mDCs promoted more robust proliferation of allogeneic Tregs, conventional CD4+ T cells (Tconv), and CD8+ T cells compared to sBcs, correlating with mDCs expressing higher levels of HLA and costimulatory molecules compared to sBcs. Both mDCs and sBcs produced large amounts of the chemokines CCL22 and CCL5, whereas sBcs also produced a high amount of CXCL10. Neither APC type produced much TNFα, IL-1α, IL-1β, IL-6, IL-12p70, or IL-23 that can destabilize Tregs. Additionally, mDCs, but not sBcs, produced IL-1R antagonist. TCR sequencing analysis showed that TCR repertoires of sBc- and mDC-expanded Tregs were diverse. The two repertoires had less than 20% overlap, suggesting the two APC types may stimulate distinct repertoires of alloantigen-reactive T cells. Additional experiments are ongoing to determine repertoires of mDC- and sBc-stimulated Tconv and CD8+ T cells and their relatedness to graft-infiltrating T cells in kidney rejection biopsies. These results will provide important information on which APC type provides more accurate recall of pathogenic and tolerogenic alloimmune responses in transplant patients.

F.92. Tolerance Induction with Hematopoietic Stem Cell for Kidney Transplantation

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Tolerance induction is considered to be a final goal in the field of organ transplantation. We have performed simultaneous bone marrow and kidney transplantation in MHC mismatched patients to induce transient mixed chimerism and donor-specific tolerance. From December 2011 to May 2014, seven MHC mismatched patients received simultaneous bone marrow and kidney transplantation. Median follow-up was 34 months after transplant.

The preconditioning regimen consisted of cyclophosphamide, fludarabine, rituximab (375mg/m^2, -7 and -2 days before transplantation), antithymocyte globulin (1.5mg/mg/day,-2~0 days) and thymic irradiation. Maintenance immunosuppressions were tacrolimus and steroids.

Immunosuppression tapering was usually initiated when the patient sustained stable graft function for at least 12 months post-transplant. Immunosuppression was slowly tapered over 6~12 months. Four out of the seven patients were successfully tapered off immunosuppression. One patient is currently undergoing immunosuppression tapering. Two patients failed at immunosuppression withdrawal; Because of acute rejection and severe BK virus nephritis. 5 patients experienced BK viremia during their post-transplant period.

We analyzed T cell reconstitution after bone marrow and kidney transplantation, showed a major expansion of CD8+ effector memory (CD45RA-CCR7-), end-stage effector (CD45RA+CCR7-) T cells, and late-differentiated CD27-CD28- cells. We screened BMTKT patients for the expression of an inhibitory receptor such as PD-1, 2B4, CD160, BTLA, TIGIT, Tim-3 and LAG-3. They were increased an expression of 2B4 and PD-1. And, we analyzed the functionally distinct

subpopulations in regulatory T cells. Patients with experienced acute cellular rejection were more elevated CD45RA-Foxp3^{low} non-suppressive Treg cells (Fr-III) than CD45RA-Foxp3^{high} effector Treg cells (Fr-II).

We have performed seven cases of simultaneous bone marrow and kidney transplantation in MHC mismatched patients. Four out of the seven patients achieved immunosuppression withdrawal and revealed increased CD45RA-Foxp3^{high} effector Treg cells (Fr-II).

T.89. Increased Expression of CEACAM1 in Human Orthotopic Liver Transplantation Is Cytoprotective During Ischemia-reperfusion Injury

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T cell exhaustion has recently been proposed as an alternative mechanism to prevent memory development and tissue damage arising during ischemia-reperfusion injury (IRI) in murine orthotopic liver transplantation (OLT). In our cohort of human OLT patients, IRI$^+$ recipients had more TIM-1$^+$CD4$^+$ and fewer TIM-3$^+$CD4$^+$ T cells 3 months post-transplant than IRI$^-$ recipients. Monocyte/macrophage and hepatocyte carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1) has been identified as a key ligand for TIM-3-mediated negative immune regulation in the liver. Therefore, we sought to understand donor and recipient contributions to CEACAM1 expression in human OLT-IRI. Pre-reperfusion biopsies from IRI$^-$ recipients showed normal hepatic lateral surface CEACAM1 protein expression on bile canaliculi that was dramatically increased in post-reperfusion biopsies. This was in stark contrast to IRI$^+$ recipients whose hepatocytes were not CEACAM1$^+$ at either time point. Additionally, IRI$^+$ biopsies contained larger infiltrates of myeloid cells post- vs pre-transplant; however, only myeloid cells found in IRI$^-$ biopsies expressed CEACAM1. Finally, blood from either group of recipients obtained just before reperfusion through the donor allograft increased the frequency of third party CEACAM1$^+$ monocytes after 7 days in culture, however blood from IRI$^+$ recipients obtained just after initial contact with the allograft induced only half the frequency of CEACAM1$^+$ monocytes as IRI$^-$ post-reperfusion blood. These results suggest that 1) loss of hepatic CEACAM1 in the pre-transplant donor allograft contributes to IRI in OLT, and 2) increased expression of CEACAM1 in human OLT is cytoprotective during IRI, potentially regulating T cell exhaustion, thereby improving IRI-related outcome in recipients.

T.90. MHC-II Donor Peptides Activates human CD8+CD45RClow Tregs Secreting IFNg, IL-10, IL-34 and TGFb to Inhibit Human Transplant Rejection

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Introduction: We previously reported the suppressive properties of rat CD8$^+$CD45RC^{low}Treg cells. To date, human counterparts have never been studied for their relevance as a cell-based therapy.

Materials and Methods: Cytokine secretion assay kits were used to sort IFNg/IL10 secreting Tregs from healthy volunteers PBMCs by FACSAria. Tregs were expanded for 14 days with anti-CD3/CD28 mAbs, allogeneic APCs or syngeneic APCs and peptide and analyzed for TCR repertoire. Suppressive function was assessed *in vitro* on syngeneic CD4$^+$CD25$^-$T cells stimulated by alloAPCs, and *in vivo* into NSG mice infused with PBMCs and grafted or not with allogeneic human skin for allograft survival and xenogeneic GVHD studies.

Results: We demonstrated that human $CD8^+CD45RC^{low}$T cells contain natural regulatory cells expressing Foxp3 and GITR and secreting IFNg, IL-10, TGFbeta and IL-34. $CD45RC^{low}CD8^+$ Tregs inhibited allogeneic T cell proliferation, more efficiently than classical $CD4^+$Tregs. Cytokines and a preferential contact with pDCs were required for $CD8^+CD45RC^{low}$ Tregs suppressive activity, but no cytotoxicity. We developed a protocol to expand $CD8^+$Tregs using IL-2, IL-15 and donor antigens and obtained up to 2000 fold expansion. Expanded Tregs displayed a biased TCR with restricted and private alpha and beta chain repertoire. We showed that $CD8^+$ Tregs recognized a dominant 16aa peptide derived from donor MHC class II molecules, expanding and activating their suppressive function. Expanded $CD8^+CD45RC^{low}$Tregs were highly suppressive *in vitro* and *in vivo* significantly delayed GVHD development and allogeneic skin graft rejection in humanized mice.

Conclusions: We identified and characterized a new natural regulatory T cell population as a promising candidate for cell therapy.

T.91. Pretransplant Expression of CD45RC on Blood T Cells to Predict Acute Allograft Rejection and Cancer in Kidney Transplantation: A Long-Term Analysis

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Pretransplant expression of CD45RC, expressed on $CD8^+$ T cells has been associated with the risk of developing acute rejection (AR) at five years of follow-up in kidney transplant recipient (KTR). The objective of the present study was to analyze the relationship between pretranspalnt CD45RC expression on T cells and the post-transplant outcomes, including AR and cancer in the long-term follow-up.

Methods. Pre-transplant expression of CD45RC on $CD4^+$ and $CD8^+$ circulating blood T cells was determined in a cohort of 89 consecutives, first time KTR. AR, cancers and deaths were retrospectively registered and characterized. The relationship between frequencies of both $CD4^+$ and $CD8^+$CD45RC T cells subsets and post-transplant events was analyzed.

Results. AR, cancer and patient death occured in 20 (22.5%), 25 (28.1%) and 14 (15.7) patients, respectively after a mean follow-up of 11 years. Pre-transplant frequency of $CD8^+CD45RC^{high}$ T cells >54.3% was strongly associated with AR conferring a 4-7 fold increased risk after adjustment on classical risk factors. Cancer was associated with a decreased proportion of $CD4^+CD45RC^{high}$ T cells, with a frequency < 51.9% conferring a 3.5-5.5 increased risk of malignancy. Frequencies of $CD4^+$ and $CD8^+CD45RC^{high}$T cells were positively correlated ($p < 0.0001$) suggesting that recipients at high AR risk display a low cancer risk.

Conclusion. Pre-transplant T cell expression of CD45RC is strongly associated with AR and cancer development in KTR. Thus, our results suggest that CD45RC appears as a double-edged sword biomarker of promising interest to assess both AR and cancer risk before kidney transplantation.

T.92. Interleukin-34, a New and Potent Regulatory Cytokine Involved in Treg function and Transplant Tolerance

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Cytokines are powerful tools to regulate immune responses. Interleukin-34 (IL-34) is a cytokine that binds to CSF1R (the MCSF receptor) and PTPz and involved in differentiation and survival of myeloid cells. Until recently, no link with T cell biology or transplantation had ever been reported for IL-34.

We showed that IL-34 was expressed by rodent $CD8^+CD45RC^{low}$Tregs and human $FOXP3^+CD45RC^{low}CD8^+$ and $CD4^+$ Tregs and we demonstrated that IL-34 was involved in the suppressive function of both $CD8^+$ and $CD4^+$Tregs and markedly inhibited alloreactive immune responses. We demonstrated that rat IL34 overexpression associated with a suboptimal dose of rapamycin potently induced tolerance to cardiac allograft in rat with a total inhibition of alloantibody production. In this model, IL-34 promoted allograft tolerance through modulation of macrophages that migrated early into the graft and induced $CD8^+$ and $CD4^+$ Tregs. We demonstrated that human IL-34 protein administration into NSG mice infused with human PBMCs efficiently delayed in a dose dependent-manner the xenogeneic GVHD when associated with a suboptimal 10-days dose of rapamycin. *In vitro*, we characterized a subpopulation of macrophages more efficiently differenciated by IL-34 and showed that human macrophages cultured in the presence of IL-34 for 6 days more efficiently expanded both $CD8^+$ and $CD4^+$ $FOXP3^+$ Tregs compared to allogeneic APCs or anti-CD3/CD28, with a potentiation of suppressive activity since lower ratios of IL-34-expanded Tregs were sufficient to delay GVHD development in humanized mice compared to polyclonally expanded Tregs.

We demonstrate here the clinical relevance of IL-34 in transplantation as a potent tolerance inducer.

T.93. Identification of Plasma Protein Biomarkers of Acute Renal Allograft Rejection

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Upon presentation of clinical symptoms, renal transplant recipients routinely undergo needle core biopsy to diagnose acute graft rejection. This procedure is invasive and associated with patient morbidity. We aimed at validating a panel of plasma proteins as a minimally invasive and cost-effective set of biomarkers to rule out renal allograft rejection. Based on a mass-spectrometry-based pilot study, 8 proteins were selected to be tested by ELISA in 164 adult renal transplant recipients, including 91 non-rejectors and 73 rejectors. The rejectors were sampled at the time of rejection (-7/+3 days of a positive biopsy) or after the event (>30 days after the positive biopsy). The non-rejectors were sampled +/- 30 days from the negative biopsy. We were able to confirm statistically significant differences both cross-sectionally and longitudinally in Apolipoprotein A1 (ApoA1), as we previously published. In addition, we found statistically significant differences in alpha-2-Macroglobulin (A2M), C1-inhibitor and ITIH4 cross-sectionally, and in Alpha-1-Antichymotrypsin and ApoA1 longitudinally. Next, we sought to determine whether combinations of these markers could be used to classify patients as rejecting or non-rejecting. Multivariate logistic regression and CART analyses demonstrated that a panel including ApoA1, A2M and C4 had the best classification performance (AUC = 0.785). Similar results were obtained in a cohort of pediatric renal transplant recipients (AUC = 0.787). In conclusion, a combination of plasma proteins could be used to rule out acute renal allograft rejection. Our data warrant further validation of this new method in a larger independent renal transplant cohort, both adult and pediatric.

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