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Allergy/asthma

F.35. Physicochemical Characterization of Philippine Grass Flora for Diagnosis of Respiratory Allergies

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As a tropical country, grass pollen grains are the important causes of respiratory allergies in the Philippines. There are different flora in the Philippines compared to that of the western countries where the pollen standards in clinical practice are imported for diagnosis of respiratory allergies. The use of local pollen extracts are important for personalized treatment. Since these pollen extracts have not yet characterized, they have to be processed to improve the extracts' quality. The grass pollen extracts from *Cyanodon dactylon* (bermuda), *Axonopus compressus* (carabao), *Imperata cylindrica* (cogon), and *Saccharum spontaneum* (talahib) were separated by gradient SDS-PAGE and immunoblotted against IgE. Bands were visualized with Fluorchem C2 aided with Alpha View software. The total protein extracts ranged from 281.3-986.6 ug/ml. The protein sizes ranged from 14.4-66.3 kDa: carabao grass, 3.5-66.3 kDa; cogon, 3.5-200 kDa; and talahib, 21.6-66.3 kDa. A single IgE-binding protein band at 55.4 kDa corresponding to known groups of grass pollen allergens was identified in the serum sample in the patient with respiratory allergies. The study showed the varying concentrations of grass pollen extracts as well as the pollen profile of their sizes and these correspond to known groups of grass pollen allergens, and found that the clinical diagnosis is consistent with the identification of the known allergen of the patient with the aid of the local protein extracts.

Keywords: Philippines, *Cyanodon dactylon* (bermuda), *Axonopus compressus* (carabao), *Imperata cylindrica* (cogon), and *Saccharum spontaneum* (talahib), diagnostic protein extract, clinical practice

F.53. IL-10 Producing Lung Resident Memory TR1-like Cells do not Protect Against Allergic Airway Inflammation

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Recent studies have identified allergen-specific tissue resident memory T cells that mediate allergic airway inflammation. However it remains unknown whether an analogous regulatory population exists. Given that previous studies have demonstrated a clear association between IL-10 induction and the long-term efficacy of immunotherapy, we hypothesized that an analogous population of IL-10 producing tissue resident memory T cells could also be present in the lungs of previously sensitized mice. Using a house dust mite induced model of allergic airway inflammation we characterized the cellular sources and the temporal and spatial aspects of endogenous IL-10 production. We find that in the setting of allergic inflammation TR1-like CD4⁺ Foxp3⁺ T cells are the main producers of IL-10. Notably, these cells accumulate in the lungs and persist long after the resolution of inflammation as part of the CD44^{hi} CD62^{low} resident memory T cell pool. However, these tissue-resident memory TR1 cells are not sufficient to prevent inflammation in response to allergen re-challenge. Local depletion in the lung had no effect on airway inflammation and adoptive transfer of these cells into naïve mice was insufficient to protect against allergen re-challenge. Together these data demonstrate that although endogenous TR1-like resident memory cells are induced upon allergen sensitization, they are insufficient to regulate inflammation upon subsequent allergen re-challenge. Future studies focused on increasing the local pool of TR1-like resident memory cells may provide a means of raising the threshold of allergic activation to protect against further allergic challenges.

F.99. Duration of attenuation of allergic responses after modulation of idiotypic regulatory network

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We showed that co-injection of antigen-specific plus anti-idiotypic antibodies could suppress OVA-induced IgE production (JIMR: 2017). Antigen-specific antibodies were produced by conventional immunization of mice (using tetanus toxoid or skin allografting). Anti-idiotypic antibodies were derived by absorption of antigen-specific antibody. Both antibodies with complementary specificity are hypothesized to stimulate two populations of T lymphocytes. Prior to using this treatment approach in larger animals and humans, we have first assessed the longevity of the attenuation afforded by this approach.

Mice received 5 weekly im injections of a mixture of the antibodies described above, and at the same time began immunization with OVA in alum (two injections 14d apart). Control animals received normal mouse Ig only along with OVA immunization. Following the second OVA injection all mice were maintained on exposure to allergen by drinking egg white solution with 1% glucose. 14 and 80 days after the final injections of the antibody mixture groups of mice received a final OVA immunization in alum and were sacrificed 10d later. Serum was collected for assays of OVA-specific IgG and IgE responses, and splenocytes stimulated *in vitro* with ConA or OVA to assess mitogen and OVA-induced IL-4 and IL-2 production.

Attenuation of OVA-IgE serum levels, and of OVA-induced IL-4 production *in vitro*, was seen even in mice receiving a boost in OVA-immunization 80d after the last modulating dose of mixed IgGs was given, and tested 10d later (>13 weeks after therapy). This approach may be used to treat chronic allergic responses in many species.

F.115. Airway Epithelium from Asthmatic Patients have Altered Immune Responses to Human Rhinovirus Infection

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Human rhinovirus (HRV) infection is a common viral trigger of asthma exacerbation. Airway epithelial cells express ICAM1, the entry receptor for most strains of HRV and are the first cell-type to encounter HRV upon infection. Epithelial cells play an important role in general tissue homeostasis, repair of tissue injury, and responses to inflammatory stimuli. Additionally, epithelial cells from asthmatic patients have been shown to be structurally dysfunctional compared to healthy epithelia. We hypothesize that the airway epithelial response to HRV infection in asthma patients is altered compared to controls. Airway epithelial cells were obtained from control and asthmatic pediatric patients, grown at air-liquid interface (ALI) and left uninfected or infected with HRV16. RT-PCR was used to analyze gene expression 24 hours post-infection. ALI cultures from control and asthmatic patients expressed elevated *IFNA* and *CXCL10* in response to HRV16 infection indicating an antiviral response. *TSLP* and *IL33* expression was elevated at baseline in ALI cultures from asthmatic patients and *IL33* was further elevated in response to HRV16 while *TSLP* was not. The IL-33 receptor (*IL1RL1*) and the TSLP receptor (*CRLF2*) were both elevated in response to HRV16 in ALI cultures from asthmatic patients, but not controls. Interestingly, Vimentin(*VIM*) expression was elevated in ALI cultures from asthmatic patients in response to HRV16 compared to controls suggesting that HRV16 may induce epithelial-mesenchymal transition in asthmatic epithelia. These data suggest that epithelia from asthmatic patients have altered responses to HRV compared to controls which may contribute to the development of subsequent asthma exacerbations.

T.7. A Novel Role of Human Lung Endothelial Cells in Allergic Airway Disease by Producing and Responding to IL-33

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Allergic asthma affects more than 300 million people worldwide and is characterized by airway hypersensitivity and eosinophilia. A prevalent feature of allergic asthma is increased serum IL-33 levels, and asthma GWAS have demonstrated that SNPs in the IL-33 locus are significantly associated with disease. While IL-33 and its downstream type 2 responses have been extensively studied in murine models of asthma, less is known about the regulation of human *IL33*. Analysis of the mouse and human *IL33* loci reveals little genomic conservation between the two species in non-coding regions. We generated a novel BAC transgenic mouse strain containing the human IL-33 locus with a reporter to interrogate the regulation and expression of human IL-33. Surprisingly, the mice expressed human IL-33 primarily in endothelium, whereas murine IL-33 was expressed primarily in epithelium. These results mirror the expression profiles of IL-33 in primary human lung cells from the LungGENS database, demonstrating that our novel mouse model faithfully replicates human IL-33 expression. To understand how human IL-33 in the lung is regulated and expressed during inflammation, we examined BAC mice challenged with house dust mite (HDM) or poly(I:C). In contrast to murine IL-33, expression of human IL-33 was reduced during allergic inflammation. We tested whether IL-33 is able to autoregulate itself via a negative feedback loop. Indeed, IL-33 administration downregulated the expression of human IL-33 in lung endothelial cells. Together, these data emphasize a distinct and novel role in humans for lung endothelium in allergic airway disease by producing and responding to IL-33.

T.20. NHERF1 Mediates the Pathogenesis of Airway Inflammation in a Murine Model of House Dust Mite-Induced Asthma

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Na⁺/H⁺ exchanger regulatory factor 1 (NHERF1) is a class I PDZ binding protein that regulates desensitization and down-regulation of agonist-bound G-protein coupled receptors. In addition, NHERF1 also functions as a scaffolding protein that recruits and trans-activates non-canonical signaling pathways, thereby possibly contributing to the pathogenesis of various diseases. Previously, we have shown that NHERF1 promotes the release of pro-inflammatory mediators from mast cells, an immune cell that causes allergic diseases in humans. There is little information, however, regarding the role of NHERF1 in the pathogenesis of asthma and airway inflammation. Here, we provide compelling *in vivo* evidence that NHERF1 sub-serves a deleterious role in asthmatic inflammation. Using a house dust mite-induced mouse model of chronic airway inflammation, we demonstrate a significant reduction in airway inflammation in NHERF1^{+/-} mice, relative to wild-type controls. Specifically, serum IgE levels, airway leukocytosis and lung Th₂ cytokines (i.e., IL-4 and IL-13) are reduced in NHERF1^{+/-} mice. Overall, these results suggest that NHERF1 levels contribute to the severity of airway inflammation in asthma. By this token, we suspect that NHERF1 may serve as a novel druggable target in the treatment of asthma and allergic airway disease.

T.21. The Effect of Fenretinide on Regulatory B Cell Development and Semaphorin 4C in Allergic Asthma Model

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Allergic asthma is a chronic respiratory disease characterized by robust Th₂ immune responses. In this Th₂-driven inflammation, B cells secrete allergen-specific IgE that leads to hypersensitivity reactions. Independent of antibody secretion, regulatory B cells (Bregs) are a subset of B cells capable of suppressing inflammation through multiple mechanisms, including IL-10 production. Despite their importance in regulating immune responses, no known lineage-

marker has been identified. Our laboratory discovered that B cell expression of Semaphorin 4C (S4C) was important for Breg development, and that S4C was downregulated in cells from our patients with either common variable immune deficiency or severe asthma. We have previously demonstrated that Fenretinide (FEN), a strong vitamin A derivative antioxidant, is capable of downregulating inflammation without affecting IgE levels in the blood. Here, we investigate the mechanism of FEN-induced regulatory immune responses using house dust mite mouse model of allergic asthma. We showed that FEN treatment *in vivo* improved lung resistance, increased regulatory B and T cell populations, and decreased IL-4-producing cells. Interestingly, FEN also induced S4C expression and a Foxp3⁺ plasmablast subset, which has not been previously characterized. Preliminary *in vitro* results showed that primary splenic B cells cultured in the presence of FEN increased the IL-10⁺ population compared to untreated controls. Elucidating the specific role of S4C in Breg development at the signaling level and phenotyping the functional role of the Foxp3⁺ plasmablast subset using FEN are currently in progress.

T.22. A Role for Early Oral Exposure to House Dust Mite Proteases in Food Allergy Susceptibility

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With dramatic increases in the burden of infant food allergy, there is a mounting imperative to understand the factors affecting gut mucosal immune ontogeny, particularly those adversely affecting the capacity to develop oral tolerance. We recently demonstrated that allergens from house dust mite *Dermatophagoides Pteronyssinus* (Der p) are present in human milk and thereby could potentially affect gut mucosal immunity in offspring. Here, we observed that mice exposed to Der p allergens through breast milk showed increased gut permeability, IL-33 secretion, group 2 innate lymphoid cells activation and Th2 cell differentiation in the small intestine lamina propria at 2 weeks of life. This pro-Th2 gut mucosal environment hindered the induction of antigen specific FoxP3 regulatory T cells upon oral exposure to egg-derived antigen ovalbumin (OVA) through breastmilk. In the long term, the Der p-induced imbalance in gut mucosal immunity abolished the possibility to prevent food allergy by OVA exposure through breast milk. Neutralization of Der p protease activity indicated that this enzymatic activity was necessary and sufficient for Der p-induced gut mucosal immune dysregulation and increased food allergy susceptibility. Finally, in a birth cohort of 100 infants, the relative risk of developing IgE-mediated egg allergy prevalence at one year was 3.4 when comparing infants exposed to Der p through breast milk to infant exposed to OVA through breastmilk. The evidence that proteases from house dust mite could profoundly affect gut immune ontogeny and risk for food allergy, should promote research for new strategies to prevent food allergy in early life.

W.40. Local Immunoglobulin E Production in Colorectal Polyps of Patients with Food Allergy

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Juvenile polyps (JP) are common in children. The aetiology of JP remains largely unknown and few reports have characterized its stroma in atopic patients. We previously showed that 70 % of patients older than 1 yo, with rectal bleeding and JP had high levels of serum IgE specific to cow milk proteins (CMP). This finding prompted us to study the relation between the aetiology of polyps and the atopic condition of patients.

Resected polyps (PT) and surrounding mucosa (SCT) were studied (n=10) by histopathology (H&E), confocal microscopy and RT-qPCR; cytokines were assessed by CBA and ELISA; germinal centers were isolated by dissection laser microscopy and analyzed by PCR.

We found in 7 patients with serum CMP-specific IgE a cell infiltrate in the stroma of polyps dominated with eosinophils, mast cells, IgE-producing plasmatic cells, ST2⁺, TSLP⁺ and IL33⁺ cells. SCT showed fewer frequencies of IgE⁺ cells (30±10 cells vs 2±1 polyps vs mucosa). Furthermore, we found higher transcription levels of il-4, il-5, il-33, ST2s (p⁺, Ki67⁺, AID⁺, and CD57⁺ (T_{FH})). We demonstrated by PCR that IgE is produced through a direct and sequential class switch (μ to ϵ , μ to γ and to ϵ).

In conclusion, we found in colorectal polyps an allergic inflammatory cell infiltrate with hyper eosinophilia and local production of IgE, which might be related to the atopic condition of patients.

W.89. Elevated Local and Systemic Allergen-Specific IgA Following Timothy Grass Sublingual Immunotherapy

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In a recent double-blinded, placebo-controlled study in adults with Timothy grass (TG) allergy, 2-years of subcutaneous immunotherapy (SCIT) elicited higher levels of circulating allergen-specific IgG4 and sIgG4/IgE ratios compared to sublingual immunotherapy (SLIT). However, inhibition of IgE-Facilitated Antigen Binding (FAB) and allergen-induced basophil activation was nearly identical between SCIT and SLIT groups. We assessed whether SLIT immunotherapy induced higher local concentrations of potential blocking antibodies or higher systemic concentrations of non-IgG4 potential blocking antibodies. Levels of TG-specific antibodies were measured in serum (IgG1 and IgA1/2 at baseline, year 1, year 2) and nasal fluid (IgA1/2, IgE, IgG4 at baseline and year 2) of 84 per protocol subjects (placebo=30, SCIT=27, SLIT=27) with clinical histories of grass pollen-induced allergic rhinitis. Nasal fluid sIgG4 increased for both SCIT and SLIT, but was higher at year 2 for SCIT (mean SCIT=397.2 vs. 152.7 AU/mL SLIT, p<0.05). Serum sIgG1 levels were elevated following immunotherapy, but there were no significant differences between SCIT and SLIT at any time point. By year 2, compared to SCIT, SLIT elicited significantly higher levels of nasal fluid sIgA1 (mean SLIT=156.8 vs. 27.7 AU/mL SCIT, p<0.01) and sIgA2 (mean SLIT=156.4 vs. 80.6 AU/mL, p<0.01). Serum sIgA1 was also elevated in SLIT compared to SCIT and peaked at year 2 (mean SLIT=860.3 vs. 729.9 AU/mL, p<0.01). SLIT-induced IgA may contribute to allergen blocking activity and may help explain why SCIT and SLIT were equally effective at reducing clinical symptoms to grass allergen by the second year of therapy.

W.113. Progressive Increase in Allergen Concentration Abrogates Immune Tolerance in Ovalbumin-Induced Murine Model of Chronic Asthma

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Persistent inflammation and remodelling of airways are the major hallmarks of asthma. Though airway inflammation diminishes in ovalbumin(OVA)-based mouse model of chronic asthma owing to immune-tolerance linked with repeated allergen exposure, which limits the application of the disease model. Accordingly, the present study was designed to develop a murine model of chronic asthma which presents persistent airway inflammation coupled with remodelling traits. Herein, OVA-sensitized BALB/c mice were challenged with increasing (modified protocol) or constant concentration (conventional protocol) of the allergen for 6 weeks; 3 times/week. The results, indeed, revealed that mice subjected to modified protocol demonstrate an improved response to the allergen as reflected by the significant increase in inflammatory cells particularly, eosinophils in bronchoalveolar lavage fluid compared to conventional protocol. The increase in inflammation was further accompanied by a marked increase in mucus production, collagen deposition, and the expression of allied factors (Muc5ac, Col1 α 1, and α -SMA). Interestingly, pre-treatment of dexamethasone, a corticosteroid (0.5 mg/kg b.wt., i.p.), suppressed the allergen-induced airway inflammation and mucus production without altering collagen deposition. Failure of dexamethasone seems to be related to their ineffectiveness to modulate the expression of TGF- β , MMP-9, COL1 α 1, and α -SMA. Overall, our results strongly suggest that mice underwent modified chronic model bears more resemblance with asthmatics as it imitates persistent airway inflammation allied with steroidrefractory remodelling traits; hence, may be useful for the evaluation of new/alternative drugs in steroid-refractory asthmatic conditions.

Autoimmune neurologic diseases

F.17. Increases in Eomes-Expressing Th Cells in Secondary Progressive Multiple Sclerosis Reveal Patients at Risk of Increased Disability

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Multiple sclerosis (MS), an autoimmune disease of the central nervous system (CNS), frequently manifests with a relapsing/remitting course (RRMS); in many patients this disease shifts to a progressive course with accompanying chronic neuroinflammation. The pathogenesis of this secondary progressive MS (SPMS) is poorly understood, lacks effective therapies, and the clinical boundary for diagnosis is unclear. Using a novel mouse model, we have previously identified cytotoxic-like CD4⁺ T helper (Th) cells that are associated with chronic CNS autoimmune disease. These unusual T cells express the transcription factor Eomes, are pathogenic, and may generate disease by directly inducing neuronal cell death. Further, we found that similar eomes⁺ Th cells are increased in the blood and in cerebrospinal fluid (CSF) of patients with SPMS, but not RRMS (Raveney *et al.* Nat. Comm. 2015).

Further characterization of eomes⁺ Th cells in SPMS patients revealed a unique cell surface phenotype, including the co-expression of CX3CR1 and granzyme B. Such markers allowed further detailed expression and functional analysis of this cell population in SPMS patients. Strikingly, analysis of clinical features revealed that the presence of high proportions of eomes⁺ Th cells in SPMS patients was linked to actively progressing disease (p=0.0013, OR=5.7) and was highly predicative of patients with worsening symptoms following the eomes⁺ Th PBMC evaluation (ROC-AUC=0.84). Thus eomes⁺ Th cells could act as a biomarker indicating transition to SPMS as well as provide monitoring information on patient disease status. Our studies into eomes⁺ Th cells may provide new therapeutic targets for SPMS treatment.

F.19. From T Cell Receptor to Antigen, Systems Approach to Discovering T Cell Antigen(S) in Multiple Sclerosis and Experimental Autoimmune Encephalomyelitis

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The long-standing quest in MS and experimental autoimmune encephalomyelitis (EAE) is to determine key T cell antigen(s), which drive the initial response. By using single cell paired TCR sequencing, we have analyzed both MS patients and in mice with EAE with respect to their overall immune system and the specific T cell types. We found significant T cell clonal expansion of brain homing, activated CD4, CD8, and gd T cells from the blood of recent onset untreated MS patients compared to healthy controls. Using yeast displayed peptide-HLA (p-HLA) library, we have screened expanded CD4 TCRs from HLA-DR1501 MS patients and found ligands which are not myelin. Yeast library derived peptides can activate CD4 T cell clones from MS patients. Similarly, In EAE, we observe successive waves of specific, clonally expanded CD4, CD8, and gd T cells in the blood and in the central nervous system (CNS). This parallels our previous results with the induction of celiac disease, suggesting that this mobilization of different T cell types is a general phenomenon across species and autoimmune diseases. Examining the antigen specificity of these T cells, we find that while many CD4⁺ T cells are specific for the MOG₃₅₋₅₅ peptide, most of the CD8 T cells are not specific for myelin proteins, nor are they cytotoxic. Furthermore, peptide mimetics for two of these CD8 T cells inhibit the EAE, by suppressing the proliferation of MOG-specific CD4 T cells. This confirms and extends previous reports describing a novel class of regulatory CD8 T cells.

F.29. Recirculating Intestinal IgA-Producing Cells Regulate Neuroinflammation During EAE in Mice

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Plasma cells (PC) are found in the central nervous system (CNS) of Multiple Sclerosis (MS) patients, however the source and role PC play in MS pathogenesis remain unclear. Studying an animal model of MS (Experimental Autoimmune Encephalomyelitis, EAE) we could find that a significant portion of PC in the CNS of EAE mice produce IgA. Moreover, we show that IgA⁺ PC are dramatically reduced in the gut of EAE mice. Moreover, we found that IgA⁺ B cells primed in the gut could be mobilized out of the intestine into extra-intestinal sites including the bone marrow, the lung, and the inflamed CNS. Removal of Blimp-dependent PB/PC resulted in exacerbated EAE that was normalized by the introduction of gut-derived IgA⁺ PC, and mice with an over-abundance of IgA⁺ PB/PC were resistant to EAE. Although IgA itself was dispensable in dampening EAE, expression of IL10 and iNOS in Blimp⁺ B cells promoted EAE resistance. Our data show that IgA⁺ PB/PC mobilized from the gut play an unexpected role in suppressing neuroinflammation and could be important for future directions in MS pathogenesis.

F.56. Dysregulation of Peripheral Lymphocytes in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome

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[Objective] Myalgic encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) is a severely debilitating disease whose symptoms include profound fatigue with various neurological and autonomic impairments. Although the etiology of ME/CFS is still unknown, B cell depletion therapy by rituximab was reported to ameliorate symptoms in about two thirds of ME/CFS patients. However, the mechanism of rituximab treatment is unclear. The purpose of this study is to evaluate abnormalities of peripheral lymphocytes in ME/CFS patients.

[Methods] 60 Japanese ME/CFS patients (age $\pm$ SD=39.5 $\pm$ 11.5 years old: Male: Female=11:49) fulfilled all of Fukuda criteria, Canadian criteria and international consensus criteria and 19 healthy individuals as controls were selected. Peripheral blood mononuclear cells (PBMCs) were isolated from blood samples and T and B cell subsets and their activation status were evaluated by flow cytometer. The B cell receptor (BCR) repertoires in PBMCs were analyzed with high-throughput sequencing and the diversity and clonality of BCR repertoire were evaluated.

[Results] The frequency of regulatory T cells (Tregs) was significantly decreased and the frequency of HLA-DR positive central memory T cells was markedly increased in ME/CFS patients compared with healthy subjects. The mean fluorescence intensity of CD86 was significantly elevated on memory B cell in ME/CFS patients compared with healthy subjects. A significant increase in clonality of the BCR repertoire in ME/CFS was observed. These results imply that patients with ME/CFS exhibit alterations in T and B cell immunity shared by autoimmune diseases.

[Conclusion] Reduction of Tregs and activation of T and B cells may be involved in the pathogenesis of ME/CFS.

F.75. Myelin-Specific CD8⁺ T cells Recruited to the Central Nervous System During CD4⁺ T Cell-Induced Autoimmunity Exacerbate Brain but not Spinal Cord Disease

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Multiple sclerosis (MS) is a demyelinating autoimmune disease of the central nervous system (CNS) that is widely studied using the animal model experimental autoimmune encephalomyelitis (EAE). While research has focused primarily on myelin-specific CD4⁺ T cells that initiate EAE, data from MS patients implicate a role for CD8⁺ T cells in MS. Nothing is known about mechanisms by which CD8⁺ T cells influence MS. We generated a model to investigate how myelin-specific CD8⁺ T cells recruited to the CNS influenced CD4⁺ T cell-induced EAE. We hypothesized that the CD8⁺ T cells will alter disease via cytokine production or lytic functions. We induced EAE by transferring CD4⁺ myelin oligodendrocyte glycoprotein (MOG)-specific T cells into mice that had received naïve TCR-transgenic CD8⁺ T cells specific for myelin basic protein (MBP). Recruitment of MBP-specific but not control CD8⁺ T cells increased the incidence and severity of symptoms associated with brain, but not spinal cord, inflammation. This activity was independent of IFN γ and perforin. These findings contrast with our observation that disease induced directly by the MBP-specific CD8⁺ T cells is dependent on IFN γ . Interestingly, MBP-specific CD8⁺ T cells increased the number of pathogenic MOG-specific CD4⁺ T cells that expressed GM-CSF and TNF α in the brain but not the spinal cord. In addition, MBP-specific CD8⁺ T cells enhanced chemokine expression and increased numbers of myeloid cells early in disease in the brain. These data suggest that the interplay between CD4⁺ and CD8⁺ T cells is critical for determining the manifestation of CNS autoimmune disease.

F.88. Acute and Chronic Damage by Autoantibodies in a Model of Neuropsychiatric Systemic Lupus Erythematosus

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Over 50 % of Systemic Lupus Erythematosus (SLE) patients suffer from Neuropsychiatric systemic lupus erythematosus (NPSLE), which includes symptoms ranging from cognitive impairment to mood disorders. Symptoms can be debilitating

for patients, and without a pathophysiology, adequate treatments for these patients have remained elusive. Our laboratory has previously identified a subset of autoantibodies cross-reactive both dsDNA and the N-methyl-D-aspartate receptor (NMDAR), which are seen in approximately 30-40% of SLE patients. We have shown that these autoantibodies not only cause excitotoxic damage to neurons within the hippocampus, but that this damage also correlates to spatial memory deficits, another hallmark of disease seen in NPSLE patients. We now show that these autoantibodies not only cause acute neurotoxicity, but also lead to the development of a chronic inflammatory state leading to increased levels of complement protein C1q and overactivation of microglia. C1q targets microglia to synapses and dendrites, leading to excessive synaptic pruning and decreased dendritic complexity. Structural damage correlated with deficits in spatial memory on behavioral testing and enlarged placefields of hippocampal neurons on electrophysiology analysis. Finally, we show that ACE inhibitors prevent both synaptic pruning and the development of spatial memory deficits. ACE inhibitors are a possible treatment for NPSLE and should be considered for clinical trial in NPSLE.

F.94. Induction of Self-Tolerance in Chronic Experimental Autoimmune Encephalomyelitis Using Mixed Chimerism and Allogeneic Neural Stem Cell Transplantation

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Introduction: Previous work has shown a potential for induction of self-tolerance in experimental autoimmune encephalomyelitis (EAE) by allogeneic mixed hematopoietic chimerism. Here we show that allogeneic mixed chimerism alone is insufficient to induce long term self-tolerance. However, inducing tolerance of donor matched neural stem cells (NSCs) with allogeneic mixed chimerism leads to long term self-tolerance in chronic EAE.

Methods: We induced EAE with proteolipid protein 139-151 in 6-8 week old female SJL mice. Upon entering the first disease remission we induced mixed chimerism using CD8 T-cell depletion, 300 cGy total-body irradiation, anti-CD40L antibodies, and 30x10⁶ bone marrow cells (BMT) from C57BL/6 (allogeneic) or SJL (syngeneic) donors. Following BMT the two groups were given C57BL/6, SJL, or a sham NSC transplant.

Results: Only mice that received allogeneic BMT and allogeneic NSCs remained relapse free for the duration of the study. All other groups relapsed within 70 days post-BMT (n > 5/group).

Allogeneic bone marrow recipients had detectable chimerism even during relapses and remained tolerant to donor antigens, implying that alloantigen tolerance alone is insufficient to induce self-tolerance. Combined allogeneic BMT and allogeneic NSC recipients had a greater percentage of splenic Tregs and their splenocytes were unresponsive to peptide restimulation, in contrast with other groups.

Conclusions: These data indicate that inducing tolerance to allogeneic NSCs can also induce self-tolerance to EAE antigens. Alloantigen tolerance is necessary but not sufficient to induce self-tolerance, which is associated with increased Tregs in our model. These results may provide new insights into tolerance induction in autoimmunity.

F.119. TOLERance Induction with Autologous Tolerogenic Dendritic Cells Treated with VITamin D3 and Loaded with Myelin Peptides in Multiple Sclerosis (TOLERVIT-MS trial): The Design of a Multicenter, Dose-Escalation Phase I Clinical Trial

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Background: Tolerogenic dendritic cell (tolDC) therapy is a promising strategy for the attenuation of pathogenic T cells in autoimmune diseases such as multiple sclerosis (MS). Our group has developed an autologous antigen-specific cell therapy based on vitamin D3 (VitD3)-tolDC loaded with myelin peptides.

Objective: To describe the design of a multicenter, open-label, dose-escalation Phase I clinical trial to evaluate feasibility, safety, tolerability and preliminary efficacy of intranodal administration of VitD3-tolDC in active MS patients.

Methods: *In vitro* studies have demonstrated a potent immunoregulatory activity of vitD3-tolDC reducing lymphocyte proliferation and IFN-g production and increasing IL-10 levels, in co-culture experiments. Moreover, *in vivo* studies in the animal model of MS revealed a beneficial effect of VitD3-tolDC ameliorating the severity of the disease. Considering these pre-clinical results, a clinical trial was designed.

Results: Active MS patients will be included in a dose-escalation best-of-five design: Cohort 1 (5×10^6 VitD3-tolDC), Cohort 2 (10×10^6), Cohort 3 (15×10^6). A fourth Cohort of patients under IFN-beta treatment receiving the selected dose of VitD3-tolDC will be included. The trial protocol has been approved by the Spanish regulatory authorities (AEMPS) (<https://clinicaltrials.gov/ct2/show/NCT02903537>). Each cohort will received 6 administrations of tolDC (first 4 every 2 weeks and last 2 every 4 weeks). Clinical, MRI and immunological monitoring of 12 patients will be performed for 24 months. Each patient will be its own pre- and post-intervention control.

Conclusions: Positive outcomes of this phase I clinical trial may lead to a phase II trial to investigate the efficacy of this therapy in MS patients.

T.6. A Novel Self-Regulatory Mechanism of Th17 Cells Controls Autoimmune Uveitis Through Interleukin-24

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The Th17 response is critical in driving inflammation and is associated with many autoimmune diseases. However, clinical trials targeting the Th17 signature cytokine, IL-17A in some autoimmune diseases, including uveitis, have been disappointing. We investigated the role of IL-17A in an experimental autoimmune uveitis (EAU) model. Unexpectedly IL-17A deficiency in these mice did not reduce the severity of their uveitis. We found that IL-17A deficient polarized Th17 cells produced elevated amounts of other Th17-related cytokines, i.e. IL-17F, GM-CSF and IL-22. RNAseq analysis and the follow up in-vitro experiments revealed that IL-17A exerts a negative feedback on Th17 cells by inducing them to produce IL-24, which in turn suppresses the production of Th17-related cytokines in an autocrine fashion. *In vivo* results showed that IL-24 treatment of recipients ameliorated Th17-induced adoptive EAU, and conversely, silencing IL-24 expression in retina-specific Th17 cells increased their pathogenicity. Mechanistic studies confirmed that IL-17A activates the NFκB signalling pathway in Th17 cells, while site mutagenesis at the NFκB binding sites in IL-24 promoter abrogates the IL-17A-induced IL-24 expression. Importantly, we also observed a similar IL-17A/IL-24 interaction in human polarized Th17 cells that resulted in self-inhibition. In conclusion, IL-17A exerts a negative feedback on Th17 cells by inducing IL-24, which limits their expression of other Th17-lineage cytokines and dampens their pathogenicity.

T.16. Immunological Dysregulation in Neuromyelitis Optica and its Recovery Following Anti-IL-6 Receptor Monoclonal Antibody Tocilizumab Treatment

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Neuromyelitis optica (NMO) is a disabling autoimmune neurological disease, often resistant to therapy. Recent studies have indicated a pivotal role of IL-6 in the pathogenesis of NMO and the potential efficacy of the anti-IL-6 receptor antibody tocilizumab (TCZ) in NMO. However, the mechanism underlying the beneficial effects of TCZ against NMO remains unclear.

An intravenous injection of 8 mg/kg of TCZ was administered monthly as an add-on therapy to 19 anti-AQP4-IgG-positive patients with NMO/NMO spectrum disorders who were resistant to canonical interventions. Peripheral blood was collected prior to each infusion, and peripheral lymphocyte subsets, gene expression profiles of whole blood, and neutrophil mRNA were studied.

Despite intensive therapy, the enrolled participants had experienced relapses of NMO before TCZ induction. Alterations of multiple lymphocyte subsets were evident in their peripheral blood. After initiating TCZ therapy, the annual relapse rate reduced markedly and counts of lymphocyte subsets with regulatory function (transitional B cells, CD56^{high} natural killer cells, CD45RA-FoxP3^{high} regulatory T (Treg) cells) increased. Furthermore, we confirmed the functional recovery of CD45RA-CD25^{high} Treg cells. Although neutrophil granule-related genes, especially those related to azurophil granules, were significantly upregulated upon entry, we observed significant reduction in the expression of these genes after one year of TCZ treatment.

The efficacy of TCZ in clinical improvement of NMO was confirmed in the 19 enrolled participants, who were resistant to steroids and immunosuppressive drugs. The beneficial effects of TCZ may be achieved by recovering regulatory lymphocyte subsets and suppressing neutrophil functions by blocking IL-6R signaling.

T.31. Schwann Cell-Derived Periostin Promotes Autoimmune Peripheral Polyneuropathy Via Macrophage Recruitment

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Chronic inflammatory demyelinating polyneuropathy (CIDP) and Guillain-Barre syndrome (GBS) are inflammatory neuropathies that affect humans and are characterized by peripheral nerve myelin destruction and macrophage-containing immune infiltrates. In contrast to the traditional view that the peripheral nerve is simply the target of autoimmunity, we report here that peripheral nerve Schwann cells exacerbate the autoimmune process through extracellular matrix (ECM) protein induction. In a spontaneous autoimmune peripheral polyneuropathy (SAPP) mouse model of inflammatory neuropathy, the ECM protein periostin (Postn) was upregulated in affected sciatic nerves and was primarily expressed by Schwann cells. Postn deficiency delayed the onset and reduced the extent of neuropathy, as well as decreased the number of macrophages infiltrating the sciatic nerve. In an *in vitro* assay, Postn promoted macrophage chemotaxis in an integrin (Itg)- α M and Itg α V-dependent manner. The PNS-infiltrating macrophages in SAPP-affected nerves were pathogenic, since depletion of macrophages protected against the development of neuropathy. Our findings show that Schwann cells promote macrophage infiltration by upregulating Postn, and suggest that Postn is a novel target for the treatment of macrophage-associated inflammatory neuropathies.

T.57. A Molecular Characterization of Meningeal Inflammatory Infiltrates in the Progressive Multiple Sclerosis Brain

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The presence of lymphoid-like immune cell aggregates in the leptomeninges is suggested to promote damage to the cerebral cortex and play a role in accumulating disability in multiple sclerosis. To explore the molecular mechanisms that drive their formation, cryosections were cut from five cortical blocks per case from 55 SPMS and 14 control brains. Meningeal tissue was dissected and RNA extracted. Affymetrix HTA 2.0 GeneChips were used to obtain the meningeal transcriptome and gene expression determined using R package Limma. Differentially expressed genes with FC>2 and FDR>0.05 were used to perform gene set enrichment analysis using WebGestalt and gene networks constructed using R package WGCNA. When comparing controls with highly inflamed MS cases, alterations were mainly found in expression of homing chemokines and receptors and in cytokines that enhance B cell survival, proliferation and antibody and IFN γ production, such as IL10 and IL18. Modifications in genes involved in the development of lymphatic vessels (LYVE1) and cell motility, survival and antigen presentation (HLA-B) were prominent. Functional pathway analysis identified significant involvement of pathways associated with *Th17*, *Th1/Th2 cell differentiation*, *haematopoietic and lymphoid organ development*, *cell adhesion and leukocyte migration*. Gene network analysis revealed 5 network modules whose eigengenes were highly correlated with disease status and lymphocytic infiltration. Functional enrichment yielded a list of functions, including cell adhesion, protein folding and pro-inflammatory processes. We have identified molecular cues that mediate meningeal inflammation in MS that suggest a dysregulation of pathways that are critical for B-cell trafficking and recruitment into the CNS.

T.67. TIGIT Regulates CD4 Autoreactive T Cells in Multiple Sclerosis

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In multiple sclerosis (MS), activated autoreactive T cells recognizing myelin antigens traffic into the CNS mediating the disease. While a similar frequency of MS myelin-reactive T cells has been reported between patients and controls, myelin-reactive T cells secrete Th1/Th17 cytokines while myelin reactive T cells in healthy subjects secrete IL-10. By competing for the same ligand CD155, the co-stimulatory receptor CD226 and the co-inhibitory receptor TIGIT can modulate Th1/Th17 immunity and production of IL-10. Genetic variants in both the *CD226* and *CD155* locus have been associated with MS, and blocking this pathway impacts experimental autoimmune encephalomyelitis. We investigated whether alterations in the balance between the inhibitory function of TIGIT and the pro-inflammatory function of CD226 contribute to dysregulation of myelin-reactive T cells in MS. We generated T cell libraries from circulating CD4 T cells of healthy donors expressing TIGIT and/or CD226, and observed that combinatorial expression of these molecules identifies T cells with distinct and stable properties. TIGIT[−]CD226⁺ T cell libraries secrete predominantly IFN γ , IL-17 and GM-CSF and less IL-10, while TIGIT⁺CD226[−] cells secrete predominantly IL-10. Functional analysis of myelin-reactive T cell libraries comparing MS patients and healthy controls revealed that myelin-reactive T cells expressing TIGIT secreted Th1/Th17 cytokines in MS patients over controls. Conversely, production of pro-inflammatory cytokine by TIGIT[−] libraries was similar between the two groups. This observation suggests that TIGIT signalling fails to restrain these responses in MS. Thus, the TIGIT/CD226 pathway may contribute to the dysregulation of immune responses in patients with MS.

T.81. Cbl-B Deficiency in the Myeloid Cell Lineages but not T Cells is Responsible Heightened Th17 Response

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E3 ubiquitin ligase Cbl-b is involved in the maintenance of a balance between immunity and tolerance. Loss of Cbl-b facilitates the development of EAE, which is believed to be mediated in part by pathogenic T_H17 responses. However, we previously demonstrated that Cbl-b does not regulate T_H1 and T_H17 cell differentiation, but it does inhibit T_H2 cell differentiation by targeting Stat6 for ubiquitination. Therefore, how Cbl-b regulates T_H17 cell development and T_H17-mediated autoimmunity is currently unknown. In this study, we utilized adoptive transfer and cell type-specific *Cblb* knockout strains to define the role of Cbl-b in T_H17 cell development and autoimmunity. We found that mice lacking Cbl-b in the myeloid cells but not T cells develop severe EAE, with a heightened antigen-specific T_H17 response. *Cblb*^{-/-} but not wild-type macrophages facilitate T_H17 cell differentiation *in vitro* using a co-culture system. Further analysis showed that loss of Cbl-b results in the down-regulation of IL-10 in macrophages which inhibits pro-inflammatory cytokines such as IL-6. Therefore, we demonstrate that Cbl-b expression in the myeloid cells potentiates the production of IL-10 which restrains T_H17-mediated autoimmunity.

T.97. Fingolimod Modulates T Cell Phenotype and Regulatory T Cell Plasticity *in vivo*

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Fingolimod is an approved therapeutic option for patients with relapsing-remitting multiple sclerosis that primarily functions by sequestering T cells in lymph nodes inhibiting their egress to the central nervous system. However, recent data suggests that Fingolimod may also directly affect the immune cell function. Here we examined the *in vivo* effects of Fingolimod in modulating the phenotype and function of T cell and Foxp3 regulatory T cell populations in patients with multiple sclerosis under Fingolimod treatment. Besides decreasing the cell numbers in peripheral blood and sera levels of pro-inflammatory cytokines, Fingolimod inhibited the expression of Th1 and Th17 cytokines on CD4⁺ T cells and increased the expression of exhaustion markers. Furthermore, treatment increased the frequency of regulatory T cells in blood and inhibited the Th1-like phenotype that is characteristic of patients with multiple sclerosis, augmenting the expression of markers associated with increased suppressive function. Overall, our data suggest that Fingolimod performs other important immunomodulatory functions besides altering T cell migratory capacities, with consequences for other autoimmune pathologies characterized by excessive Th1/Th17 responses and Th1-like regulatory T cell effector phenotypes.

W.4. Subsets of Micro-Rnas May Serve as Novel Biomarkers and Therapeutic Targets in Multiple Sclerosis

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Multiple sclerosis (MS) is an autoimmune disease characterized by inflammation and demyelination of the neurons in the central nervous system (CNS), affecting approximately 400,000 Americans and 2.3 million people globally. A significant proportion of MS patients fails to respond adequately to the presently available drugs. Moreover, biomarkers for MS used in the clinics have inherent limitations. Thus, there is an unmet need for both new therapeutics as well as biomarkers to monitor disease activity and therapeutic response. Considering changes in the expression of a large panel of immune pathways-related genes during MOG-induced experimental autoimmune encephalomyelitis (EAE) model of MS in our study, we reasoned that micro-RNAs (miRNAs), which are ssRNA about 19-21 nucleotides long and highly conserved, play a vital role in disease development and might also serve as biomarkers. Using the MOG-EAE model, we determined the miRNA expression profile of immune cells (splenocytes), with or without treatment with a triterpenoid compound that we showed to inhibit EAE progression. Analysis by miRNA-microarray revealed that 142 miRNA elements (129 increased, 13 decreased) were markedly altered in EAE. By comparison, 46 miRNA elements (20 increased, and 26

decreased) were distinctly altered following triterpenoid therapy. Analysis by IPA and Targetscan software highlighted 10 miRNAs and their 3'-UTR (untranslated regions) gene targets that influence Th17/Treg differentiation, cytokine responses, neuroinflammation, neuronal growth, and neuroprotection involved in EAE/MS pathogenesis. We believe that a subset of these 10 miRNAs might serve as biomarkers of disease activity and therapeutic response, while others may be targeted for MS therapy.

W.5. The Expression Signature of Very Long Non-Coding RNA In Myalgic Encephalomyelitis / Chronic Fatigue Syndrome

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Myalgic encephalomyelitis/ Chronic fatigue syndrome (ME/ CFS) is a chronic debilitating disease with huge social-economic impact. It has been suggested that nitrosative stress, oxidative stress, and immune activation might contribute to disease pathogenesis. However, the etiology of CFS remains largely unclear, and the diagnostic/ prognostic disease markers are lacking. Several long noncoding RNAs (lncRNA, >200bp) have been reported to play roles in immunological diseases or in stress responses. In our study, we recruited 44 CFS patients (diagnosed according to the Canadian Consensus Criteria), and examined the expression signature of 10 very long lncRNAs (>5kb, CR933609, His-RNA, AK124742, GNAS1-AS, EmX2OS, MIAT, TUG1, NEAT1, MALAT1, NTT) in their peripheral blood mononuclear cells (PBMCs). LncRNAs NTT, MIAT and EmX2OS levels were found to be significantly elevated in CFS patients as compared with healthy controls. Furthermore, NTT and EmX2OS levels also positively correlated with disease severity. Lower expressions of lncRNAs CR933609, GNAS1-AS, TUG1, and higher expression of NEAT1 were only detected in mild female CFS patients (with Bell score >30, disease duration 2O₂ (oxidative stress) and poly (I:C) (double strand RNA, representing viral replication byproduct) increased the expression levels of NTT and MIAT. Our study revealed CFS disease severity-associated very long lncRNA expression signatures, which might implicate the regulatory responses in CFS patients to oxidative stress and to chronic viral infection. Further investigations need to be done to uncover the functions and roles of these lncRNAs in CFS.

W.9. Detection and Characterization of Novel Myelin-reactive CD8⁺ T Cell Populations in Multiple Sclerosis

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CD8⁺ T cells are thought to play an important role in the pathogenesis of multiple sclerosis (MS), yet their function remains poorly defined. Understanding the antigenic targets of CD8⁺ T cells is vital to determining which CD8⁺ T cells are relevant to MS and what role they may play. Myelin antigens are putative auto-antigens implicated in MS. Although myelin-reactive CD8⁺ T cells have been previously identified, their detection and characterization has been hampered by their low frequency. We constructed a panel of candidate myelin peptide MHC I tetramers and were able to detect low frequencies of myelin tetramer-positive CD8⁺ T cells from the peripheral blood of a small cohort of MS patients. In order to validate the antigen-specificity of such low frequency populations, tetramer-positive CD8⁺ T cells were enriched by cell sorting followed by expansion with mitogen. Myelin-specificity was then confirmed by demonstrating functional reactivity to cognate myelin antigen. Using this approach, we have been able to identify several previously unidentified myelin CD8⁺ T cell epitopes in humans. We have begun studies to compare the precursor frequencies and phenotypes of

these newly described myelin CD8⁺ T cell populations in MS patients compared to healthy controls in order to determine their potential relevance to MS.

W.46. Exosomal Let-7i Inhibits the Differentiation of Regulatory T Cells in Multiple Sclerosis

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Exosomes deliver functional miRNAs from cells to cells, which is a form of intercellular communications. MiRNAs are involved in differentiation and function of helper T cells, which play a fundamental role in the pathogenesis of multiple sclerosis (MS). In this study, we found that the frequency of regulatory T (Treg) cells (IFN γ -IL17A-Foxp3⁺CD4⁺ T cells) decreased among T cells after culture in the presence of exosomes from patients with MS. The profile of exosomal miRNAs was different between patients with MS and healthy controls (HC). Among the differentially expressed miRNAs, *let-7i*, which was increased in patients with MS, suppressed the induction of Treg cells. *Let-7i* was also increased in circulating exosomes from mice with experimental autoimmune encephalomyelitis (a model of MS). Further experiments suggested that *let-7i* exerted the effect by decreasing expression of insulin-like growth factor 1 receptor (IGF1R) and transforming growth factor-beta receptor 1 (TGFBR1) on naïve CD4⁺ T cells. The expression of these receptors was lower on naïve CD4⁺ T cells in the peripheral blood from patients with MS than HC. The expression was positively correlated with the frequency of Treg cells in the circulation. Moreover, persons with a higher amount of *let-7i* in the circulating exosomes had lower frequency of Treg cells. Collectively, this study indicates that circulating exosomes are involved in the pathogenesis of MS by suppressing the induction of Treg cells via *let-7i* - *TGFBR1/IGF1R* axis.

W.54. Mass Cytometry Assisted Phenotyping Identified Structural Rewiring of Immune System in Refractory Epilepsy

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Refractory epilepsy (RE) is a chronic neurological disease with unknown aetiology that encompasses epilepsy patients non responsive to anti-epileptic drugs. Inflammatory processes have been demonstrated to play a role in seizures in autoimmune encephalitis (AE) and RE patients but its is unknown if and how immune mechanisms contribute to RE. In the present study, we used high dimensional mass cytometry to study the immune in refractory epilepsy and compare it with AE and normals.

A system analysis of the immune cell networks showed surprising analogies between AE and RE and profound differences with the healthy control, with higher modularity and centralisation and a loss of negative regulatory mechanism. Statistical analysis showed expansion in pro inflammatory CD4⁺ subsets. Contraction in regulatory CD8⁺ subsets in RE and AE compared to healthy subject was also observed. We found NK cell subsets that is specifically associated with RE but not with AE.

In conclusion, our comprehensive deep immuno-phenotyping uncovered systemic rewiring of immune cell network with

loss of regulatory checks leading to highly modular networks causing pathogenic pro-inflammatory mechanisms to prevail in RE and AE. These findings may have direct translational implications related to the pathogenesis of these diseases and to novel ways to treat them

W.69. Abatacept Selectively Modulates CD4⁺ Treg, Tfh, and CD38-expressing T Cells in the Periphery of Patients with Relapsing-Remitting Multiple Sclerosis

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The ITN ACCLAIM trial was a phase II, randomized, double-blind, placebo-controlled, multicenter study of abatacept (CTLA-4Ig) in adult patients with relapsing-remitting multiple sclerosis (RRMS). To determine abatacept's mechanism of action on specific T cell populations in RRMS, viable, cryopreserved PBMC collected before, during, and after treatment were analyzed using flow cytometry, epigenetic and RNAseq technologies. Compared to placebo-treated participants, abatacept selectively reduced percentages of circulating CD45RO⁺ CD4 Treg (p<0.0001), ICOS⁺-PD-1⁺ Tfh (p<0.0001), and CD38-expressing T cells (p<0.05). In addition, abatacept decreased surface expression of ICOS on circulating PD-1⁺ Tfh (p<0.0001) and in parallel, reduced percentages of CD38⁺CD24^{lo}IgD-CD27⁺ plasmablasts (p<0.005) in blood. Epigenetic analysis (Epiontis) of the FOXP3 Treg-specific demethylation region confirmed that abatacept significantly reduced circulating CD4 Treg. RNAseq analysis of bulk sorted CD4 Treg and PD-1⁺ Tfh cells confirmed changes in activation status, and identified common and unique molecular pathways modulated by abatacept in these populations. After discontinuation of abatacept, all T cell parameters evaluated returned to pre-therapy levels. Importantly, abatacept-induced changes in T cells were validated in placebo-treated participants who later received abatacept as part of the crossover trial design. These data expand upon the mechanism of action of abatacept reported in other autoimmune diseases, and show that abatacept reduces frequencies and activation of PD-1⁺ Tfh and CD4 Treg in RRMS. The reductions observed in both effector T cell and regulatory T cell subsets emphasize the complexities of costimulatory blockade therapy, and highlight the need for further studies regarding how best to exploit this pathway to achieve tolerance.

W.73. iNKT Cell Ligand OCH Induces Anti-Inflammatory Immune Responses in Patients with Multiple Sclerosis: Results of Investigator-Initiated, First-In-Human Phase 1 Study

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Multiple sclerosis (MS) is a putative autoimmune disease of the central nervous system (CNS). Previous works have demonstrated that regulatory cell populations, including iNKT cells, MAIT cells and regulatory T cells (Treg), are altered in MS. We previously reported that oral administration of a sphingosine-truncated analog of α -galactosylceramide, OCH, would selectively induce IL-4 production from iNKT cells, thereby preventing the development of experimental autoimmune encephalomyelitis (EAE) (Nature 2001). To develop OCH as a therapeutic agent for MS, we have completed a first-in-human phase 1 study of OCH in 15 healthy subjects (HS) and 9 patients with MS. To evaluate the

effect of OCH *in vivo*, flow cytometer and DNA microarray analyses were conducted for peripheral blood samples obtained at various time points after oral OCH treatment. High concentrations of OCH than anticipated were detected in the recipients' blood, indicating absorption of OCH from gut is better in human than rodents. We have observed several changes in the blood of the HS and MS, which included (i) a significant increase of T reg [CD45RA-Foxp3⁺ effector/activated regulatory T cells] at 6 h, (ii) upregulation of immunoregulatory genes (MAFB and IL411) at 6 and 24 h, (iii) downregulation of immune-activating genes (NR4A2, FOS, and FOSB) at 6 and 24 h, and (iv) downregulation of GZMB and killer cell immunoglobulin-like receptors. Three of the patients received weekly oral administration of OCH for three months without any adverse events. The results are promising and would validate a need for phase 2 study.

W.82. Ocrelizumab does not Modulate Peripheral T Cell Functionality or Prevalence in a Small Subset of Relapsing MS Patients Enrolled in OPERA I, A Phase III Double-Blind Doubledummy Interferon Beta-1a-Controlled Study

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Ocrelizumab is a humanized, anti-CD20 specific, cytolytic antibody that depletes B cells, and is indicated for the treatment of relapsing and primary progressive forms of multiple sclerosis. B cells modulate T cell activity through antigen presentation, cytokine production, and support secondary lymphoid organ structure. The goal of these studies was to evaluate the effect of ocrelizumab treatment on peripheral blood T cell prevalence and/or function in a subset of relapsing MS patients enrolled in the OPERA I study at UCSF. A novel mass cytometry (CyTOF) assay was developed to immunophenotype a diverse array of B and T cell subsets, and used in a blinded fashion to compare PBMC from patients before and after ocrelizumab or interferon beta-1a treatment. To test the ability of T cells to elicit cytokine responses, cells were stimulated and assayed for intracellular cytokine secretion. Despite dramatic reduction of all B cell subsets in blood after ocrelizumab treatment, no significant modulation in the frequency of T cell subsets was observed. Permutation testing identified CD19⁺ B cell subsets as the only cells significantly differentiated. Furthermore, an unsupervised t-distributed stochastic neighbor embedding (t-SNE)-based visualization revealed B cell depletion and modulation; while overt changes in T cell populations were not apparent. Our findings suggest that the peripheral blood T cell compartment remains largely unaltered in RMS patients treated with ocrelizumab. The treatment also did not appear to affect the ability of T cells to elicit a functional cytokine response to stimulation. These results may be relevant for safety purposes.

W.110. Immunoglobulin A is a Critical Regulator of Demyelinating Neuroinflammation

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Recent data from our lab and other groups suggests that gut dysbiosis contributes to pathogenesis in autoimmune central nervous system (CNS) demyelination. Immunoglobulin A (IgA) is a key regulator at the gut mucosal interface, contributing to the maintenance and expansion of regulatory T cells and modulation of systemic inflammation. Here, we investigated the role of IgA and IgA-producing cells in gut dysbiosis and along the gut-brain axis in multiple sclerosis (MS) and experimental models of autoimmune demyelination.

We analysed IgA-bound bacteria in the gut as well as serum and CSF IgA-binding of gut bacteria by combining flow cytometry with sorting and 16S rRNA gene sequencing (IgA-SEQ) in MS patients and healthy controls. Remarkably, IgA-SEQ of gut bacteria revealed differential targeting of bacteria with a pro-inflammatory phenotype in MS participants compared with controls. Quantification of serum- and CSF-binding to autologous gut microbiota showed high prevalence of gut bacteria-specific IgA along the gut-brain axis in MS patients. Ongoing studies investigating the role of IgA and IgA-producing cells in MS and experimental demyelination suggest a recruitment of IgA-producing cells to the inflamed CNS.

These data suggest that IgA and IgA-producing cells play a critical role in regulation of inflammation in autoimmune CNS demyelination highlighting a potential interaction between gut microbiota and humoral / B cell-mediated immunity.

Autoimmune rheumatologic diseases

F.5. Multi-Cohort Transcriptome Analysis Implicates Novel Biological Mechanisms in 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. Therapeutic options for SLE treatment are limited, with only one new FDA-approved lupus therapy in the last 50 years. To identify robust molecular changes associated with SLE, we performed an integrated, multi-cohort analysis of 7,471 whole transcriptome profiles from 40 independent studies. We identified a 93-gene signature that is significantly altered in the blood of individuals with SLE compared to 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. The SLE signature correlated significantly with disease activity and clinical measures of inflammation. We prospectively validated the SLE signature in an independent cohort of pediatric SLE patients using a custom, microfluidic RT-qPCR array, demonstrating the robustness of the signature. Based on meta-analyses of 16 studies of interferon stimulation and sorted immune cells, we found that many genes were independent both of interferon and neutrophils. Pathway analysis uncovered unexpected links to nucleic acid biosynthesis and immunometabolism. We have further refined a neutropoiesis signature and identified novel transcripts related to NK and NKT cells, alopecia, and heavy metal clearance by metallothioneins. In conclusion, the SLE signature has potential to aid clinicians in the diagnosis and monitoring SLE, and implicates novel genes in SLE pathogenesis.

F.7. Persistence of Transcriptomic Signature in CD4 Memory T Cells in Polyarticular JIA Patients despite Anti-TNFA Biologics Therapy

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The majority of JIA patients demonstrate clinical remission with anti-TNFA therapy. 50-80% of patients will relapse upon therapy discontinuation. Drug toxicities and costs have driven the need to find predictors for successful drug withdrawal. We have identified by CyToF a cluster of inflammatory CD4 memory subset (CD45RA-TNFA⁺IL-6⁺PD1-CTLA4⁻, $p < 0.05$) in patients who are in clinical remission but are destined to flare upon therapy withdrawal. The presence of these

cells is predictive of flare. These cells expands aggressively upon therapy withdrawal. The data inspire two questions ; (a) what is the transcriptomic profile of these therapy-resistant T cells before and after withdrawal of clinically successful therapy. Addressing this question may provide new therapeutic targets, (b) if this cell subset and its transcriptomic profile is present in yet untreated patients with active disease. Addressing this question would strengthen the pathogenic and biomarker relevance of this T cell subset. We subjected to Nanostring RNA analysis sorted T cells from 16 JIA patients (4 active paired naïve/post treatment, and 6 who relapsed or 6 remain inactive after anti-TNF withdrawal) and 3 age matched normals. We found significantly enriched pathways ($p < 0.05$) that were strikingly present in both patients groups, (a) TCR activation, (b) TNFA signalling, (c) Apoptosis, (d) NF- κ B signalling, (e) MAPK signalling. The results suggest that the presence of pro-inflammatory CD4 memory subsets could be pivotal in defining clinical fates of patients in therapeutic management, thus providing a dual translational value.

F.16. Development of Citrullinated-Vimentin-Specific CAR for Targeting Tregs to Treat Autoimmune Rheumatoid Arthritis

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Rheumatoid arthritis (RA) is one of the most common chronic autoimmune diseases, characterized by an aberrant inflammation of the synovial membrane that causes irreversible joint and bone damage. Regulatory T cells (Tregs) are defective in RA patients while adoptive transfer of expanded autologous Tregs efficiently reversed disease in collagen-induced arthritis, an animal model of RA. These studies strongly suggest that Treg therapy may be effective in the treatment of RA patients by reducing joint inflammation and inducing immune tolerance. However, clinical treatment with polyclonal Tregs may lead to general immunosuppression. Thus, the development of precise RA Ag-specific Tregs would increase specific activity, promote selective migration to the site of the abnormal inflammation and enhance their function. Thus, we engineered chimeric antigen receptor (CAR)-expressing Tregs specifically targeting an antigen present in the joint of RA patients to induce a localized and effective immunosuppressive response. We developed a single chain Fv directed against a posttranslational modified intermediate filament protein, citrullinated vimentin (CV), an antigen found, almost exclusively, in the extracellular matrix of the synovial tissue of the RA patients and implicated in the pathogenesis of the disease. The scFv was grafted into a functional CAR construct, transduced into human Tregs and shown to react with CV expressed in RA patient synovial fluid and CV-expressing cells. Studies are underway to demonstrate the functional activity of these CAR-expressing Tregs in *in vivo* mouse models a validation step in the development of a promising therapeutic tool to treat RA and potentially other autoimmune disorders.

F.28. Selective Inhibition of NF- κ B Inducing Kinase (NIK) is Therapeutically Efficacious in IFN γ -Accelerated Lupus Nephritis Prone Mice

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Systemic lupus erythematosus (SLE) is often considered a disease driven by autoreactive B cells and anti-nuclear antibodies. However, therapeutic targeting of B cells, for example through BAFF blockade, has been only partially effective in SLE. NF- κ B Inducing Kinase (NIK) mediates non-canonical NF- κ B signaling downstream of disease relevant TNF family members, such as BAFF, TWEAK, CD40, and OX40. We hypothesized that NIK inhibition might be more efficacious than BAFF blockade in lupus, and therefore generated a highly selective and potent small molecule inhibitor

(SMI) of NIK to test this hypothesis. In cellular assays, this molecule inhibits non-canonical NF- κ B signaling downstream of multiple TNFRSF family members. In order to differentiate the effects of NIK inhibition and BAFF blockade *in vivo*, we compared NIK inhibition with BAFF blockade in the context of NZB/W F1 lupus prone mice. As predicted, NIK inhibition recapitulated the pharmacological effects of BAFF blockade. Furthermore, NIK inhibition, but not BAFF blockade, affected T cell parameters in the spleen and pro-inflammatory gene expression in the kidney, the latter of which is partly attributable to TWEAK signaling. Finally, NIK inhibition resulted in improved survival, ameliorated renal pathology, and lower proteinuria scores. Collectively, our data suggest that NIK inhibition affects multiple disease-relevant pathways and may therefore have superior efficacy compared to BAFF inhibition in SLE.

F.38. Computational Analysis of Clinical Data in a Well-Phenotyped Lupus Cohort Points to Disease Subtypes

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Systemic Lupus Erythematosus (SLE) is a chronic autoimmune disease characterized by a wide range of clinical manifestations which differ between racial groups. In order to better understand this clinical heterogeneity, we identified race associated clinical phenotypes in a multiethnic cohort of 265 patients from the California Lupus Epidemiology Study (CLUES). As compared to Caucasian patients, African-American, Asian and Hispanic patients had significantly higher incidence of anti-dsDNA autoantibody, lupus nephritis, and leukopenia and lower incidence of oral ulcers and photosensitivity (FDR<0.1). African-Americans had higher incidence of discoid rash as compared to Caucasians, Asians, and Hispanics (FDR<0.1).

We also found significant (FDR<0.1) pairwise associations between several clinical and demographic variables; for instance we identified positive relationships between anti-dsDNA and lupus nephritis (polychoric correlation $r = 0.4$), female sex and lupus nephritis ($r = 0.4$), discoid rash and anti-Smith ($r = 0.3$), leukopenia and anti-Smith ($r = 0.3$) and negative associations between age with lupus nephritis ($r = -0.3$), anti-dsDNA ($r = -0.5$), and anti-Smith ($r = -0.3$), which suggests potential clusters of clinical disease subtypes. Electronic Medical Record (EMR) data provides a unique opportunity to study disease heterogeneity across a large patient population. The UCSF EMR consists of 920,000 patients from 2012-present including approximately 800 SLE patients identified by having two ICD-10 codes, 30 days apart, with both structured and unstructured data available including diagnosis codes, lab values, medication history, and clinical notes. We are currently leveraging the UCSF EMR to validate our findings. Identification of clusters based on clinical presentation may aid in characterizing distinct SLE subtypes with different underlying disease mechanisms.

F.43. Gene Expression Analysis Reveals Different Ancestry Related Cellular Contributions to Systemic Lupus Erythematosus (SLE)

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SLE is a complex autoimmune disease more prevalent in African-Americans (AA). To determine whether the molecular pathways were different in AA and European American (EA) SLE patients, analysis of gene expression was undertaken. Three independent SLE datasets, comprising 232 AA and 1133 EA patients, were downloaded from GEO. Male subjects were excluded to minimize variability and comparable age, disease activity and anti-dsDNA titers were confirmed. AA and EA patients were found to differentially express (DE) specific sets of genes. As a control, random subsets of EA were compared to each other and no DE transcripts were found. Iscope, a tool to identify leukocyte subsets in microarray datasets, demonstrated increased plasmablast/plasma cells (PC) and lymphocyte populations in AA SLE patients and increased myeloid cell transcripts in EA SLE patients. Gene Set Variation Analysis (GSVA) showed increased PC, B, and T cell transcripts in the majority of AA patients and increased myeloid cell transcripts in most EA patients. CXCL8 was uniformly increased in EA patients, corroborating the increased myeloid cell transcripts in

most EA patients. IPA upstream regulator analysis showed an increased magnitude of the IFN signature in AA compared to EA patients and this finding was confirmed by GSVA. IPA canonical pathway and upstream regulator analysis confirmed the different cell populations predominating in AA versus EA SLE patients. Differences in the preponderant cell types and upstream regulators in AA versus EA patients suggest that AA and EA patients may respond distinctively to various SLE therapies.

F.44. Integration of BCR, TLR, BAFF Receptor and TACI Signals Promotes Autoantibody Production by Transitional B Cells in BAFF Transgenic Mice

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Although originally characterized as a negative regulator of B cell activation, TACI signals were recently shown to be critical for BAFF-driven autoantibody formation. Mechanistically, we showed that splenic B cells with a CD21^{low} transitional surface phenotype upregulate TACI expression and directly contribute to BAFF-driven autoantibodies. To confirm that immature, transitional B cells are the predominant source for class-switched autoAb in BAFF-Tg mice, we developed a CD21-Cre-ROSA-YFP reporter strategy. By irreversibly labeling B cells that have expressed CD21, we confirmed that transitional B cells that had not yet differentiated beyond the T2 stage spontaneously produce class-switched autoantibodies in BAFF-Tg mice. We then sought to determine the signals required for TACI upregulation on T1 transitional B cells. Surprisingly, despite established roles for dual BCR and TLR signals in autoantibody production in SLE, signals downstream of these receptors exerted distinct impacts on transitional B cells. Whereas loss of BCR signals prevented transitional B cell TACI expression and resulted in loss of serum autoantibodies across immunoglobulin isotypes, lack of TLR signals exerted a limited impact on autoantibody class switch recombination without impacting transitional B cell TACI expression. Finally, in parallel with the protective effect of TACI deletion, loss of BAFF-R activation signals protected against BAFF-driven autoimmunity. In summary, we highlight how distinct signaling pathways integrate to promote class-switched autoantibody production by transitional B cells, findings with implications to the understanding of SLE pathogenesis and other humoral autoimmune diseases characterized by elevated serum BAFF, most notably immune reconstitution following hematopoietic stem cell transplant.

F.52. Extracellular Vesicles in Systemic Juvenile Idiopathic Arthritis

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Systemic Juvenile Idiopathic Arthritis (sJIA) is a chronic, childhood inflammatory disease of unknown etiology. Extracellular vesicles (EVs) are submicron particles secreted by most cells under physiologic conditions and increased during inflammation. EVs participate in cellular communication and may play an important role in inflammatory diseases. We are investigating EVs in the plasma of sJIA patients during disease activity and quiescence, in comparison to plasma of pediatric controls. We use an Influx cell sorter (BD Biosciences), which allows a limit of detection close to 100 nm; to further separate EVs from instrument noise, labeling of EVs with BODIPY FL dye is used. To determine the concentration of EVs in plasma, we use TruCount tubes (BD Biosciences). We have analyzed plasma from 10 active sJIA patients, 7 quiescent and 10 controls. Plasma from sJIA active patients have higher number of CD41⁺ EVs and CD146⁺ EVs compared to controls; these markers identify EVs derived from platelets and endothelial cells respectively. Plasma from sJIA quiescent patients did not differ from controls and tended to have lower concentration of these two EVs subpopulations than samples obtained at flare. The number of CD235⁺ EVs, likely from erythrocytes, was similar between sJIA and controls. These results implicate a change in the cellular source(s) of EVs as a component in the active phase of sJIA. Further studies are needed to elucidate the specificity of these EV for sJIA and their biological role(s) in disease and to determine if they can serve as biomarkers of disease activity.

F.58. Characterization of a Novel Epigenetic Regulator Required for T Helper 17 Differentiation

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Epigenetics is characterized by factors that alter gene expression without changing the underlying genetic code. Two well-characterized epigenetic modifications are histone methylation and DNA methylation. Histone methylation occurs in a variety of flavors with some marks inducing gene activation while others induce gene repression. The main histone marks of gene repression are H3K9me3 and H3K27me3. Recently, H3K27me3 has been found to be important for T cell differentiation. For this reason, we *hypothesized* that H3K9me3 would similarly be critical for T cell differentiation. To explore this possibility, we created a conditional knockout mouse for the activating transcription factor 7 interacting protein (ATF7ip), a protein that is a well-characterized cofactor in a protein complex that is essential to create the H3K9me3 mark. Interestingly, T cell specific deletion of ATF7ip results in a specific defect in T helper 17 (Th17) differentiation without affecting other T cell lineages. In vivo studies of Th17 function showed that ATF7ip is required for colitis. Furthermore, RNA-seq analysis of CD4-Cre ATF7ip^{fl/fl} T cells revealed that these cells produce increased IL-2 and mechanistic studies with H3K9me3 ChIP-seq showed that ATF7ip is required for H3K9me3 deposition at the *Il2* locus. IL-2 is a known inhibitor of Th17 differentiation, thus IL-2 overproduction is a likely mechanism for the defect in Th17 differentiation. Low dose IL-2 therapy is in clinical trials for a variety of autoimmune diseases so therapeutic targeting of ATF7ip in the future may be of clinical utility.

F.65. The Transcriptional Co-Activator BOB.1 Inhibits Plasma Cell Differentiation and Induces Costimulatory Capacity of B Cells in GC-Like Environment

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Recently we identified the transcriptional co-activator BOB.1 as specifically overexpressed in rheumatoid arthritis (RA) synovium, where its levels strongly correlated with the presence of germinal centers (GCs). In accordance with human data, mice lacking BOB.1 failed to mount GC response and were resistant to the experimental model of RA. Immunofluorescence staining of inflamed RA synovium demonstrated the presence of aggregates of BOB.1-positive cells in a pattern similar to the normal tonsillar tissue. Further analysis revealed that expression of BOB.1 in RA synovial B cells was significantly higher than in tonsillar B cells. Furthermore, expression of BOB.1 was elevated in lymph nodes of RA patients compared with healthy controls. To investigate how high levels of BOB.1 impact phenotype and function of B cells in a GC-like environment we overexpressed BOB.1 in human primary B cells cultured with CD40L and IL-21. In these conditions B cells rapidly differentiate into plasma cells, however, the percentage of plasmablasts was significantly reduced in BOB.1-overexpressing cells. Instead, these cells expressed higher levels of costimulatory receptors CD40, CD80 and PD-L2 involved in B-T cell interactions and demonstrated increased BCR-induced calcium flux. Accordingly, T_{FH} cells co-cultured with SEB-pulsed BOB.1-transduced B cells exhibited a higher rate of proliferation and increased production of CXCL13. These data suggest that increased levels of BOB.1 in B cells in GCs suppress their terminal differentiation and enhance expression of costimulatory molecules and BCR signaling strength. Whether this is sufficient to drive/accelerate autoimmune disease will be evaluated in a B cell-specific BOB.1-tg mouse model.

F.74. Dermal Lymphatic Function and Photosensitivity in a Lupus Model

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Lupus erythematosus is characterized in part by photosensitivity, whereby ultraviolet radiation (UVR) from sunlight can induce inflammatory skin lesions. The pathophysiology of photosensitivity in lupus is poorly understood. Lymphatic vessels normally function to limit the magnitude and duration of tissue inflammation by transporting fluid and inflammatory mediators and cells out of tissue. Chronic inflammatory states such as obesity are associated with lymphatic dysfunction that can exacerbate tissue inflammation. The functional state of lymphatic vessels in lupus is not known, and we hypothesize that there is lymphatic dysfunction in a lupus model that could contribute to photosensitivity, and whether improving lymphatic flow can reduce photosensitivity. Here, we characterize UVR-induced changes in lymphatic flow using Evan Blue lymphangiography and have begun to characterize lymphatic flow in MRL/lpr mice and controls. UVR exposure results in an immediate increase in ear thickness that peaks at day 7 and partially resolves by day 28. At 72 hours post UV, there is a substantial decrease in skin lymphatic flow in both MRL/lpr and controls, with a suggestion of greater decrease in MRL/lpr mice. Our results suggest that there may be impaired local lymphatics in the MRL/lpr lupus strain, raising the possibility that improving lymphatic function may ameliorate photosensitivity.

F.78. A Protective Langerhans Cell-Keratinocyte Axis is Dysfunctional in Lupus Photosensitivity

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Photosensitivity, or skin sensitivity to ultraviolet radiation (UVR), is a feature of lupus erythematosus (LE) and other autoimmune and dermatologic conditions, but the mechanistic underpinnings are poorly understood. Here, we identify a Langerhans cell (LC)-keratinocyte axis that limits UVR-induced keratinocyte apoptosis and skin injury via keratinocyte epidermal growth factor receptor (EGFR) stimulation. We show that absence of LCs in Langerin-DTA mice leads to photosensitivity and that, *in vitro*, mouse and human LCs can directly protect keratinocytes from UVR-induced apoptosis. LCs express EGFR ligands and ADAM17, the metalloprotease that activates EGFR ligands. Deletion of ADAM17 from LCs leads to photosensitivity and LC ADAM17 is activated by UVR, suggesting that LCs protect by providing activated EGFR ligands to keratinocytes. Photosensitive systemic LE (SLE) models show reduced epidermal EGFR phosphorylation and LC defects, and topical EGFR ligand reduces photosensitivity. Human SLE skin shows reduced epidermal EGFR phosphorylation and reduced LC numbers. Together, our data establish a tissue-protective function for LCs, reveal a mechanistic basis for photosensitivity, and suggest EGFR stimulation as a treatment for photosensitivity.

F.89. Immune Cells Avoid Death from Immunoproteasome Inhibitors by Switching to Conventional Proteasome

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The pan-proteasome inhibitor bortezomib has demonstrated sustained clinical efficacy in off label trials in SLE patients by depleting pathogenic immune cells including highly secretory plasmablasts and plasmacytoid dendritic cells. But bortezomib also effects non-immune cells including neurons which raises significant safety concerns thus limiting the feasibility of pan-proteasome inhibitors as a treatment for autoimmune disease. As an alternative, targeting the immune cell specific immunoproteasome has been proposed as a promising therapy to deplete pathogenic immune cells while avoiding toxicity issues. Using highly specific immunoproteasome inhibitors, we show that this strategy does not lead to immune cell death as anticipated. We generated a large panel of small molecule inhibitors with varying specificity to the $\beta 5$ subunit of the conventional and immuno- proteasome, and found that effects on viability of plasmablasts and pDCs correlate with the inhibition of the conventional proteasome, not the immunoproteasome. Our data indicates that the immune cells upregulate the conventional proteasomal subunits and prevent the accumulation of ubiquitinated protein. Widely used immunoproteasome inhibitors also block the conventional proteasome which, our data would indicate, is key to their ability to deplete immune cells.

F.100. BAFF Promotes Progressive Lupus Nephritis Via Activation of the B Cell Surface Receptor TACI.

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B cells are known to promote the pathogenesis of systemic lupus erythematosus (SLE) via the production of pathogenic anti-nuclear antibodies. However, the signals required for autoreactive B cell activation and the immune mechanisms whereby B cells impact the lupus nephritis pathology remain poorly understood. The B cell survival cytokine B cell activating factor of the TNF Family (BAFF) has been implicated in the pathogenesis of SLE and lupus nephritis in both animal models and human clinical studies. Although BAFF receptor (BAFF-R) has been predicted to be the primary BAFF family receptor responsible for BAFF-driven humoral autoimmunity, in the current study we identify a critical role for signals downstream of Transmembrane Activator and CAML Interactor (TACI) in BAFF-dependent lupus nephritis. Whereas transgenic (Tg) mice over-expressing BAFF develop progressive membranoproliferative glomerulonephritis, albuminuria and renal dysfunction, TACI deletion in BAFF-Tg mice provided long-term (~1 year) protection from renal disease. Surprisingly, disease protection in the context was not explained by complete loss of glomerular immune complex deposits. Rather, TACI deletion specifically reduced endocapillary, but not mesangial, immune deposits. Notably, although excess BAFF promoted widespread breaks in B cell tolerance, BAFF-Tg antibodies were enriched for RNA- relative to DNA-associated autoantigen reactivity; and these RNA-associated autoantibody specificities were specifically reduced by TACI or Toll-like receptor 7 (TLR7) deletion. Thus, our study provides important insights into the autoantibody specificities driving proliferative lupus nephritis, and suggests that TACI inhibition may be novel and effective treatment strategy in lupus nephritis.

T.35. MicroRNAs as Novel Biomarkers of Disease Development and Therapeutic Response in Autoimmune Arthritis

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Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by swelling and destruction of the synovial joints. Approximately 1 percent of adults worldwide are afflicted with this disease. Current drugs for RA are effective at mitigating the disease, but up to 40 percent of RA patients fail to respond well to them. Moreover, current RA biomarkers have inherent limitations. Therefore, there is a need for additional biomarkers that can monitor both disease activity and therapeutic response. We proposed that micro-RNAs (miRNAs) might fulfill this role. These are ssRNA about 19-21 nucleotides long, which are highly conserved in nature. Using the rat adjuvant-induced arthritis (AA) model of RA, we determined the miRNA profile of arthritic rats as well as arthritic rats treated with celastrol, a natural triterpenoid. We performed miRNA-microarray analysis of RNA from immune (lymphoid) cells of these rats. A total of 903 miRNA elements were significantly altered in the arthritic rats. Of these, 748 were upregulated, while 155 were downregulated.

In comparison, in celastrol-treated rats, a total of 1,336 miRNA elements were significantly altered, with 105 upregulated, and 1,231 downregulated. IPA and Targetscan software analysis identified about 20 miRNAs and their target genes involved in various pathways important for arthritis. These include T cell differentiation, bone remodeling, IL-17 signaling, and others. We believe that a subset of these miRNAs play a crucial role in the development and progression of arthritis, and that some of these miRNAs might serve as biomarkers of disease activity and therapeutic response.

T.37. High Dimensional Analysis of Immunome in Systemic Sclerosis Reveals Abnormalities in Frequency and Function of MAIT Cells

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Systemic sclerosis (SSc) is an autoimmune connective tissue disease characterised by excessive fibrosis of skin and internal organs, and vasculopathy. Association of T and B cells have been reported in SSc, however there is a lack of systematic study of immune cells in this disease. Here we report high dimensional mass cytometry and RNA-seq based analysis of immune cells from peripheral blood of SSc patients. Mononuclear cells from blood of 20 SSc patients and 10 healthy controls were analysed by mass cytometry using a 36 marker panel. Unsupervised clustering analysis revealed significant differences in the frequencies of T and B cell subsets. Most strikingly we identify a 3-fold decrease in frequencies of Va7.2⁺CD161⁺ mucosal associated invariant T cells (MAIT) in SSc patients and increase in total B cells, particularly CD19⁺CD27⁻ naïve cells. CXCR3⁺ memory CD8 T cells were found to be increased in SSc patients as compared to healthy controls. Transcriptome analysis of sorted B and T cell subsets showed decrease in genes related to survival and increased expression of apoptotic genes in CD4, CD8 T and MAIT cells from SSc patients. Genes related to exhaustion and leukocyte migration were highly expressed in T cells from patients.

This study provides an in depth analysis of systemic immune composition in SSc with the potential to delineate mechanisms of pathogenesis and identify diagnostic and/or therapeutic targets. This is the first demonstration of MAIT cell dysfunction in SSc and further functional characterisation in this context is required.

T.40. A Natural Killer Cell Based Disease Activity Score for Rheumatoid Arthritis Patients: Novel Mathematical Intervention in Rheumatic Disease Biology

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The present study looks into role of oxidative stress and immunobiology of NK and NKT cells in designing a mathematical model for assessing disease severity in Rheumatoid Arthritis patients. Improved disease severity prediction markers are need of the hour to aid early prognosis and personalized therapeutic approach.

Methods: Following ethical approval, 50 RA patients were recruited from the Rheumatology Clinic of PGIMER Chandigarh. Fifty age and sex matched healthy volunteers were also enrolled. The biochemical parameters investigated in serum samples were Lipid Peroxidation, Reduced Glutathione, Catalase, Superoxide dismutase, Glutathione peroxidase, IL-18 and TNF- α . NK and NKT cells related intracellular parameters (DNA damage, caspase-3, perforin,

granzyme A and B, IL-4, IFN- γ and IL-8 expression) were measured using multicolour flow cytometer. Mathematical model equation was developed for predicting RA blood based disease activity score (RABBDAS) using multiple linear regression analysis. **Results:** Oxidative balance was deregulated in RA patients. Granzyme A, perforin and IL-8 were independently associated with RABBDAS as:

$$\text{RABBDAS} = -1.115 + 0.054X_1 + 0.032X_2 + 0.14X_3 \quad (r^2 = 0.95)$$

Where X_1 : NK_GranA; X_2 : NKT_Perf; X_3 : NKT_IL-8

Discussion: There is a state of profound oxidative stress in RA patients. Immunobiology of NK and NKT cells were severely compromised.

T.44. Dysregulation of Innate, Adaptive and TNF-Superfamily Immune Pathways Alongside Autoantibody Accrual Informs a Biological Surrogate for Clinical Disease Activity in Lupus

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Systemic lupus erythematosus (SLE) is a complex autoimmune disease marked by immune dysregulation. Yet, how immune system changes influence clinical disease activity is largely unknown. This study evaluates SLE-linked immune mediators and autoantibodies in 311 SLE plasma samples procured on dates of low (<4, range 0-3, n=132) or active (≥ 4 , range 4-30, n=179) clinical disease activity measured by the hybrid SLEDAI (hSLEDAI; SELENA-SLEDAI with SLEDAI-2K-defined proteinuria) vs. matched healthy control (HC) samples (n=48). No difference in age, ethnicity, or gender was noted between low or active clinical disease activity samples. After adjusting for multiple comparison (Bonferroni corrected $p < 0.0018$), only IL-6, IL-1 α , IP-10, and IL-8 remained significantly correlated with hSLEDAI scores (Spearman $r = 0.179$ - 0.253), yet 22/32 soluble mediators significantly correlated with the number of SLE-associated autoantibodies accrued, including mediators listed above, SCF, IFN- α , IFN- γ , IL-17A, IL-10, MIG, MIP-1 β , TNFR2, and BlyS ($r = 0.318$ [IL-17A]- 0.468 [IP-10]). The regulatory mediator IL-10 was highest in autoantibody-negative, disease activity-low samples ($p < 0.05$), while all other mediators were highest in autoantibody-positive, active disease samples and lowest in autoantibody-negative, disease activity-low and HC samples ($p < 0.001$). We leveraged these findings to inform a surrogate immune mediator score (IMS) calculated utilizing normalized (case vs. control) soluble mediator levels (n=32) weighted by the number of SLE-associated autoantibodies in each individual. The IMS significantly correlated with hSLEDAI scores in SLE patients ($r = 0.226$, $p < 0.0001$) and identified patients with renal organ involvement ($p = 0.002$). Immunological profiles may be useful for delineating clinical disease pathogenesis, guiding therapy and clinical trial design, and detecting subtly-presenting serious autoimmune disease.

T.45. New Insights in the Pathogenesis of Immune-Mediated Necrotizing Myopathies: Role of Auto-Antibodies and Complement *in vitro* and *in vivo*

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Background and objective. Immune-mediated necrotizing myopathies (IMNM) are a newly recognized group of severe acquired myopathies associated to auto-antibodies (aAbs) directed against the signal recognition particle (SRP) or 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR). Besides their usefulness as diagnostic biomarkers, it remains to determine whether IMNM-associated are directly pathogenic.

Methods. Recombinant human SRP or HMGCR was used to measure aAb levels by ALBIA. Immunostaining and reverse

transcription PCR were performed on muscle biopsies and primary muscle cell cultures. Atrophy and regeneration were evaluated based on the myotube surface area as well as gene and cytokine profiles, after incubation with aAbs. IgGs from patients were transferred to normal, Rag2^{-/-} or complement C3^{-/-} mice, supplemented or not with human complement. Muscle strength was evaluated by grip test and *in situ* muscle contraction upon sciatic nerve electrostimulation.

Results. aAb titers correlated with CK levels and/or disease severity. SRP and HMGCRC were detected on altered myofibers and on myotubes. Sarcolemmal complement deposits correlated with myofiber necrosis. *In vitro*, anti-SRP and anti-HMGCRC Abs provoked myotube atrophy, and increased transcription of atrophy-related genes, inflammatory cytokines and reactive oxygen species. aAbs myoblast fusion and muscle regeneration. *In vivo*, patients IgG decreased muscle strength transiently or permanently in immunocompetent or immunodeficient mice, respectively. Pathogenicity was reduced in C3^{-/-} mice while increased by human complement. Immunization with SRP or HMGCRC provoked a muscle deficit.

Conclusion. Anti-SRP and anti-HMGCRC aAbs are complement-dependent pathogenic effectors that provoke atrophy and impair regeneration, prompting to evaluate complement- and B-cell targeting therapies in IMNM.

T.46. Immune Cells From Sisters Of Lupus Patients Highlight Important Pre-disease Epigenetic States And The Factors That Induce Them

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Systemic lupus erythematosus is a highly heterogeneous autoimmune disease with largely unknown etiological bases. Here, we epigenetically profile cells from the sisters of SLE patients, as these individuals share both genetic and environmental burden with their affected sibling, and often share endophenotypes that are hallmarks of SLE disease pathogenesis. We find cell type-specific open chromatin and RNA-seq profiles consistent with activation of disease-associated pathways. In particular, sisters that have an IFN signature have differential open chromatin near important GWAS loci. We found IRF8 and IRF9 transcription factors in monocytes and B cells, respectively, to be implicated in modulating the epigenetic landscape in sisters with IFN signatures. Importantly, we found sisters with IFN signatures to have differential open chromatin overlapping a rare, high effect SLE-associated SNP near MAP3K8, implicating this predispose state to induce chromatin changes that lead to disease pathogenesis. These data support a model for step-wise SLE disease progression in relatives of SLE patients.

T.48. Prolactin and Autophagy in Systemic Lupus Erythematosus: Clinical Significance of Correlation Between PRL-Receptor and Atg14⁺ Expression on B and T Reg Cells.

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Autophagy, a highly conserved protein degradation pathway, is essential for removing protein aggregates and misfolded proteins in cells and its defects contributes to SLE pathogenesis. Objectives: Analyze the relationship between PRL receptors (PRL-R) on B cells and markers of autophagy on T regulatory cells and the association, if any, with clinical characteristics of SLE. Methods: The expression of PRL-R on B cells CD19⁺, and autophagy-related key regulator protein ATG14⁺, on T regulatory cells CD25⁺, were measured by flow cytometry, and expressed in percentages of SLE

patients and healthy controls. Results: A total of 40 SLE patients and 20 healthy controls were included. Mean age of patients and controls was 30.67 ± 4.16 . Twenty patients were active (SLEDAI 8.45 ± 1.9) and of these, lupus glomerulonephritis was observed in 13 patients (65%). The expression of PRL-R on B cells of active SLE was higher than in inactive SLE (50.5% vs 26.5%). In the relation of autophagy, the mean expression of ATG14+ in 20 active SLE patients was 11.19% in comparison with inactive SLE patients, 7.13%, ($p = 0.04$), and in healthy donors, 7.445% ($p = 0.0281$). Our study suggest: In active SLE patients the expression of PRL-R and autophagy-related key regulator protein ATG14+ are very high in B cells and T regulators respectively. These novel findings suggest the interaction between PRL-R and autophagy in order to promote clinical/immune activation with overproduction of autoantibodies. PRL-R and ATG14+ may be a new target of SLE treatment.

T.54. Molecular Mechanisms of Autophagic Memory in Pathogenic T Cells in Human Arthritis.

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One of the key elements of immune pathogenesis of human autoimmune arthritis is the resilience of pathogenic T cells. We have previously reported higher autophagy in CD4+ T cells from RA patients compared to healthy equivalents. Here, we explored at epigenetic and transcriptional levels the concept of persisting increased autophagy as the consequence of "autophagic memory", as one of the mechanisms conferring resilience to pathogenic T cells, in particular to a subset of CD4+ T cells (CPL: Circulating Pathogenic-like Lymphocytes), which are significantly more represented in patients with active arthritis and resistant to therapy with biologics.

First, we demonstrated elevated autophagic levels in CD4+ memory T cells when compared to naïve CD4+ T cells. Second, we showed that autophagic levels are increased in naïve and CD4+ T cells from RA patients compared to healthy controls. Using next generation RNA-sequencing, transcription factor gene regulatory network (TF-GRN) and methylation analyses, we identified MYC as key regulator of autophagic memory in a human T cell line. Transcriptome and network analysis of RNA-seq data from patients'; CPLs confirmed MYC as key modulator of autophagy. Importantly, inhibitor of MYC increases autophagy

The present study suggests that autophagic memory is retained both at the transcriptional and epigenetic levels as an integral part of mechanisms of efficient activation and survival of memory T cells. This mechanism is particularly relevant to the immunopathogenesis of autoimmune diseases, such as arthritis. These studies have a direct translational valency as they identify autophagy and its metabolic controllers as a novel therapeutic target.

T.61. Elevated Neopterin Levels are Associated with Dyslipidemia in Juvenile Dermatomyositis

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Background: Our previous studies of older patients who had Juvenile Dermatomyositis (JDM) in childhood documented increased atherosclerosis, associated with dyslipidemia (Emier 2011). The hypothesis of the current study is that, in JDM, dyslipidemia is associated with systemic inflammation. We focused on the association of neopterin and dyslipidemia because TNF alpha, a proinflammatory cytokine produced by activated macrophages, can lead to dyslipidemia; activated macrophages also produce neopterin.

Methods: This IRB approved retrospective study was conducted at the Ann and Robert H. Lurie Children's Hospital of Chicago. All JDM patients (n= 144, mean age of 11.9 years, 74% female, 69% white, 37% had P155/140+ve autoantibody) who had fasting lipid profile, disease activity score (DAS), and neopterin level at the same visit were included. Increased neopterin was defined as >10 nmol/liter.

Results: 24% of the children had elevated neopterin levels. Those with elevated neopterin levels had mean TG, HDL, LDL levels of 126, 38 and 81 respectively. In contrast, the mean TG, HDL and LDL were 110, 51, and 86 respectively in the JDM group with normal neopterin. The difference in the HDL level was statistically significant, $p < 0.0001$ by T-test. Pearson Correlation of neopterin and HDL was -0.41 ; $p < 0.0001$. Disease Activity Scores (DAS-Skin, Muscle and Total) were higher in patients with elevated neopterin levels (10 ± 5.4 vs 3.3 ± 3.6 $p < 0.0001$). (Normal DAS = 0)

Conclusion: Elevated neopterin levels are associated with: 1) increased DAS in JDM, and 2) decreased HDL levels, suggesting that macrophage activation may contribute to the dyslipidemia of JDM.

T.68. Integrative Analysis of Clinical and Molecular Data on a Well-Phenotyped Lupus Cohort Identifies Molecular Associations with Disease Manifestations

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Systemic Lupus Erythematosus (SLE) is a serious multiorgan autoimmune disease involving the skin, joints, kidney, and central nervous system. We aimed to define how molecular differences underlie this clinical heterogeneity through an integrative approach leveraging methylation, genetic and phenotypic data from a multiethnic cohort of 265 patients from the California Lupus Epidemiology Study (CLUES). We applied Weighted Gene Correlation Network Analysis (WGCNA) to methylation data obtained using the Illumina EPIC chip (adjusted for age, sex, ancestry, cell type composition, and disease duration) and correlated network modules to specific SLE clinical presentations. This analysis revealed a module consisting of 157 CpG sites in 51 genomic regions, whose eigengene was significantly associated with anti-Smith autoantibody presence (FDR<0.01). The module was enriched for Gene Ontology terms relating to Type I IFN signaling, IFN-gamma, and innate immune response. This signature suggests that epigenomic differences in immune-related genes may underlie the clinical heterogeneity of SLE. We then performed a meQTL analysis with the 157 CpG probes selected by WGCNA which identified 22 significant *trans*-meQTLs and 55 significant *cis*-meQTLs (FDR<0.01). Both *cis* and *trans* meQTLs were found in BST2, EIF2AK2, and XAF1, genes previously found to be upregulated in SLE patients. In summary, this result indicates that genetic variation in these three genes modulates both proximal and distal DNA methylation which in turn affects anti-Smith antibody incidence. Identification of individual immune disease correlates may help improve patient stratification in future clinical trials of complex autoimmune diseases and design of personalized therapeutics.

T.69. Vitamin D reduces the Th17-phenotype in ACT1/Act1-associated Systemic Lupus Erythematosus

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Introduction: Mutations in *ACT1* (rs33980500) have been identified in Systemic Lupus Erythematosus (SLE). rs33980500 encodes a non-functional *ACT1* variant (*ACT1*-D10N) in T cells. Psoriasis patients with *ACT1*-D10N mutations display elevated Th17/IL-17A levels. It is unknown if *ACT1*-D10N SLE patients express similar Th17 hyperactivity; however, *Act1*^{-/-} mice show elevated Th17/IL-17A and develop a lupus-like phenotype. Since vitamin D (VitD) inhibits Th17 differentiation, and VitD deficiency is associated with lupus pathology, we hypothesize that VitD supplementation reduces disease in *Act1*^{-/-} mice. Additionally, we propose that *ACT1*-D10N SLE patients display elevated IL-17A and Th17/Treg ratios, susceptible to VitD regulation. **Results:** Germ-free *Act1*^{-/-} mice were recolonized while provided varying doses of VitD (0, 2 or 10 IU/g chow). After 6wks, serum VitD levels stabilized at ~5, ~18 and ~30ng/ml, respectively. At harvest, circulating Th17 cells and the Th17/Treg ratio negatively correlated with VitD concentrations, suggesting that VitD inhibits Th17 cell accumulation in *Act1*^{-/-} mice. We subsequently studied 25 SLE patients enrolled in the Cleveland Clinic Lupus Registry. Patients' disease activity ranged from inactive to moderately active (all SLEDAI<10). VitD levels were similar between patients with and without the *ACT1*-D10N mutation (p=0.26). Two patients expressed elevated IL-17A concentrations (>300pg/ml), but neither carried the *ACT1*-D10N mutation. Among the remaining 23 patients, *ACT1*-D10N carriers displayed elevated IL-17A (56.6±34.8pg/ml vs. 24.2±33.4pg/ml; p<0.05). Studies determining Th17/Treg ratios are ongoing. **Summary:** *ACT1*-D10N SLE patients express elevated IL-17A similar to *Act1*^{-/-} mice. VitD supplementation reduces Th17 cells, partially normalizing the Th17/Treg ratio in *Act1*^{-/-} mice. Thus, *ACT1*-D10N SLE patients may be susceptible to VitD supplementation.

T.73. The SLE-associated Gene *BANK1* Inhibits Macroautophagy and Restricts Plasma Cell Differentiation and Immunoglobulin Production by Human B Cells

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Dysregulation of plasma cell (PC) differentiation and/or homeostasis contributes to autoantibody production and pathogenesis in systemic lupus erythematosus (SLE). Recent findings indicate that PC differentiation and antibody production require the autophagy pathway. Using mass spectrometry-based proteomics, we detected an interaction between the SLE-associated protein, *BANK1* and the autophagy regulator, ATG3. We validated this protein-protein interaction and used immunofluorescence to show that endogenous *BANK1* co-localizes with lipidated LC3B puncta (eg LC3B-II) in human B cells. We next generated human B cell lines deficient for *BANK1* using CRISPR/Cas9-based gene editing. We found that *BANK1*-deficient B cells exhibit increased autophagic flux in response to treatment with rapamycin. To link these findings to PC differentiation, we used a human primary B cell culture system that couples CRISPR/Cas9 ribonucleoprotein complex delivery in naïve B cells (allowing gene disruption rates of ~95%) with *ex vivo* differentiation into PCs. Using this system, we found *BANK1* disruption increased both PC numbers (including long-lived cells) and antibody production. Finally, we compared the rates of PC differentiation in primary B cells isolated from healthy control subjects expressing the SLE-associated risk or non-risk coding variants in *BANK1*. Similar to what we observed in *BANK1*-deleted cells, primary B cells homozygous the risk genotype for *BANK1* exhibited increased PC differentiation and antibody production. Our findings reveal an unappreciated role for *BANK1* as an autophagy inhibitor protein that limits the differentiation of primary human B cells into antibody-secreting PCs, providing mechanistic insight into the role of *BANK1* variants in SLE susceptibility.

T.86. Specialization of Vascular Adventitial Fibroblasts Correlates with Lymphocyte Infiltration in Human Dermal Diseases

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Vascular pathology is a hallmark of the cutaneous manifestations of human systemic sclerosis, dermatitis, lupus, and other immunological diseases. Many studies have addressed changes in the endothelial and vascular smooth muscle compartments in dermal vascular lesions. Our work demonstrates that the dermal vascular adventitia also differentiates and dramatically expands. Distinct from dermal fibroblasts in healthy skin, adventitial fibroblasts express CD90 and surround blood vessels. Adventitial fibroblasts expand in systemic sclerosis, but VCAM1 expression and perivascular infiltration are rare. Elevated adventitial fibroblast VCAM1 expression in dermatitis and lupus is accompanied by the adventitial accumulation of T cells. In whole skin biopsies from DLE patients, VCAM1 RNA was highly correlated with a T cell RNA signature, as well as IL-15, CCL19, and CCL21 RNA. VCAM1 induction, T cell retention, and the correlation of VCAM1 with survival cytokine and homeostatic lymphoid chemokine RNA indicate that dermal vascular adventitial fibroblasts are beginning to resemble lymphoid organ stroma. We also observed VCAM1⁺ reticular networks enmeshing T cells around large vessels in the liver and pancreas in two murine models of autoimmunity. Expanded and activated adventitial fibroblasts help distinguish vascular unit pathology in cutaneous systemic sclerosis, dermatitis, and lupus. These differentiated stromal networks may promote perivascular leukocyte accumulation.

T.87. Enhancers in Autoimmune Disease Cells Contribute to Disease Pathogenesis

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For many autoimmune diseases the molecular mechanisms are poorly understood. To create insight into the epigenetic contribution to autoimmune diseases, we aimed to assess the epigenetic profile of autoimmune disease patient-derived cells. Juvenile Idiopathic Arthritis (JIA) was used as a model for studying autoimmune diseases, due to the unique possibility to analyze cells derived from the site of inflammation. We hypothesize that epigenetic profiling can contribute to a better understanding of autoimmune disease pathogenesis and might lead to the identification of novel therapeutic targets.

Analysis of the active enhancer profile of primary T cells and monocytes obtained from the synovial joints of JIA patients demonstrated a disease-associated (super-)enhancer profile, which corresponds to disease-associated gene expression (RNA-seq). Arthritis-associated SNPs were significantly enriched in JIA (super-)enhancers, illustrating the importance of these non-coding regions for disease pathogenesis. In addition, expression of several histone-modifying proteins was altered compared to healthy controls, suggesting that these proteins might be involved in shaping the enhancer repertoire. Furthermore, BET inhibition of JIA patient cells using JQ1 preferentially reduced disease-associated gene expression, indicating that enhancers contribute to disease pathogenesis.

These results demonstrate that enhancers contribute to disease-associated gene expression, which can be disrupted upon inhibition of enhancer activity. Together, this indicates that BET inhibition might be a potential therapeutic approach for the treatment of autoimmune diseases.

W.20. Gene Expression Analysis Delineates the Roles of Multiple Type 1 Interferons in Systemic Lupus Erythematosus

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A role for interferon (IFN) in lupus pathogenesis has been inferred from the presence of a prominent IFN gene signature (IGS). However, the identification of the major IFN species up-regulating gene expression and its relationship to SLE disease activity have not been determined. Gene Set Variation Analysis (GSVA) of cytokine and interferon signatures derived from *in vitro* stimulation of PBMC, IFNB1 treated MS patients, and IFNA2 treated HepC patients separated SLE patients from control samples and Z score calculations demonstrated a prominent role for IFNB1 and IFNW1. Analysis of 1041 active and 319 inactive SLE patients demonstrated that 76% of active and 69% of inactive SLE patients had an IGS. Weighted gene correlation network analysis (WGCNA) of WB and PBMC SLE datasets showed low (Pearson $r < .3$) or no correlation to disease activity. Longitudinal analysis of SLE patients at 0, 16 and 52 weeks showed little variation ($VAR.S < .05$) in the IGS enrichment score over time for most subjects. In contrast, 9 of 10 SLE nephritis patients treated with immunosuppressives showed changes ($VAR.S > .05$) in the IGS, although the correlation with disease activity was minimal. Notably, a significant correlation by linear regression analysis ($p < .0001$, $r^2 = .36$) was noted between the IGS and a myeloid cell signature. These results suggest that type 1 interferons contribute to SLE. Although the variation over time in the IGS is minimal in most subjects and there is little association with disease activity, a correlation with the myeloid cell signature suggests a common biology.

W.31. Identification of a Novel B Cell Subset in Systemic Lupus Erythematosus Using AMNIS Imaging Flow Cytometry

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Using Imaging Flow Cytometry (IFC) to study FOXO1 localization in peripheral blood lymphocytes from SLE patients, our laboratory has observed dramatic cytoplasmic localization of FOXO1 in B cells, specifically in the IgD-CD27- subset of B cells. So-called "Double Negative" B cells have previously been shown to be increased in SLE and enriched in autoreactive clones. We have named this newly-observed B cell subset "FcytDN" B cells for Double Negative B cells with predominantly cytoplasmic FOXO1, as opposed to "FnucDN." We find abundant FcytDN B cells in SLE patients, particular in those with more active disease, as opposed to primarily FnucDN in healthy controls. FcytDN B cells appear to have other unique features such as relatively low CD20 expression and high CD95/Fas expression. Together with cytoplasmic FOXO1, this combination of surface markers likely represent a high state of B cell activation with excess kinase signaling. We will include data from preliminary experiments on the unique gene expression profile of SLE DN B cells, with a focus on aberrant FOXO1-dependent gene regulation. We hypothesize that FcytDN B cells in SLE represent a critical stage in the expansion of pathologic, autoreactive B cells and their unique, cytoplasmic FOXO1 provides an opportunity to develop a highly targeted approach to treating SLE.

W.42. Novel Innate and Adaptive Immunity Abnormalities as Key Risk Factors for Infections in Patients with Systemic Lupus Erythematosus: Results from the GERMEN Cohort

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Introduction: Patients with systemic lupus erythematosus (SLE) have multiple innate and adaptive immune response abnormalities. It is unknown whether they play a key role in the development of infections, which was the aim of the present study.

Methods: A prospective cohort study of 59 SLE patients was undertaken. We registered clinical data and performed immunophenotyping by flow cytometry. We analyzed the number of neutrophil extracellular traps (NETs) and their LL-37 expression by confocal microscopy.

Results: Twenty-one patients (35%) developed an infection during 12 months of follow-up. At baseline, they had lower absolute numbers of B lymphocytes (88 vs 253, $P=0.028$), higher numbers of Th17 (11 vs 3, $P=0.007$) and of low density granulocytes (LDG) (105 vs 32, $P=0.006$). Patients with infections showed a trend towards lower LL-37 expression in their NETs. To encompass the variability of data over time, we calculated the delta(Δ) value of every parameter in 2 consecutive visits and performed a logistic regression ($R^2=0.77$). The RR (95% CI) for infection were: Δ Hemoglobin (8.3 (2.3-89.7, $P<0.05$), $-\Delta$ TLR2 in monocytes (0.999 (0.9995-0.9999, $P<0.05$), $-\Delta$ total monocytes (0.991 (0.9813-0.9988, $P<0.05$) and $-\Delta$ B cells (0.993 (0.9871-0.9993, $P<0.05$).

Conclusions: Patients who developed infection had baseline B cell lymphopenia and higher levels of Th17 and LDG. During follow-up, the decrease in hemoglobin, B cells, monocytes as well as their TLR-2 expression were independent risk factors for infections. These findings support the key role of innate and adaptive immunity SLE related abnormalities in the development of infections in SLE.

W.47. Enrichment of ROR γ t⁺ Innate-like T Cells in Intestinal Biopsies from Spondyloarthritis Patients

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Dysregulated IL-23/IL-17 responses have been linked to development of spondyloarthritis (SpA), a cluster of inflammatory rheumatic diseases which is frequently marked by the presence of (subclinical) gut inflammation. IL-23/IL-17 inflammation is controlled by ROR γ t, the key Th17 cell transcriptional regulator, which is also expressed by subsets of innate-like T cells including invariant natural killer T (iNKT), mucosal associated invariant T (MAIT) and $\gamma\delta$ -T cells, but their role in SpA pathology, especially with regard to intestinal manifestations is still unclear. In this study, intestinal biopsies from the ileum and colon were collected next to peripheral blood from healthy control subjects and new-onset SpA-patients. Lamina propria lymphocytes (LPL) and PBMC were isolated and analyzed by multi-color flow cytometry. Unsupervised clustering analyses revealed a marked heterogeneity of blood iNKT, MAIT and $\gamma\delta$ -T cells which seemed skewed in SpA-patients. Circulating $\gamma\delta$ -T cells consisted of a major population of $\gamma\delta$ -intermediate (TCR $\gamma\delta$ -int) and a smaller subset of $\gamma\delta$ -high (TCR $\gamma\delta$ -hi) cells, the latter displaying a distinct PLZF-Tbet^{low}GATA-3⁺ phenotype and profound IL-23 mediated Th17-like immune responses. ROR γ t⁺ innate-like T cells, including TCR $\gamma\delta$ -hi cells were clearly enriched in LPL samples from SpA-patients as compared to controls, suggesting that in SpA intestinal innate-like T cells are skewed towards a predominant pro-inflammatory Th17 profile. Finally, ROR γ t inhibition efficiently blocked IL-17 producing innate-like T cells while selectively sparing IL-22⁺ subsets, which could be of clinical importance. Overall, these findings highlight a unique diversity of human ROR γ t⁺ T cells and underscore the potential of ROR γ t antagonism to modulate aberrant type 17 responses.

W.48. Low Density Granulocytes and Neutrophil Extracellular Traps as Key Players of Disease Activity in Patients with Idiopathic Inflammatory Myopathies

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Introduction: Low density granulocytes (LDG) are a subset of neutrophils that produce neutrophil extracellular traps (NETs) and type I IFN. The latter correlates with disease activity in patients with idiopathic inflammatory myopathies (IIM). The aim of this study was to assess the role of LDG and their NETs protein cargo in patients with IIM.

Methods: We recruited 61 adult patients with IIM according to Bohan and Peter criteria. The percentage and absolute number of LDG were assessed by flow cytometry as those CD10⁺, CD15⁺ and CD14⁻ cells in the mononuclear fraction. LDG were isolated by positive selection and their NETs protein cargo was analyzed by confocal microscopy.

Results: The percentage of LDG was inversely correlated with hemoglobin (Rho -0.25, P=0.047) and prednisone dose (Rho -0.47, P=0.009). The absolute number of LDG was positively correlated with serum ALT (Rho 0.28, P=0.029), the physician's (Rho 0.38, P=0.003) and patient's (Rho 0.37, P=0.003) disease activity score according to a visual analogue scale and inversely with the MMT8 score (Rho -0.39, P=0.002). Interestingly, patients with calcinosis had a higher percentage of LDG (38.9% vs 10.9%, P=0.002) and those with anti-Ku antibodies had lower absolute number of LDG (8 vs 78, P=0.013). Furthermore, we found that NETs from LDG were enriched in LL-37 and HMGB1.

Conclusions: LDG from patients with IIM produce NETs with pro-inflammatory and interferonogenic proteins such as LL-37 and HMGB1 and are associated with disease activity. Our findings support a novel physiopathogenic mechanism of innate immunity for disease activity in IIM.

W.52. Pathological Consequences of Anti-PAD4 Antibody Binding to the Surface of Monocytes: Implications in Rheumatoid Arthritis

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Antibodies that activate peptidylarginine deiminase 4 (PAD4) are found in rheumatoid arthritis (RA) patients with the most severe joint and lung disease, implicating them in disease pathogenesis. These antibodies activate soluble PAD4 to enhance the generation of extracellular citrullinated proteins, hallmark serologic targets in RA. However, it is unknown if they have additional functional consequences beyond citrullination. Recent evidence has shown that PAD4 is expressed on the monocyte surface, suggesting that monocytes may be direct cellular targets of PAD4-activating autoantibodies in patients with RA. To dissect this potential pathogenic mechanism, we investigated the interaction of PAD4-activating antibodies with monocytes using a well-characterized panel of human anti-PAD4 and control monoclonal antibodies (mAbs). Flow cytometry studies revealed preferential binding of PAD4 mAbs to PAD4 present on surface of monocytes, with $63 \pm 14\%$ monocytes being bound by PAD4 mAbs versus $1.9 \pm 0.03\%$ by control mAbs ($p=0.0087$). Monocytes expressed surface PAD4 in an enzymatically active state that could be hyperactivated 2 to 3 fold upon PAD4 mAb binding. Importantly, PAD4 mAb binding induced 100 to 150-fold more secretion of RA-associated pro inflammatory cytokines including IL-6 and TNF than control mAbs. In conclusion, anti-PAD4 autoantibodies directly interact with PAD4 present on the surface of monocytes and enhance extracellular citrullination and cytokine secretion. This interaction may play a vital role in promoting inflammation and influencing monocyte differentiation into bone eroding osteoclasts, suggesting a novel pathogenic mechanism in RA.

W.55. Kidney Infiltrating T cells in Murine Lupus Nephritis are Metabolically and Functionally Exhausted

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While T cells are important for the pathogenesis of systemic lupus erythematosus (SLE) and lupus nephritis, little is known about how T cells function after infiltrating the kidney. The current paradigm suggests that kidney infiltrating T cells (KITs) are activated effector cells contributing to tissue damage and ultimately organ failure. Herein, we demonstrate that the majority of CD4⁺ and CD8⁺ KITs in two murine lupus models are not effector cells, as hypothesized, but rather expressed multiple inhibitory receptors including PD-1, Lag3 and Tim3, and proved highly dysfunctional with reduced cytokine production and proliferative capacity. Mechanistically this was linked directly to metabolic and specifically mitochondrial dysfunction with a vast reduction in spare respiratory capacity. This was driven by the expression of an “exhausted” transcriptional signature. The KIT phenotype and transcriptional signature is analogous to what has been described in the setting of chronic infection and the T cell infiltrates in the tumor microenvironment. Our data thus reveal that the tissue parenchyma has the capability to suppress T cell responses and limit damage to self. These findings open novel avenues for the treatment of autoimmunity based on selectively exploiting the exhausted phenotype of tissue-infiltrating T cells.

W.60. A Novel “Anti-Vaccine” for the Treatment of Collagen-Induced Arthritis

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Treatment options for rheumatoid arthritis (RA) are inadequate, highly expensive, and fail to address the autoimmunological pathogenesis of the disease. Tolerogenic dendritic cells (tDCs) are an attractive solution for treatment of RA because they target the immune cascade responsible for RA. However, *ex-vivo* stability, post-transfer survivability, and tremendous manufacturing costs inhibit the widespread use of exogenously-treated tDCs for autoimmune treatments. In an effort to circumvent drawbacks with exogenously-derived tDC therapy, an “anti-vaccine” delivering tolerogenic factors and antigen via poly(lactic-co-glycolic acid) (PLGA) microparticles has been explored as a potential system for RA treatment. **We hypothesize that a dual-sized PLGA microparticle “anti-vaccine” will increase the frequency of tolerogenic innate immune cells, particularly DCs, ultimately promoting antigen-specific tolerance and reduction of systemic rheumatic-associated inflammation.**

To test this system, 25 mice were induced with collagen-induced arthritis (CIA). Additionally, two sizes of microparticles were fabricated, small microparticles (1 µm) containing collagen II or vitamin D3, and large microparticles (30 µm) loaded with GM-CSF or TGF-β1. Once mice had become arthritic, microparticle injections (2.5 mg of each microparticle) were given 3 times in the first week followed by biweekly boosters. Terminal and mid-study assessments to establish RA progression included [18-F]DG PET imaging, histology of paws, gait analysis, immune cell phenotyping, and cytokine expression via RT-PCR.

These preliminary studies demonstrate that the microparticle “anti-vaccine” formulation can (a) modulate the immunophenotype of bone marrow derived DCs and MΦs *in vitro* (b) modulate the inflammatory environment in the paws of CIA mice towards anti-inflammation, (c) prevent the progression of collagen-induced arthritis.

W.75. Manipulation of Immune Checkpoints via an Epitope-Specific Vaccine Restores Immune Tolerance and Induces Clinical Amelioration in Human Rheumatoid Arthritis.

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Therapeutic strategies tampering with immune checkpoints like PD-1 that govern immune tolerance are gaining traction in several diseases. Here, we dissect the immune mechanisms underlying the establishment of immune tolerance by epitope-specific immunotherapy with the dnaJP1 peptide that results in clinical amelioration of RA. We hypothesize that the reactivation of immune checkpoints orchestrates immunomodulatory mechanisms that dictates immune tolerance and clinical control.

In-depth examination of the T cell immunomes of dnaJP1 responders and placebo non-responders revealed a unique subset of CD4⁺FoxP3⁺ regulatory T (Treg) cells in dnaJP1 responders displaying an upregulated expression of the inhibitory immune checkpoint receptor PD-1. The expression of PD-1 in this Treg subset contributes actively to the enhanced production of signature anti-inflammatory cytokines such TGFβ. We also observed a reciprocal dampening of the effector T (Teff) cell compartment that is characterized by a decline in the expression of pro-inflammatory cytokines such as IL-17A and IFNγ. Furthermore, epitope-specific immunotherapy induced active antigen-experienced memory T cells (CD4⁺CD45RO⁺CD69⁺TGFβ⁺) that perpetuate the longevity of the tolerogenic immune response. Lastly, our preliminary findings demonstrate that concomitant use of Hydroxychloroquine (HCQ) favours the re-establishment of immunological homeostasis by driving antigen-presenting cells (APCs) to adopt a tolerogenic phenotype which then skew T cells towards a functionally protective phenotype.

Collectively, our data presents an exciting vaccine-like therapeutic strategy capable of reprogramming the auto-inflammatory immune system and reactivating tolerogenic pathways through the manipulation of immune checkpoints.

W.111. Using Metabolomics to Understand the Immunopathogenesis of Juvenile-Onset SLE and Stratify Patient Groups

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Patients with Juvenile-onset systemic lupus erythematosus (JSLE) have more aggressive disease and increased mortality compared to patients with adult-onset SLE and all SLE patients suffer from accelerated atherosclerosis and a significantly increased risk of heart disease. Our previous work describes multiple defects in fats (such as cholesterol) that contribute to immune cell abnormalities in adult SLE patients. However, in patients with JSLE, the immune system is still developing and very little is known about disease pathogenesis, whether it is the same as adult disease and whether the same treatments available to the adults are relevant for this younger group of patients. We examined immune cell and blood fat levels in JSLE patients (n=35) compared with sex and age matched teenage healthy controls (n=30) using flow cytometry, qPCR and nuclear magnetic resonance metabolomics analysis. T and B lymphocytes from JSLE patients had significantly increased lipid (fat) expression which correlated significantly with disease activity. High disease activity was associated with increased levels of circulating blood fats (very low, intermediate and low density lipoprotein; VLDL, IDL and LDL respectively) and increased fat levels in immune cells from JSLE patients. In-depth metabolomics analysis assessing over 200 different types of blood fat identified three distinct metabolic JSLE patient groups, each group characterized by unique immunological and clinical features. We propose that metabolomic stratification based on measuring blood fats could help to pinpoint which JSLE patients would benefit from existing or in development fat-modifying therapies.

Bone marrow or stem cell transplantation

F.3. iR-HLA Cassette: An Automated and Integrated Solution for NGS Based HLA-Typing

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Based on the dimer-avoided-multiplex-PCR technology (dam-PCR), the iR-HLA cassette provides the end-user a solution to generate DNA libraries for HLA-NGS typing in an automated, disposable and fully closed format, without complex manipulations. Automation on the iR-Processor helps to prevent human error, sample contamination, and allows for significant time savings. The user deposits a minimum amount of genomic DNA (50 ng) into the cassette and loads the cassettes into the iR-Processor. Once processing is complete, the library is ready for sequencing using the Illumina MiSeq platform in 7 hours. Compared with current HLA-NGS products, the iR-HLA cassette has some significant advantages: (1) Robust amplification. The assay uses our patent pending multiplex amplification strategy, dam-PCR, simplifying library preparation while maintaining true high end-point resolution. (2) Closed system. Once the cassette is loaded with a sample and closed, all the amplification and purification procedures can be conducted within the cassette. Since amplicons will not be exposed to the laboratory environment, and the majority of PCR reagents are pre-installed at the manufacturing site, both the contamination source and the opportunity for contamination are under control. (3) Easy to use. The integrated, fully automated design minimizes requirements for specialized training and facilities for technicians who perform the test. (4) Speed. The entire NGS workflow from gDNA amplification to genotype assignment can be completed within 2 working days. With the advantages of saving time and simplifying the protocol, iR-HLA cassettes will allow laboratories to have more time and energy in analyzing and interpreting the results.

F.11. MicroRNA-23a Plays an Essential Role in Graft-versus-Host Disease

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Graft-versus-host disease (GVHD) is a major obstacle to allogeneic hematopoietic cell transplantation. GVHD is induced when donor mature T cells encounter host antigens and get activated. In this study, we investigated the role of miR-23a (miR-23a) in GVHD using miR-23a knockout T cells (KO). One million T cells from KO or wild-type (WT) C57BL/6 donors were transplanted into lethally irradiated (8.5 Gy) BALB/c mice along with 1×10^7 donor T-cell-depleted bone marrow (TCDBM) cells. While all recipients of WT T cells developed lethal GVHD and died within 35 days after transplantation, eight out of ten KO T cell recipients survived more than 60 days after transplantation ($P < 0.05$). Both clinical scores and body weights were significantly improved in the KO T cell recipients compared with those in the WT control group. Similar results were observed in a second GVHD model (C57BL/6@C3H/HeJ). Further experiment demonstrated that the effect of miR-23a was more prominent in CD4 T-cell-induced GVHD than in CD8 T-cell-induced GVHD. Mechanistically, *in vitro* and *in vivo* data demonstrated that T cell expansion in KO group was dramatically decreased comparing with WT group in response to alloantigens. Decreased clonal expansion was a result of increased ROS-mediated necrosis of alloantigen-activated KO T cells compared with WT T cells. Finally, the results from graft-versus-tumor (GVT) experiments indicated that T cells deficient of miR-23a preserve GVT effects. Taken together, we demonstrate that miR-23a is critical for the induction of GVHD and could potentially be targeted for prevention and treatment of GVHD.

F.107. Fate of Endothelial Progenitor Cells in an Altered Intracellular and Mitochondrial Calcium Homeostasis

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Endothelial progenitor cells (EPCs) are known to respond differently to physiological stress and often depends on the microenvironment. Alteration of calcium homeostasis is one of the major event occurring during the physiological stress,

leading to cascade of cellular signals that mobilizes the EPCs towards the damaged tissues. However the response of the EPCs to these intracellular calcium $[Ca^{2+}]_i$ and mitochondrial calcium $[Ca^{2+}]_m$ homeostasis, survival, differentiation and/or repair are poorly understood. In many cases pathological microenvironment are characterized by Ca^{2+} overloading, which is attributed as one of the major reasons for the failure of stem cell repair mechanisms. Hence the fate of the mobilized EPCs in the niche to the stimulus of external bolus of Ca^{2+} or their threshold to withstand such heavy physiological stress is not clearly explained. Further there is a speculation on the positive and negative Ca^{2+} homeostasis initiating the cell mobilization, grafting, homing and repair of tissues. In the current study we used EPCs as model to study the Ca^{2+} overloading downstream effects on cytokine and molecular signals with orientation to cell survival and differentiation. Physiologically EPC's are initially mobilized cells to the site of tissue injury, our results showed a dynamic Ca^{2+} dependent cell death markers and comparatively altered Ca^{2+} threshold making it susceptible to stress rather than repair. Further the current report will add a significant light on the EPCs threshold on the $[Ca^{2+}]_i$ and $[Ca^{2+}]_m$ separately on the differentiation and cell signaling.

T.47. Scatterbodies for Morphometric Profiling by Single Cell Mass Cytometry in Human Hematopoietic Cells

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The advent of mass cytometry (CyTOF) has transformed immunophenotyping, facilitating quantification of 50+ features simultaneously in millions of individual cells per experiment at an unprecedented resolution. However, CyTOF fails to quantify side scatter (SSC) – a light-based parameter that is commonly used in flow cytometry for discrimination of immune cell subsets and diagnosis of hematopoietic malignancies. By measuring the structural features and enzymatic machinery that underlie cell morphology and generate SSC, we can reliably recapitulate this parameter *in silico* with CyTOF, facilitating profiling of normal and neoplastic hematopoietic cells in a novel, “morphometric” way.

Morphologically different cells show consistent and distinct morphometric profiles, providing a framework for analysis that directly integrates into current experimental pipelines and clinical diagnostic workflows. By incorporating scatterbodies into a general-purpose panel for leukemia and lymphoma diagnosis, we can not only generate comparable biaxial plots as clinical flow cytometry, but also evaluate antigens on all identified cell populations in a way conducive to computational automation. The resolution achieved by these analyses reveals cell types and dysplasia not readily visualized with conventional cytometry. Finally, we apply machine learning to generate a morphological immune reference map that can reveal neoplasm-specific deviations, paving the way for integration of automated mass cytometry into clinical diagnostics.

T.114. Impact of HLA Disparity in the UCB Transplant Outcome of Malignant and Non-Malignant Diseases

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Donor/recipient Human Leukocyte Antigen (HLA) compatibility is regarded safe for successful transplant outcome. UCB hematopoietic stem cells are naïve and the extent of HLA disparity is not severe as observed with BM or PBMHSCs. In recent past there has been sharp increase in the number of cord blood transplantations and transplant failures vice-versa and in the current study, we have investigated the impact of HLA disparity between malignant (M) and non-malignant (NM) hematological diseases, compared to its success rate. Further our aim to assess the global figure, therefore we have restrained from retrospective single hospital based analysis. Methodologies included data mining, analysis of

reviews and original articles. Our study design included only diseases with well-established transplant therapy. The umbilical cord blood source of stem cells used for the transplantation were 54% and 46% for NM and M hematological diseases respectively. The overall HLA disparity percentage noted between the NM and M were 60:40. The average survival as disease free-state (DFS) after 48 months were better with NM and low with M. The overall rise of 9.7% for NM and 7.3% for M new cases using cord blood stem cells shows HLA disparity in MN is much tolerant than M. The growing trend to utilize umbilical cord blood stem cells as source for treating M and NM is based on its abundant availability and less severe HLA disparity complications. NM conditions are much tolerant to HLA disparity compared to M conditions where, disease resurgences are common with severe immunological complications.

W.32. Expression of Programmed Cell Death Protein-1 (PD-1) on T cells After Allogeneic Hematopoietic Stem Cell Transplantation

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Programmed cell death protein-1 (PD-1) blockade is a promising strategy to improve the antitumor effect of allogeneic hematopoietic stem cell transplantation (HSCT). However, severe and sometimes fatal Graft-versus-Host-Disease (GvHD) has been reported as a complication of anti-PD1 therapies administered for cancer relapse after allogeneic HSCT. Little is known about the dynamics of expression of PD-1 on immune effector cells after allogeneic HSCT, a crucial information for the optimization of PD-1 blockade therapies in this context. In the present study we analyzed PD-1 expression on T cells subpopulations isolated from 107 patients after allogeneic HSCT. Our analysis revealed a significant increase in the proportions of PD-1-expressing CD8 and CD4 T cells at early phases after allogeneic HSCT followed by a progressive normalization of PD-1 expression at CD8 but not CD4 T cell surface. Analysis of co-expression of other exhaustion markers, including 2B4 and CD160, failed to reveal any increase in other markers. Analyzing the association between clinical factors and the expression of PD-1 on T cells, we identified the use of *in vivo* and/or *ex vivo* T-cell depletion as the factor most strongly associated with elevated PD-1 levels on T cells. Conversely, we failed to detect any significant differences in PD-1 expression on T cells from patients with acute GvHD, chronic GvHD or disease relapse. Our results, showing that PD-1 expression is differentially regulated in CD8 and CD4 T cells after allogeneic HSCT, may have important implications for the optimization of PD-1 blockade therapies after allogeneic HSCT.

W.85. Autologous iPSC-derived Thymic Epithelial Cells as Cell Therapy to Restore Thymic Function in Stem Cell Transplant Recipients

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Hematopoietic stem cell transplantation (HSCT) outcome and recipient thymic function are intimately intertwined. The production of mature T-cells occurs in the thymus and depends on the interaction of hematopoietic progenitor cells with thymic epithelial cells (TECs). Through positive and negative selection of maturing T-lymphocytes, the thymus generates a broad and self-tolerant T-cell receptor repertoire that protects from infection and prevents autoimmunity. At odds with its importance for successful transplant outcomes, the thymus is exquisitely sensitive to a wide range of insults encountered during the transplant period, including chemotherapy, irradiation and acute graft-versus-host disease (GVHD). Thymic injury in turn, leads to susceptibility to infections, autoimmunity and chronic GVHD. Currently, no effective treatments options to target thymic injury exist.

Building on the induction of definitive endoderm from human pluripotent cells, we have developed an in-vitro-differentiation platform for the combinatorial assessment of the Wnt3a, BMP4, FGF7, FGF8, and FGF10 cytokine

signaling pathways which are all implicated in thymic development. The 56 combinations that showed the highest expression of 3rd pharyngeal pouch and thymic markers by qPCR were then compared to primary human fetal TECs using transcriptional profiling by RNAseq. Our data provides strong evidence for the impact of retinoic acid and FGF8-signaling in thymic development. Functional testing of the in-vitro derived TECs to support T-cell maturation in a humanized mouse model is currently underway. In-vitro derived autologous TECs are a novel strategy to treat thymic injury post-transplant which is expected to improve post-transplant immune reconstitution, mitigate autoimmunity and prevent the development of chronic GVHD.

W.90. Biomaterial Substrate-Mediated Three-Dimensional Culture Enhance the Immunosuppressive Action of Mesenchymal Stem Cells on Inflamed Immune Cells *In Vitro* and *In Vivo*

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With immunosuppressive character, mesenchymal stem cells become an attractive candidate used for allogeneic stem cell transplantation. Our previous study demonstrated that murine several tissue MSCs exhibit *in vitro* immunosuppressive effects on allogeneic immune cells, but not enough to reduce severity GvHD after allogeneic bone marrow transplantation. This is possibly due to *in vitro* artificial cell expansion. Self-assembly MSC spheroids has shown to create an *in vivo*-like microenvironment. However, less immunity study of MSC spheroid formed on biomaterials. In present study, we examine MSC spheroids form on different biomaterial sources and analyze their anti-inflammatory effects on macrophages and lymphocytes. Results have shown that MSC spheroids from chitosan film exhibited the better differentiated capability than 2D culture MSCs and other material 3D cultures MSC. Conditioned medium or cell spheroids from chitosan film cultured-MSCs showed better anti-inflammatory effects than that of 2D and hanging drop cultured-MSCs on activated macrophages and lymphocytes by inhibiting secretion of inflammatory cytokines, lipid mediators, ROS, co-stimulatory marker, and lymphocyte proliferation. In vivo results had showed MSC spheroids alleviate the symptom of mouse peritonitis. Mechanical studies showed that chitosan formed MSC spheroids lead the macrophage polarized to M2 type macrophage. In addition, the high levels of TSG-6, TGF- β , PGE2, and enzyme COX-2 and immune check point PD-L1 expression were detected in chitosan-cultured-MSCs spheroids; and that the anti-inflammatory activities were abolished by an COX inhibitor or PD-L1 blockade. These results suggested that MSCs cultured as 3D spheroids on chitosan substrate can strengthen their immunosuppressive capabilities on inflamed immune cells.

W.115. TIM-1 in the Donor Graft Modulates Graft-Versus-Host Disease Following Allogeneic Hematopoietic Cell Transplantation

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Graft-versus-host disease (GVHD) continues to be a common cause of morbidity and mortality after allogeneic hematopoietic stem cell transplantation (HSCT). In the acute setting, GVHD is predominately mediated by T cells and therefore immune receptors expressed by T cells can be therapeutic target to prevent or treat GVHD. The T cell immunoglobulin and mucin (TIM) proteins represent a family of molecules that act in concert with T-cell receptor and costimulatory signals to regulate expansion, differentiation and effector function of T and NKT cells. Given the immunoregulatory role of TIM-1 in other transplant models, we explored how TIM-1 influences GVHD in a murine model of allogeneic HSCT. Mice treated with an antagonistic anti-TIM-1 mAb are protected from lethal GVHD while maintaining

graft-versus-leukemia effects. Protection against GVHD appears mediated by TIM-1 expression on the donor graft as WT recipients of TIM-1 knockout donor grafts showed improved survival. Unlike every major immunosuppressive approach used clinically to prevent GVHD, we determined that TIM-1 blockade does not alter the homing and expansion of donor T cells *in vivo*, nor does it affect murine or human T proliferation *in vitro*. Instead, we found that anti-TIM-1 treatment protects by promoting an anti-inflammatory environment in the spleen and the gut tissue. Antagonistic anti-TIM-1 mAb also protects from lethal GVHD in a xenogeneic model of GVHD, making possible the direct translation of this therapy into clinical studies. Overall, these findings can form the basis for the development of novel therapeutic strategies to prevent GVHD and improve HSCT.

Cytokines/chemokines

F.62. Is Depression a Consequence of Immunological Changes among Heavy Users of Alcohol?

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Introduction: High rates of comorbidity exist between alcohol use disorder (AUD) and major depression (MD). Emerging data during the recent years suggest depression in AUD to be mediated by dysregulated innate immune response- often represented by overzealous cytokine production, impaired neurogenesis, neurodegeneration, and oxidative damage in the periphery and the CNS. This study examines the longitudinal relationships between such immune responses and depression in patients with AUD. We will test whether the precipitation and progression of major depression in AUD is mediated by neuroimmune dysregulation.

Methodology: We carry out two parallel prospective cohort studies, one each in Norway and Nepal among 150 inpatients with AUD and 50 healthy controls. Baseline interview and blood sample collection will be done with follow up after 6 weeks, 3 months and 6 months. Assessments will include sociodemographics, psychiatric interview including substance screening, mood symptoms and fatigue, physical activity, sleep, medical history and medication. Neurobiological assessments will include cytokines, liver function tests, CRP, nutritional biomarkers, neurotrophins, TLR-4, NF-κB, neopterin, cortisol, tryptophan metabolism, immune complexes (eg HMGB1/IL-1B), miRNA let-7b, microvesicles, and gut microbiota (faecal specimen). The neuroimmune changes over time will be observed and possible associations between changes in immune function and the occurrence of MD will be identified.

Results: This study will establish neuroimmune correlates of depression in AUD individuals.

Discussion: Co-morbid MD in patients with AUD may be a result of dysregulated neuroimmune functions. Such a finding will have substantial translational value. This will be a pioneering neuroimmunology research on different human populations with AUD.

F.69. Progression from NAFLD to NASH: Miscommunication Between the Intestinal Microbiota and the CD4 T-Cells as a Potential Cause

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The reason why only a fraction of patients suffering from Non Alcoholic Fatty Liver Disease (NAFLD) progress to Non-Alcoholic Steatohepatitis (NASH) is not clear. Studies on mouse models suggest a role of the intestinal microbiota in this

progression. On this basis, we hypothesise that dysbiotic intestinal microbiota promotes the capacity of intestine-derived CD4 T cells to co-produce multiple cytokines; a cellular phenomenon described as plasticity. In the liver these cells orchestrate a pathogenic immune response driving the progression from NAFLD to NASH.

To test our hypothesis, we isolated immune cells obtained from the human liver and intestine of both NAFLD and NASH patients. CD4 T cells were analysed by multi parameter flow cytometry and visualized thorough t-SNE according to their cytokine production. Of note, the CD4 T cells infiltrating the liver of patient with NAFLD or NASH are characterized by a high grade of plasticity, for example by co-expressing the signature cytokines of TH1 and TH17 cells.

To fully test our hypothesis, we set up an intestinal microbiota-driven mouse model of NASH and tested the role of these multi-cytokine producing CD4 T cells using a loss of function and gain of function approach.

In short we observed that CD4 T cells are plastic in the liver of patients and a distinct cytokine profile appears to distinguish between NAFLD and NASH patients. We aim to integrate the CD4 T cell atlas data, 16S rDNA sequencing and clinical parameters of patients in order to develop a new screening system for NASH patients.

F.117. Epigenetic Editing of FOXP3 in Human T Cells Induces Overexpression and is Sufficient to Create a Regulatory T Cell Phenotype *In Vitro*.

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Background: Regulatory T-cells (T-reg) are important suppressors T-cell activation, and deficiencies are found in many autoimmune diseases.

Hypomethylation of regions of the FOXP3 gene, particularly the TSDR region, have been associated with FOXP3 expression, but it is unknown whether demethylation of this region alone is sufficient to induce both overexpression and induce a regulatory phenotype.

Methods: Jurkat cells were electroporated with a nuclease-defective dCas9-GCN4, scFv-GFP-TET1, and guide RNA (gRNA) sequences targeting the FOXP3 promoter, TGF-beta sensor (CNS1), and TSDR (CNS2) in a Suntag arrangement. 3 days after transfection, FOXP3 expression was quantified by RT-PCR. DNA methylation of the TSDR region was quantified by pyrosequencing. Epigenetically edited cells were then mixed at a 1:1 ratio with cell-trace-dye-pulsed primary human CD4⁺CD25⁻ cells and incubated with anti-CD4/anti-CD28 beads and 30U/mL rIL-2. Cell proliferation indices were calculated based 4 days after mixing.

Results: Epigenetic editing of all three FOXP3 regions induced overexpression, with the TSDR region being the most efficient (FOXP3 vs. GAPDH relative units: vehicle: 0.7 ± 0.2 , TSDR-targeted: 230 ± 10 , $p < 1E-4$, promoter targeted: 120 ± 20 , $p = 6E-5$, CNS1-targeted: 59 ± 10 , $p = 1E-3$). An average 30% hypomethylation was found in 9/11 CpG sites within the TSDR ($p = 9E-5$). TSDR-epigenetically-edited cells suppressed expansion of primary effector T cells (20% avg. suppression, $p = 0.008$).

Conclusions: Epigenetic editing of FOXP3 induces both overexpression and a regulatory T cell phenotype. Our data are exciting but need confirmation, particularly to clarify the persistence of induced DNA methylation changes and resistance to phenotype switching. If confirmed, this approach has the potential to significantly improve upon current methods for Treg generation.

T.13. Toxoplasma Gondii Induces IL-1 β Production and Pyroptosis Independent Release from Primary Human Monocytes Through the Syk Signaling Pathway

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Monocytes contribute to host defense against *Toxoplasma gondii* infection by initiating a robust inflammatory response mediated by IL-1 β release. However, the mechanisms by which IL-1 β is produced by and released from *T. gondii*-infected monocytes are only partially defined. Previously our lab has shown that *T. gondii*-infected monocytes induce activation of the NLRP3 inflammasome, which processes IL-1 β for release from the cell. We now observe that *T. gondii* infection induces spleen tyrosine kinase (Syk) phosphorylation in primary human monocytes, and the inhibition of Syk reduces IL-1 β maturation and release from infected monocytes. Monocyte mRNA analysis demonstrated that Syk inhibitors decreased parasite-induced *IL-1 β* and *NLRP3* transcripts, suggesting that Syk functions upstream of NF- κ B-dependent transcript production in human monocytes. IL-1 β is thought to be released primarily through an inflammatory form of cell death called pyroptosis, which is driven by caspase-1 activation. However, cell viability assays indicate that *T. gondii* induction of IL-1 β release from infected monocytes was not associated with cell death. Moreover, extracellular glycine, a pyroptosis inhibitor, did not reduce IL-1 β release from infected monocytes, and treatment with a caspase-1 inhibitor reduced *T. gondii*-induced IL-1 β release without affecting cell death. Taken together, our data indicate that *T. gondii* induces a Syk-NLRP3-caspase-1 pathway of inflammasome activation and IL-1 β release in primary human monocytes, which does not involve pyroptosis. This research expands our knowledge of how innate immune cells regulate inflammation during parasite infection and reveals a role for caspase-1 activity, in the absence of pyroptosis, in the release of IL-1 β from viable monocytes.

T.70. ILC2s and Type 2 Immunity Influences Hair Follicle Stem Cell Proliferation and Skin Homeostasis

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Type 2 immunity is a major driver of inflammatory allergic skin disease. However, the fundamental role of type 2 immunity in the context of skin homeostasis and its influence on skin tissue function is not well understood. Here we show that type 2 innate lymphoid cells (ILC2s) are the predominant skin tissue-resident immune cells that secrete type 2 cytokines in homeostasis. ILC2s in the skin have a distinct subset of activating signals when compared to tissue resident ILC2s from other tissues. The activation of ILC2s and secretion of IL-13 is coupled to the stage of the hair follicle cycle. Deletion of ILC2s or the type 2 immunity signaling axis leads to increased proliferation of the hair follicle stem cells in a model of depilation-induced hair growth. Repeat depilation of mice deficient of type 2 immunity leads to stem cell senescence as evidenced by elevated p16. Our work identifies a previously uncharacterized function for type 2 immunity in homeostasis, and highlights the crosstalk between ILC2s and type 2 immunity with hair follicle stem cells.

T.118. Quantitative Bead-Based Multiplex Immunoassays for Human Bone Metabolism

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Bone metabolism is a complex equilibrium between bone formation and resorption that is maintained by specialized cells called osteoblasts and osteoclasts, respectively. This equilibrium is orchestrated by numerous factors including hormones, steroids, growth factors, and cytokines in a constant and dynamic manner throughout life. Dysregulation of this equilibrium can result in diseases such as osteoporosis, a serious public health concern in aging populations.

We have developed a multiplex panel, using fluorescence-encoded beads, which is suitable for use on commonly available flow cytometers. The panel simultaneously quantifies 12 human proteins, including Osteoprotegerin (OPG), Osteopontin (OPN), Alkaline Phosphatase Liver/Bone/Kidney (ALPL), Acid Phosphatase 5 Tartrate Resistant (ACP5), Leptin, RANKL (TRANCE), Tumor Necrosis Factor Alpha (TNF- α), Interleukin 6 (IL-6), Parathyroid Hormone (PTH), IL-1 β , Bone Morphogenetic Protein 2 (BMP-2), and Dickkopf WNT Signaling Pathway Inhibitor 1 (DKK-1). The assay was validated for specificity, dilution linearity, cross-reactivity, and inter and intra-assay precision. Additionally, serum samples from either osteoporosis or rheumatoid arthritis patients were tested using this assay. The expression profiles of each condition were different compared to those of healthy donors.

This multiplex panel is a robust tool for measuring the concentration of the 12 soluble mediators of bone metabolism and related diseases in human serum, plasma, and cell culture supernatant samples, and offers greater efficiency and broader dynamic ranges than conventional ELISA assays.

W.27. Investigating the Expression, Function and Regulation of IL-17A and IL-17F Expressing CD4⁺ T Cells in Human Health and Inflammatory Disease

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IL-17A is a well-characterised pro-inflammatory cytokine, which has been implicated in the immunopathology of inflammatory arthritis. IL-17F is less well-studied. We investigated the phenotype of IL-17F⁺ CD4⁺ T cells, what drives IL-17F expression in CD4⁺ T cells and how IL-17F may contribute to inflammation.

Healthy donor CD4⁺ T cells were cultured with IL-1 β , IL-23, anti-CD3 mAb and anti-CD28 mAb, and cytokine expression was analysed by flow cytometry. In comparison to IL-17A⁺ IL-17F⁻ CD4⁺ T cells, IL-17F⁺IL-17A⁻ and IL-17A⁺IL-17F⁺ CD4⁺ T cells contained lower proportions of cells co-expressing IL-10 (6 $\pm$ 0.8% vs. 0.4 $\pm$ 0.1% and 2 $\pm$ 0.4%, respectively) and higher proportions of cells co-expressing IFN γ (34 $\pm$ 2% vs. 52 $\pm$ 4.0% and 50 $\pm$ 3%). Titration of anti-CD28 mAb into healthy donor CD4⁺ T cell cultures revealed that strong co-stimulation increased the frequency of IL-17F⁺IL-17A⁻ and IL-17A⁺IL-17F⁺ CD4⁺ T cells, whereas IL-17A⁺IL-17F⁻ CD4⁺ T cell frequencies decreased.

Addition of human recombinant IL-17A, IL-17F and TNF α to synovial fibroblasts from patients with psoriatic arthritis (PsA) and rheumatoid arthritis (RA) resulted in significant production of IL-6. Combined blockade of IL-17A and IL-17F reduced IL-6 production to a larger extent than blockade of IL-17A alone, indicating IL-17F can contribute to inflammation.

These data indicate that IL-17A and IL-17F are differentially regulated and that strong T cell stimulation induces IL-17F which can contribute to inflammation. Our findings also suggest that IL-17A⁺IL-17F⁻, IL-17F⁺IL-17A⁻ and IL-17A⁺ IL-17F⁺ CD4⁺ T cells are functionally distinct. We are currently further characterising IL-17A/IL-17F-expressing CD4⁺ T cells via CyTOF.

Supported by a BBSRC CASE studentship with UCB.

W.30. Preparation and Experimental Evaluation of Sargassum BINDERI - "Phyto Nanovesicles" a Prospective Immunomodulatory Formulation Against STZ Induced Diabetic Wound in Wistar Rats

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Wound healing is a complex process overlapping with various phases such as inflammation, epithelialization, angiogenesis and matrix deposition. The concept of curing wounds in diabetes is highly challenging. Present treatment for severe wounds in the diabetic patient is to clean and remove infected tissue by surgical procedure and keep the wound area with adequate blood supply and moisture. Cytokines play key a role in the development of granulation tissue through recruitment of inflammatory leukocytes and stimulation of fibroblasts and epithelial cells. The discovery of novel drug moiety which has an antibacterial and immunomodulatory effect has a significant role in the modern therapeutic

system of medicine to combat bacterial colonization in the diabetic wound. Seaweeds have been proved as a potential source of bioactive molecules which is substantial in developing novel pharmaceuticals. In this study, oleic acid vesicles were prepared by entrapping petroleum ether extract of *Sargassum binderi* using film hydration technique. The purpose of this research work is to understand the efficacy of phyto nanovesicles to heal the diabetic wound. Therefore, the present study has been focused on establishing the pro-inflammatory cytokine levels as predominant markers in diabetes mellitus. The *in vivo* study has shown an excellent immunomodulatory effect on cytokine network after induction of diabetic wound using streptozocin (STZ) in Wistar rats. Thus, the study demonstrates phyto – oleic acid nanovesicle preparation as a better therapeutic agent for topical application to heal the diabetic wound.

W.100. Targeting IL-17-producing T cells Attenuates the Severity of *Pseudomonas Aeruginosa* Lung Infection

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Recent studies suggest that IL-17-producing T cells may play a key role in the response to *Pseudomonas aeruginosa* infection and their suppression may provide clinical benefit for chronic respiratory diseases. Recently, we demonstrated that IL-17A levels and IL-17-producing T cells were enriched in a mouse model of long-term lung colonization. In this context, selective bromodomain and extra-terminal domain (BET) inhibition targeting IL-17-producing T cells during the development of chronic *P. aeruginosa* colonization may reduce exaggerated inflammation and may limit exacerbations of bacterial infection. Taking into consideration these results, we are exploring the therapeutic potential of CPI203, a selective small molecule inhibitor of the BET family of proteins. CPI203 activity was evaluated at early (two days) and advanced (two weeks) stage chronic *P. aeruginosa* infection. Daily treatment with CPI203 in mice decreased incidence of chronic colonization, indicating the role of BET bromodomains in mediating host resistance to *P. aeruginosa*. In addition, CPI203 treatment reduced levels of pro-inflammatory cytokines/chemokines and infiltrating leukocytes (CD45⁺), including neutrophils (CD11B⁺, GR-1^{hi}), thus ameliorating host immunopathology, without increasing the bacterial burden. We also found out significantly reduction of CD45⁺ RORγt⁺ cells at early time point. Moreover, a detailed characterization of the RORγt⁺ T cells subsets modulated by CPI203 treatment by FACS analysis is ongoing. Overall, our results support the further evaluation of BET bromodomain inhibition as a point of potential therapeutic intervention to reduce harmful inflammatory response without compromising the host defense during chronic respiratory diseases mediated by *P. aeruginosa* infections.

W.109. An Aberrant SPDEF-CCL2 Molecular Mechanism Promotes Metastasis of Prostate Cancer

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Since castration-resistant prostate cancer (CRPC) in part still relies on functions of the androgen receptor (AR), novel therapeutics inhibiting either steroid metabolic enzymes or the AR have improved the survival rates in CRPC patients. However, none of those are curative, and resistance eventually develops. The inflammatory response is involved in many aspects of malignant progression, however, it is still not clear how ADT promotes the inflammatory response of prostate cancer. We identified CCL2 as a key inflammatory response that is negatively regulated by AR-SPDEF signaling in prostate cancer. Following downregulation using small interfering RNA approach and CCL2 inhibitor, CCL2 was shown to be essential in metastasis and inflammatory response of prostate cancer cells after androgen withdraw. In prostate cancer cell lines, CCL2 are suppressed by AR signaling partly through a SPDEF-mediated function. SPDEF is an androgen-inducible factor and a member of the ETS transcription factor family. We found that SPDEF transcriptionally represses CCL2 and that ADT-treated patients have reduced SPDEF and increased CCL2. Our findings suggest that an aberrant SPDEF-CCL2-mediated molecular mechanism is proposed to explain the inflammatory progression following

androgen/AR-targeted therapies.

Diabetes and other autoimmune endocrine diseases

F.2. Aire-dependent Thymic FoxP3⁺ Regulatory T Cells in Type 1 Diabetes

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Aire expression within specialized mTECs has been found to be important for enforcing negative selection of T cells, however, mounting evidence has demonstrated the importance of Aire in Treg differentiation and development. Autoimmune diseases like T1D arise from the failure to restrain immune responses against self. Utilizing a novel mouse model, where the Aire locus drives the selected expression of the high affinity DT receptor, revealed a significant reduction in the number of FoxP3⁺ Tregs within the thymus following treatment. Thus, we hypothesize that alterations in the thymic self-antigen expression and presentation via ablation of Aire-expressing mTECs, will affect the repertoire and function of Aire-dependent FoxP3⁺ Tregs. New high-throughput platforms and single-cell DNA barcoding technology have allowed us to assess several thousand individual Tregs that have undergone Aire-dependent selection in the thymus. The identification of these unique clones and gene signatures will be further interrogated retrogenically, allowing for qualitative assessment of their function within the model of T1D. The antigen specificities of the Aire-dependent TCRs will be assessed via screening against peptide libraries using MHCII molecule strongly associated with NOD mouse T1D. We anticipate identification of novel sequences that are recognized by Tregs, which may lead to discovery of new self-peptides that contribute to T1D. Our proposed study aims to expand our knowledge on TCR repertoire of Aire-dependent FoxP3⁺ Tregs and the antigen-specificity in T1D, improving our understanding of the respective function of thymic-derived antigen-specific Treg suppression in peripheral tissues associated with autoimmune T1D.

F.34. Peripheral Blood Gene Expression Profiling to Identify Biomarkers of Disease Susceptibility in Type 1 Diabetes

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Type 1 diabetes (T1D) results from the gradual autoimmune destruction of pancreatic beta cells in genetically susceptible individuals. The etiology of this disease is not well understood. While serum auto-antibodies (AAs) are currently the best predictors of disease progression, only 15% of single AA⁺ individuals progress to T1D within a 10 year period and ~85% of T1D patients present without a family history of T1D. In this study, we extracted RNA and performed gene expression analysis by RNA sequencing of whole blood samples of AA- first degree relatives of T1D patients (FDRs) and non-T1D related controls to identify candidate gene expression biomarkers of disease risk. Non-T1D related control samples were collected at Stanford, while AA- FDRs were obtained from the TrialNet sample repository and collected at the TrialNet site at Stanford University (Pathway to Prevention Study). Our preliminary data demonstrate that a subset of AA- FDRs express high levels of interferon-stimulated genes, possibly indicating a higher risk to seroconvert. LASSO (Least Absolute Shrinkage and Selection Operator) analysis was performed to identify candidate genes that may be used to separate AA- FDRs from healthy controls. This analysis identified <20 genes whose expression could be used to distinguish the majority of AA- FDRs from controls. These findings suggest that a panel of genes could serve as

candidate biomarkers of disease risk, and if validated, would allow us to identify individuals who are at risk to develop T1D, early during the disease process before the appearance of a serum autoantibody.

F.47. A Novel Mendelian Model of Autoimmune Diabetes

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Germline gain-of-function (GOF) mutations in signal transducer and activator of transcription 3 (STAT3) are associated with multiorgan autoimmunity including neonatal type 1 diabetes. To investigate the role of STAT3 hyperactivity on type 1 diabetes, we engineered a knock-in (KI) mouse model incorporating the highly diabetogenic K392R mutation on the STAT3 gene, in the non-obese diabetic (NOD) mouse strain. Mice heterozygous for K392R STAT3-KI on the NOD background developed diabetes significantly earlier than their STAT3-WT siblings supporting STAT3 GOF as the cause for early diabetes onset. Insulinitis present in STAT3 GOF mice supported an autoimmune etiology of diabetes rather than a developmental beta cell defect. Additionally, male mice developed diabetes with similar incidence to female mice, a result not seen in classical NOD populations. Immunophenotyping revealed significant increases in memory CD4⁺ and CD8⁺ T cells, in addition to Tregs. Furthermore, Th1 and TFh populations were significantly increased in mice heterozygous for STAT3 GOF suggesting enhanced T cell activation and/or survival as a cause for earlier autoimmune disease. Together, these results show STAT3 GOF in a genetically susceptible background represents a new Mendelian model for autoimmune diabetes and helps define the critical role of STAT3 in the development of T1D.

F.54. Hyaluronan Levels are Increased in Serum and Muscle of Human Subjects with Type 2 but not Type 1 Diabetes Independently of Glycemic Control

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Aims/hypothesis: Hyaluronan (HA), an extracellular matrix polysaccharide, is implicated in the pathogenesis of both type 1 diabetes mellitus (T1DM) as well as type 2 diabetes mellitus (T2DM) through effects on local immune dysregulation and insulin resistance, respectively. Here, we have examined the tissue distribution of HA in human subjects with T1DM and T2DM and in mouse models of these diseases.

Methods: Serum was collected from T1DM subjects and T2DM subjects matched for age, gender, and hemoglobin-A1c, and from non-diabetic controls (n = 20 for each group). Muscle was collected from discarded surgical specimens. Serum and muscle were likewise collected from age- and gender-matched DORMO mice (a model of T1DM), dbdb mice (a model of T2DM), and non-diabetic BalbC and C57Bl6 mice (controls).

Results: Serum HA is increased in T2DM but not T1DM. Serum HA concentrations are independent of donor hemoglobin-A1c, C-peptide, body mass index, or time since diabetes diagnosis. HA is likewise increased in skeletal muscle in T2DM subjects relative to non-diabetic controls. Similar increases in serum and muscle HA are seen in dbdb mice relative to C57Bl6 controls, but not in DORMO mice relative to BalbC controls.

Conclusions/interpretation: These data indicate that HA content is increased in multiple tissue compartments in T2DM but not T1DM, independently of insulin levels or glycemic control. Serum HA may have value as a biomarker of systemic inflammation in T2DM.

F.63. Circulating CXCR5-PD1hi Peripheral T Helper Cells are Increased in Children with T1D and in Autoantibody-Positive Children At-risk for T1D

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A novel subset of T cells, CD4⁺CD45RA⁺CXCR5⁺PD1^{hi} peripheral helper T cells (Tph) capable of promoting B cell responses and antibody production in inflamed tissues, has recently been observed to be associated with rheumatoid arthritis. It is currently unclear, however, whether Tph cells play a role in the development of type 1 diabetes (T1D).

We first analyzed the phenotype and function of circulating human CXCR5⁺PD1^{hi} Tph cells by flow cytometry. We confirm that circulating Tph cells share several features with CXCR5⁺PD1^{hi} circulating T follicular helper (Tfh) cells, such as the high expression of ICOS and IL-21. However, they display a higher expression of chemokine receptors associated with trafficking to inflamed tissues, such as CCR2, CX3CR1 and CCR5, and produce more IFN- γ than Tfh cells. Tph cells are also able *in vitro* to activate memory B cells into antibody-secreting plasma cells as efficiently as Tfh cells. Next, we compared the frequency of Tph cells in blood samples from children with newly-diagnosed T1D (n=42) or in autoantibody-positive children at-risk for T1D (n=39) to that in healthy age- and HLA-matched children (n=81) in a pairwise manner. The frequency of circulating Tph cells was increased in children with newly-diagnosed T1D but also in autoantibody-positive children at-risk for the disease when compared to healthy children.

Our results suggest that Tph cells may play a role in the development of T1D and could have potential both as a biomarker of disease progression and as a target for immunotherapy.

F.67. Follicular Regulatory T Cells Expand in the Peripheral Blood of Patients with Type 1 Diabetes and Express Reduced Levels Of PD-1 in the Pancreatic Lymph Nodes

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Follicular helper (Tfh) and follicular regulatory (Tfr) T cells are involved in islet-specific autoantibody (AAb) development and the onset of type 1 diabetes (T1D). Hence, we investigated their frequency in the peripheral blood, spleen and pancreatic lymph nodes (PLN) from diabetic patients and subjects with risk for developing T1D. Pediatric patients with new onset T1D had higher percentage of blood Tfh2, activated Tfh and highly functional Tfh as compared to healthy controls. Adult diabetic patients presented higher frequency of activated Tfh in the blood but also in the spleen and PLN. Diabetic children and adult subjects with high risk for developing T1D, i.e. those with multiple AABs, exhibited higher frequency of blood Tfr compared to low risk individuals, i.e. AAb- and single AAb⁺ donors. Although Tfr frequency in the spleen and PLN did not change according the disease status, the expression of PD-1 on Tfr was reduced in diabetic patients as compared to healthy controls. Together, our findings suggest a role for Tfh and Tfr cells in the pathogenesis of human islet-specific AAB development and T1D and imply that early targeting of these cells could have important therapeutic implications.

F.71. KLRG1⁺TIGIT⁺ CD8 T Cells are a Hyporesponsive Cell Type Varying in Quantity across Individuals and Disease Conditions

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T cell exhaustion is deleterious in cancer but beneficial in autoimmunity. We previously identified a partially exhausted CD8 T cell subtype: KLRG1⁺TIGIT⁺ (double positive, DP) cells with an EOMES-associated transcriptional signature which expanded in type 1 diabetes (T1D) responders to anti-CD3 therapy. Here, we further define the phenotype, function, and quantity of DP cells across disease settings. DP cells were enriched in chronic viral (CMV and EBV) antigen-specific T cells but were less frequent in compared to acutely activated influenza-specific CD8 T cells. DP cells expressed higher levels of Ki67 *ex vivo*, relative to double negative memory cells, indicative of basal *in vivo* proliferation. Yet, DP cells failed to proliferate upon re-stimulation *in vitro* suggesting a hyporesponsive state. Thus, to better characterize hyporesponsiveness of DP cells, we developed an *in vitro* assay. DP cells labelled and mixed-back into total PBMC were stable for 14 days following stimulation with anti-CD3 and retained co-expression of PD-1 and EOMES along with low CD127 and cytokines as determined by FlowSOM clustering. Thus, DP cells are a stable cell type with many features of exhaustion. In peripheral blood of renal cell carcinoma patients, DP cells that co-express CD160 and lack CD127 were enriched as compared to age-matched controls. However, when comparing T1D subjects to age- and gender-matched healthy controls, we found no differences. Future experiments will address whether the frequency of DP cells associates with delayed progression in T1D. In addition, determining factors that drive expansion of DP cells may reveal novel tolerogenic therapeutic targets.

F.84. Hypo-responsiveness is the Dominant Phenotype Of Memory T Cells Following Alefacept Treatment of New Onset T1D.

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In the ITN T1DAL trial, Alefacept (an LFA3-Fc fusion protein targeting CD2) treatment preserved endogenous insulin production in 30% of recent onset T1D patients, a year following therapy cessation. Longitudinal flow cytometric analysis was performed to elucidate Alefacept's mechanism of action on CD2-expressing lymphocytes in blood. Treated subjects experienced marked depletion of CD2hi CD4 and CD8 memory T cells and CD56hi NK cells, along with preservation of CD2lo Tregs. Further investigation revealed that spared non-Tregs display phenotypes associated with hypo-responsiveness. CD127loCD8 effector memory CD45RA⁺ T cells persisted in complete responders after treatment cessation although these are not CD2lo. In CD4 non-Tregs, an increase of TIGIT⁺ and PD-1⁺TIGIT⁺CD4 effector memory T cells occurred following treatment. In addition to lacking KLRG1⁺ expression, functional assays indicate that PD-1⁺TIGIT⁺CD4 T cells secrete less inflammatory cytokines than KLRG1⁺ CD4 T cells. In vitro cultures with Alefacept replicated CD2hi lymphocyte depletion and an increase of PD-1⁺TIGIT⁺ in CD2lo CD4 effector memory T cells indicated that alefacept may function in part through selective expansion of CD2loPD-1⁺TIGIT⁺CD4 T cells. T cell receptor repertoire and proliferation assays focused on distinguishing whether Alefacept selectively induces or spares these subsets are ongoing, along with CyTOF studies to determine whether CD127loCD8 effector memory CD45RA T cells display features of exhaustion. Collectively, our data suggest that through differential effects on CD4 and CD8 T cells,

Alefacept not only depletes pathogenic T cells, but also promotes immune tolerance by sparing CD4 Tregs and supporting hypo-responsive subsets well over 1 year after therapy cessation.

F.113. Reversal of Autoimmune Diabetes and Restoration of Self-Tolerance via a Cell-Based Therapy in NOD Mouse Model

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Type 1 diabetes mellitus (T1D) is a T cell-mediated autoimmune disorder of pancreatic β cells. Complete alleviation of T1D necessitates both reestablishment of self-tolerance and regeneration of β cells. Tryptophan catabolism plays a crucial role in self-tolerance. Indoleamine, 2,3 deoxygenase (IDO) is the major regulator of tryptophan catabolism within the immune system. Here, we report that reinforcing IDO activity in non-obese diabetic (NOD) mice through a cell-based therapy results in restoration of immune tolerance toward β cells with subsequent reversal of hyperglycemia.

To this end, genetically modified IDO-expressing fibroblasts were injected to NOD mice at commencement of hyperglycemia. Animals were then monitored for hyperglycemia and immune markers.

Hyperglycemia was reversed in 80% of NOD mice received IDO-expressing cells while all control animals remained diabetic. Histological examination revealed clearance of insulinitis and functional islets in IDO treated group. Splenic CD4⁺CD25⁺FOXP3⁺ Tregs were increased from $4.68\% \pm 1.01\%$ at the onset of diabetes to $16.04\% \pm 1.14\%$ ($p < 0.001$) following IDO cell therapy while decreased to $3.94\% \pm 1.00\%$ in the control group. The same pattern was found in the pancreatic lymph node Tregs. On the other hand, β cell specific autoreactive CD8⁺ T cells -detected by NRP-V7 MHC I tetramer binding- were significantly decreased in both spleen and pancreatic lymph nodes of IDO treated mice compared to those of control mice.

In conclusion, we showed that enhancement of IDO activity in NOD mice efficiently reinstated self tolerance and treated hyperglycemia. This promising finding opens new insights into development of a cure for T1D.

F.114. Major Impact of 'Aging' of Adaptive Immunity on High-Fat Diet-Induced Insulin Resistance and Nonalcoholic Steatohepatitis

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Despite the knowledge about the association between obesity, chronic low-grade inflammation and insulin resistance, many murine models failed to translate promising treatments to clinical studies, as relevant aspects of the human immune system are unappreciated by specific pathogen-free (SPF) mice. SPF mice express a immune system like young children with low if any number of effector/memory T cells. Recently, we could show that mice housed in antigen-exposed (AE) conditions express higher frequencies of effector/memory T cells associated with poorer bone fracture healing, as seen also in patients with higher number of effector/memory T cells (Reinke et al Sci Transl Med 2013). Here, we compared diet-induced obesity in SPF mice with those housed in antigen-exposed (AE) conditions and hypothesised faster progression of T2 diabetes. In fact, AE conditions induced significant rise in memory/effector T cells. Surprisingly, AE mice fed a high fat western diet (HFD) maintained increased insulin levels to compensate for insulin resistance, which was reflected in islet hyperplasia and improved glucose tolerance compared to SPF mice. In contrast,

we observed higher proportions of memory/effector T cell subsets in blood and liver of AE HFD mice accompanied by the development of nonalcoholic steatohepatitis-like liver pathology (NASH). Thus, our data demonstrate the impact of immune aging on metabolic alterations and suggest that using AE mice could provide a tool for investigating therapeutic targets for future immune-based interventions for type 2 diabetes patients. It also show that our preclinical models have to be adapted to be closer to clinical challenges.

T.19. A Single Dose of Siltuximab can Transiently Modify T-effector Resistance to Suppression in T1D

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IL6 signaling is implicated in the pathogenesis of multiple autoimmune diseases, including T1D; STAT1/STAT3 responses to IL6 are increased in T1D subjects. Here, we tested whether a single dose of anti-IL6 (siltuximab) could modify downstream pSTAT signaling, or modulate other immune-mediated readouts. We enrolled 10 participants with T1D (disease duration 0.4-7.9 years) in an open-label mechanistic endpoint study. Peak drug concentration varied 1.7-fold between lowest and highest peak concentration (194-327 ug/mL). No change was detected in the primary endpoint (IL6-stimulated pSTAT3 at week 12). 9/10 subjects experienced reduced neutrophil counts at 28 days post-infusion. In 2/10 subjects this decline was clinically significant. Phosphoflow, intracellular cytokine staining, and cell surface immunophenotyping was performed at each visit (n=468 immune parameters). These data were filtered to identify parameters that were stable between preinfusion timepoints, and then further filtered to find parameters showing consistent, directional change after siltuximab. Two-way hierarchical clustering of these filtered parameters (n=102) grouped cell populations by expression patterns, and clustered subjects by peak drug concentration. We also examined whether T effector resistance to Treg suppression could be modified by siltuximab. At 2 weeks after infusion, resistance of effector T cells to suppression by Tregs was reversed in 9/10 subjects. Suppression correlated with peak drug concentration. Improved Treg suppression was sustained to 12 weeks post-infusion in 5/9 subjects. In summary, siltuximab modified T effector resistance to suppression, and lowered neutrophil count, in the majority of T1D subjects.

T.28. Immune Checkpoint Inhibitor Diabetes Mellitus: An Atypical Presentation of Type 1 Diabetes Mellitus?

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Background: Immune checkpoint inhibitors (ICI) have revolutionized cancer care but cause immune-related adverse events (irAEs). One irAE is acute insulinopenic diabetes mellitus (DM). While thought to be autoimmune pancreatic beta cell destruction consistent with type 1 DM (T1DM), there is little research comparing the pathophysiology of “conventional” and “ICI-induced” DM.

Results: Between UCSF and Yale, 24 patients have ICI-DM. Eleven were treated with anti-PD1 alone, 1 with anti-PDL1 alone, and 12 with combination anti-PD1/anti-CTLA-4. Over 95% of subjects had no significant hyperglycemia until within 14 days of ICI-DM diagnosis. Thirteen were in DKA; the average A1c was 7.7. Of 22 subjects tested for auto-antibodies by clinical or research labs, 36.4% had at least one positive auto-antibody. Anti-GAD65 positivity was most common at 31.8%; the other T1DM autoantibodies were positive in 7 to 20%. Of 16 subjects who underwent HLA typing, 81.3% were positive for HLA-DR4, 18.2% were positive for HLA-DR3, and none were DR3/DR4 heterozygotes. Among melanoma subjects, 85% of those on only anti-PD1 at ICI-DM diagnosis had an objective response compared to 33% of

those on combined anti-PD1/anti-CTLA-4 ($p=0.053$) (reported objective response rate for nivolumab alone is 43.7% and for combined Ipilumab/Nivolumab is 57.6% (Larkin 2015)).

Conclusion: Anti-PD-1/PD-L1 therapy (but to date not anti-CTLA-4) leads to ICI-DM in a subset of patients. Clinical features including rapid progression of hyperglycemia and low rates of auto-antibodies are in contrast to T1DM. The high prevalence of HLA-DR4 is notable but the lack of DR3/DR4 heterozygosity is atypical for T1DM patients.

T.30. Discovery of a Novel Human Anti-IL-2 Antibody that Potentiates Regulatory T Cells by a Structure-Based Mechanism

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Interleukin-2 (IL-2) has been shown to suppress immune pathologies by preferentially expanding regulatory T cells (Treg). However, this therapy has been limited by off-target complications due to pathogenic cell expansion. Recent efforts have been focused on developing a more selective IL-2. It is well documented that certain anti-mouse IL-2 antibodies induce conformational changes that result in selective targeting of Treg cells. We report the generation of the first fully human anti-IL-2 antibody, F5111.2 that stabilizes IL-2 in a conformation that results in the preferential STAT5 phosphorylation of Tregs *in vitro* and selective expansion of Tregs *in vivo*. When complexed with human IL-2, F5111.2 induced remission of type 1 diabetes in the NOD mouse model, reduced disease severity in a model of experimental autoimmune encephalitis, and protected mice against xenogeneic Graft-versus-Host-Disease (GvHD). These results suggest that IL-2-F5111.2 may provide a new immunotherapy to treat autoimmune diseases and GvHD.

T.49. Combined Features of T and B Cells Define Distinct Immunotypes of Adult T1D Subjects

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Cellular phenotypes associated with T1D include multiple cell types which together, may offer clues to pathogenesis and guide therapeutic choices. Yet, defining and ranking phenotypes in T1D has been challenging to date. We designed a large ($n=100/\text{group}$), age and gender matched, cross-sectional study of control and T1D subjects including >280 reproducible T- and B-cell parameters using 3 steady-state and 4 cytokine stimulation flow cytometry panels. T-cell activation (HLA-DR, CD25), cytokine (CXCR3, CD126), and early B-cell markers (IgD) along with CD21 on plasmablasts were most prominent in single parameter analyses. Clustering significant parameters across all T1D subjects we defined four distinct cohorts, or 'immunotypes': an IL-6/plasmablast, a Treg/transitional B-cell, a cytokine/T-cell, and a heterogeneous immunotype. These features did not define clusters in healthy controls or aged subjects and were not associated with known genetic, clinical, or demographic parameters. Together, our findings move beyond single parameter analyses of disease, offering a more comprehensive understanding of the immune phenotypes associated with T1D. These combinatorial findings reveal immunologic heterogeneity and cellular association in T1D that may lead to better selection of targeted immune-based treatments for individuals.

T.77. Pro-inflammatory Tissue-Resident Fibroblastic Reticular Cells Promote Destructive Immune Responses in Type 1 Diabetes.

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Fibroblastic reticular cells (FRC) play an important role in maintaining immune homeostasis and orchestrating immune responses by producing chemokines and cytokines that promote immune cell migration, organization, function, and survival in secondary lymphoid organs. However, little is known about their function in the development and maintenance of inflammation in non-lymphoid tissues. We show that FRC-like cells are found in inflamed islets of Langerhans in type 1 diabetes-prone NOD mice. No FRC are found in non-inflamed islets and crucially their number increases with severity of islet infiltration. Islet FRC share many characteristics of lymph node FRC, including CCL19 and IL-7 expression. However, in contrast to lymph node FRC, islet FRC are a major source of IFN γ -induced CXCL9 in inflamed islets, indicating their more proinflammatory phenotype. Additionally, co-culture of islet FRC with activated T cells results in increased expression of proinflammatory molecules including granzymes, perforin, and IFN γ by T cells. Increased expression of granzyme A by islet CD8⁺ T cells is dependent on IL-6 secretion by islet FRC. Finally, co-transplant of islet FRC but not lymph node FRC with healthy islets into diabetic mice accelerates the transplant destruction resulting in renewed hyperglycemia. These data indicate FRC-like cells in inflamed tissue actively contribute to the local inflammatory responses and may act as a viable therapeutic target for type 1 diabetes treatment.

T.83. Modulation of Cytokine Production by Dietary Fatty Acids in T Cells from Type 1 Diabetes-Susceptible NOD Mice

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Type 1 Diabetes (T1D) is an autoimmune disorder in which autoreactive T cell subsets coordinate an attack on pancreatic β -cells causing a loss of insulin production. A genetic component to the disease exists but globally there has been an increased incidence of T1D in individuals with low to moderate risk alleles, pointing to environmental factors impacting disease onset. One specific environmental factor that has been suggested as a risk factor for T1D is dietary intake of polyunsaturated fatty acids (PUFAs). The goal of this project is to understand how polyunsaturated fatty acid levels impact T cells from mouse models with different Type 1 Diabetes genetic susceptibilities. We tested the main hypothesis that cell-intrinsic differences between T cells from diabetes-prone vs -resistant mouse strains determine their response to specific PUFAs. Data obtained show that the polyunsaturated fatty acid linoleic acid increases pathogenic cytokine expression in specific T cell subsets from diabetes prone mice. Gene expression analysis has revealed a decreased expression of fatty acid desaturases in T cells from diabetes-prone mice versus diabetes-resistant mice upon stimulation with linoleic acid. Data obtained demonstrates that specific dietary PUFAs may promote pathogenicity of autoreactive T cells by altering the balance of pro- and anti-inflammatory cytokines. Further studies will provide more insight in the regulation of T cell subsets and T1D pathogenesis by fatty acids present in the organismal environment, with potential implications for the development of new therapeutics to prevent T1D.

W.1. Glucokinase is Recognized as an Autoantigen in Subjects with Type 1 Diabetes

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Type 1 diabetes (T1D) is caused by the destruction of pancreatic beta cells. Autoantibodies and autoreactive T cells that recognize multiple antigens accumulate leading up to disease. During progression, beta cells also exhibit dysfunction, evidenced by altered insulin secretion kinetics. In this work we investigated immune recognition of glucokinase to establish a potential link between beta cell dysfunction and autoimmunity. Glucokinase is a glucose sensor in pancreatic beta cells and catalyzes glucose-stimulated insulin secretion. Through mass spectrometry analysis we observed that glucokinase can be citrullinated at 19 distinct residues. Looking in serum samples from human subjects, anti-citrullinated glucokinase antibodies were present at significantly higher levels in T1D patients vs healthy controls ($p < 0.05$). Likewise, antibody levels were elevated in pre-diabetic NOD mice ($p < 0.005$). Glucokinase antibodies were weakly associated with anti-ZnT8 ($p = 0.057$), but not with other conventional autoantibodies (Insulin, GAD, IA2). Using class II tetramers, we verified that T cell responses to citrullinated glucokinase peptides are detectable in the peripheral blood of subjects with T1D. We then isolated T cell clones and lines specific for a subset of these peptides to facilitate further studies. Glucokinase expressed in beta cells versus liver cells is distinguished by a unique promoter and first exon. However, our findings indicate that citrullinated residues and epitopes primarily occur within the c-terminal domains of the protein. In total our results suggest that monitoring antibody and T cell responses to citrullinated glucokinase could provide important new insights.

W.2. HLA-dependent Association of Systemic Antibody Responses to Intestinal Commensal Bacteria with Islet Autoimmunity in Pediatric Cohorts

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The intestinal microbiota influences numerous aspects of host immunity. Recent advances in microbial community sequencing suggest that changes in microbiota composition are associated with autoimmunity. However, the mechanisms through which the gut microbiota influence autoimmune responses to distal anatomical sites have not been elucidated. To address this knowledge gap we used a flow cytometry-based platform to quantify and isotype systemic antibody responses against human gut commensal species and investigated whether patterns of these anti-commensal antibody (ACAb) responses differed between individuals with and without type 1 diabetes (T1D).

Cultured commensal bacterial strain pools representing defined species were used as targets incubated with serum samples from pediatric TrialNet cohort subjects who were discordant for subsequent progression to diabetes. We investigated whether ACAb response patterns observed prior to T1D diagnosis were associated with later disease development, or with subject HLA genotype. Serum IgG2 antibodies against *Roseburia faecis* and against a 32-strain commensal bacteria consortium, were associated with future T1D diagnosis in an HLA DR3/DR4-dependent manner. Moreover, in TrialNet subjects who later became diabetic we found DR3/DR4-dependent associations between anti-*R.faecis* and anti-*Streptococcus gallolyticus* IgG2 responses and the presence of anti-insulin and insulinoma-2 antigen antibodies respectively.

These results identify an interaction between HLA genotype, immune responses to commensal gut bacteria and the development of islet autoimmunity. Furthermore, our findings define a platform for development of biomarkers to bridge the gap between the intestinal microbiota and host autoimmune responses against a distal target tissue, applicable to understanding this interface in multiple autoimmune diseases.

W.6. Single-cell RNAseq Identifies Expanded Clones and Specific Characteristics of Islet Antigen-Reactive Memory T Cells in Peripheral Blood of T1D

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Islet antigen-reactive CD4 T cells contribute to the development and progression of type 1 diabetes (T1D), but these cells can also be detected in some healthy individuals. To identify unique characteristics and clonality of islet antigen reactive memory CD4 (IARM-CD4) T cells that relate to disease progression and response to therapy, we combined index sorting and single-cell RNA sequencing of islet peptide stimulated cells. We studied healthy control subjects and established T1D from the BRI registry and TrialNet LIFT study, and recent onset T1D subjects treated with Alefacept (LFA-3Fc) from the ITN T1DAL study. We found greater TCR clonotype expansion in IARM-CD4 T cells from subjects with disease than healthy subjects, suggesting T cell clonal expansion during disease progression. Further, expanded TCR clonotypes were stable over time. Among the expanded clonotypes, we identified IGRP specificity for five different expanded TCR clonotypes from four patients, implicating this molecule as an antigenic trigger of IARM-CD4 T cell expansion. Interestingly, transcriptional differences between IARM-CD4 T cells from healthy subjects and T1D were minimal. However, when TCR expansion was introduced as a variable, we identified distinctive profiles from the most expanded clones. Transcriptional and surface expression of expanded IARM-CD4 T cells demonstrated phenotypic heterogeneity amongst subjects. Our findings demonstrate that IARM-CD4 T cells with unique specificities and phenotypes are expanded during disease progression and can be detected by single-cell analysis of peripheral blood. We are currently using this approach to investigate the response of IARM-CD4 T cells to therapy by the biologic agent Alefacept.

W.8. Islet Antigen-Specific CD8 T Cells Within Individual Type 1 Diabetes Subjects Exhibit Multiple Distinct Phenotypes Which Include Features of Both Early Differentiation and Exhaustion

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Islet antigen-specific CD8 T cells are thought to play a role in the development and progression of type 1 diabetes (T1D) as their frequency in the peripheral blood correlates with disease. Yet, substantially less is known about their phenotype. We developed a 35 parameter mass cytometry (CyTOF) panel including markers of differentiation, activation, and exhaustion, as well as pooled metal-conjugated MHC class I tetramers (Tmr) loaded with 5 islet-specific peptides and for comparison, 2 chronic viral peptides. Using recent-onset (n=9) and established T1D (n=11) subjects with a range of clinical features, we quantified the distinct phenotypes of islet-specific CD8 T cells within subjects using a novel analytical approach. Tmr⁺ cells were mapped onto a generalized landscape of total CD8 T cell phenotypes determined through unbiased clustering. Using a chi-square statistic, we determined that islet-specific cells were enriched within multiple (2.9 ± 0.7) of these total CD8 clusters. Often, one or more of the islet-specific clusters were distinct from chronic virus-specific CD8 T cell clusters within the same subject. Analysis of islet-specific CD8 T cell clusters from multiple subjects revealed several shared islet-specific phenotypes across individuals ranging from activated effector memory cells marked by CD45RO, CXCR3 and NKG2D expression to exhausted cells marked by PD1, Tim3, and TIGIT along with unique intermediate phenotypes. Thus, we found that islet-specific CD8 T cells are not homogeneous but instead exhibit several distinct phenotypes. Future studies will quantify variability of phenotypes across disease state and treatment response, ultimately guiding selection of targeted therapies that promote protective phenotypes.

W.16. The Leader Region of Preproinsulin Contains Common Epitopes for Islet-Infiltrating CD8 T-Cells Derived from Organ Donors with Type 1 Diabetes

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The goal of our study is to identify antigenic epitopes that can be used as T-cell biomarkers for type 1 diabetes (T1D). Given evidence that CD8 rather than CD4 T-cells in pancreatic islets share a larger proportion of T-cell receptors (TCRs) with those in peripheral immune organs, antigens targeted by islet-infiltrating CD8 T-cells may be useful biomarkers for T1D. Preproinsulin is an abundant protein specifically expressed by pancreatic beta cells, therefore we hypothesized that preproinsulin peptides are targets for islet-infiltrating CD8 T-cells. We chose TCR alpha and beta pairs identified from 35 CD8 T-cells that were detected on multiple T-cells isolated from the islets of three independent organ donors with recent-onset T1D and HLA-A*02:01 (A2). We generated T-cell transductants expressing these TCRs to test the response to one hundred preproinsulin peptide pools in the presence of antigen-presenting cells exclusively expressing A2. Among 35 CD8 T-cells analyzed we identified a total of six TCRs that responded to peptide pools containing positions 2-15 and 12-25 of preproinsulin. These results suggest that preproinsulin peptides 2-10, 6-14, and 15-24, which are in the leader region of preproinsulin and have been identified as epitopes for CD8 T-cells in the peripheral blood of T1D patients, are targets for islet-infiltrating CD8 T-cells as well. Importantly, the six preproinsulin-reactive TCRs were detected in islets of multiple T1D organ donors. Thus, the leader region of preproinsulin contains common targets for islet-infiltrating CD8 T-cells, and therefore may serve as T-cell biomarkers for T1D.

W.37. PD-1 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. As there is a clear IFN signature in thyroid autoimmune tissue, we have investigated the expression of PD-L1, induced with IFN γ , and its receptor PD-1 in thyroid autoimmune glands.

Cryostat sections of GD, Hashimoto thyroiditis, multinodular goiter 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 γ and stained at 24h-48h to assess HLA-I/II and PD-L1 induction.

In most GD glands PD-L1 was detected at very low levels in thyroid follicular cells (TFCs) confined to follicles close to lymphoid infiltrates, much less extensive than HLA class II. In multinodular goiter glands only traces of PD-L1 staining was visible in TFCs close to infiltrates. Importantly, PD-1 was highly expressed by the infiltrating T cells in GD. PD-L1 expression could be readily induced by IFN- γ in TFC cultures in a time and dose dependent manner, as assessed by FACS and RT-PCR.

Despite of the IFN signature previously detected in autoimmune thyroid tissues, PD-L1 expression is lower than expected, and does not correlate with the high expression of PD-1 seen in infiltrating lymphocytes. Overall these results point out a possible dysregulation in signaling through PD-1/PD-L1 axis in autoimmune thyroiditis.

W.41. Targeting Chronic Inflammation in Ageing and in Type 2 Diabetes Patients Using Metformin

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Background: - Population ageing has become a concern for both developed and developing nations. The number of individuals over the age of 60 was 900 million in 2015 and this number is expected to rise to 2 billion by the year 2050. Ageing is the main risk factor for developing Diabetes and other age-related diseases. One of the most common features of most age-related comorbidities including diabetes is the presence of low-grade chronic inflammation. Not much has been done to identify inflammatory markers of pathological ageing and chronological ageing.

Objective: - Our main objective is to identify major inflammatory biomarkers in ageing and in diabetes patients.

Method: - We used clinical data and biological parameters to subdivide our cohort into five groups, then we measure 102 different cytokines and chemokines and mapped these molecules to different groups

Result:- Diabetes patients show an increased level of sTNFR-II, sICAM-1, and TIMP-1 when compared to Healthy, Non Diabetes, and Prediabetes individuals. These inflammatory molecules are also associated with insulin resistance and metabolic syndrome. By further analysis of different treatments within diabetes patients, we show metformin monotherapy decrease inflammatory molecules especially the TNFa/sTNFR pathway when compared to other monotherapies. Five years follow up data indicate a higher proportion of death occurred in individuals taking other monotherapies compared to metformin monotherapy.

Conclusion:- Increased chronic inflammation is associated with an increase in age and in diabetes patients. Here we use Diabetes patients as a model and showed metformin could decrease chronic inflammation.

W.83. Sustained Delivery of IL-2 Using an Injectable Hydrogel Prevents Autoimmune Diabetes

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IL-2 is a promising therapy for autoimmune type 1 diabetes (T1D), but the short half-life of injected IL-2 (less than 6 minutes) necessitates frequent injections and limits effective tissue exposure to IL-2. We have developed an injectable hydrogel for delivery of sustained release IL-2. This platform integrates clinical grade, commercially available materials, including collagen, hyaluronan, and heparin, that are thiolated and crosslinked into a hydrogel, to deliver IL-2 over time. This hydrogel degrades slowly over a two-week period *in vivo* while releasing IL-2 throughout this time. Moreover, we find that heparin or heparan sulfate binding of IL-2 potentiates IL-2 activity and promotes Foxp3⁺ regulatory T-cell (Treg) expansion *in vitro* in the setting of antigenic signals and TGF-beta. In the Non Obese Diabetic (NOD) mouse model of T1D, 3x/week IL-2 injections at a variety of concentrations failed to prevent autoimmune diabetes while once weekly IL-2 hydrogel delivery prevented disease in 70% of mice at 21 weeks of age. This treatment was associated with increased CD4⁺Foxp3⁺ Treg induction but no increase in CD8⁺ T-cells or activated CD4⁺FoxP3-CD25⁺ T-cells. Together these data suggest that IL-2 hydrogels could be a potent and valuable tool for use in IL-2 delivery protocols.

General autoimmunity

F.26. Elucidating the Function of Mosaic, a Novel Gene Mediating Autoimmunity

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A novel candidate gene, *Mosaic* (Multi-Organ System Autoimmunity in Canines), was identified as the culprit causing an early and severe multiorgan autoimmunity in a unique subset of dogs. Little is known about its function. It is conserved across all known vertebrate species including humans and mice, and it is highly expressed in immune cells based on BioGPS. We hypothesize that the disruption of *Mosaic* function results in a breach in T cell tolerance leading to autoimmunity. To evaluate the function of *Mosaic*, we generated two mouse models. First, a *Mosaic* knockout model was generated using a B6 ES cell line by deleting a 2.5kb region containing the start codon and inserting a reporter cassette. Second, a floxed *Mosaic* mouse model was generated using CRISPR-Cas9 by inserting two LoxP sequences flanking exon 3 which contains the start codon. Using the reporter cassette, *Mosaic* was identified to have the highest expression level in memory T cells and T regulatory cells. Global deletion of *Mosaic* resulted in partial embryonic lethality. However, viable knockout pups showed decreased naïve and increased memory T cells. Analysis of the *Mosaic*^{fl/fl}CD4-Cre mice also showed a reduced naïve and increased memory T cell compartment. Furthermore, *Mosaic*-deficient T cells showed increased proliferation with CD3 or CD3+CD28 stimulation. Together, the data suggest a role for *Mosaic* in T cell function. Further investigation will reveal the molecular mechanism behind *Mosaic*'s role in immune tolerance.

F.57. Different Cell Types Contribute to Unique Components of the Interferon Signature in SLE PBMCs

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Systemic lupus erythematosus (SLE) is a systemic autoimmune disease with loss of tolerance towards nucleic acids. SLE displays a prevalent interferon (IFN) signature, which correlates with disease activity in only a fraction of patients. Little is known about the functional heterogeneity across SLE patient PBMCs at the single cell resolution. Here we report profiling of 117k single cells from 17 pediatric SLE and 5 matched healthy children, resulting in 17 cell clusters. In SLE, three cell populations are expanded including subsets of CD14⁺ monocytes, CTLs and B-cells, while five are decreased including subsets of B-cells, CTLs, NK cells, T cells and pDCs. A cell-type frequency based analysis stratified SLE patients into four main groups. One of these groups includes a subset of CD14⁺ CD83⁺ monocytes expressing both “IL1B”, and IFN-stimulated genes (ISG), suggesting a loss of IL1B and type I IFN cross-regulation. SLE PBMC subsets express ISGs in a cell-specific manner: “IFITM1” is found in both T and NK cells; “IFITM2” is ubiquitous, while “IFITM3” is preferentially expressed in monocytes. Thus, blood single cell RNA-seq analysis has the potential to decode the origin of gene signatures that are associated with complex diseases such as SLE.

F.61. The Role of Thymic Tolerance in the Pathogenesis of the COPA Syndrome

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Our lab recently co-discovered the COPA syndrome, an autoimmune disease characterized by high-titer autoantibodies, inflammatory arthritis and interstitial lung disease. The disease is caused by dominant mutations in the *Coatomer subunit alpha* (COPA) gene, which encodes the alpha subunit of coat protein complex I (COP1). In eukaryotic cells, COP1 complex mediates membrane and protein trafficking from the cis-Golgi complex to the endoplasmic reticulum (ER). Interestingly, the COPA mutations identified so far all occur within the same domain called WD40-a highly conserved domain involved in selecting cargo proteins for retrograde transportation.

To dissect the immunological mechanisms by which mutant COPA leads to a loss of self-tolerance and autoimmunity, we generated a germline point mutant knock-in mouse bearing the exact same E241K mutation as COPA syndrome patients. Our preliminary data demonstrates that *Copa*^{E241K/+} mice spontaneously develop mononuclear organ infiltrates and an examination of peripheral lymphoid tissues reveals a significant increase in effector memory T cells. Detailed study of developing thymocytes shows a significant increase in mature CD8⁺ and CD4⁺ single positive (SP) cell populations, findings suggest alterations in thymocyte development or selection. *Copa*^{E241K/+} mice bred to a T cell receptor transgenic mouse system revealed a defect in the negative selection of CD4⁺ T cells. Reciprocal bone marrow chimera experiments map the selection defect to E241K COPA expression in the thymic stroma. Taken together, our findings provide evidence that altered thymic selection is a critical precursor to autoimmune disease in the COPA syndrome.

F.79. Regulatory T Cells Mediate the Selection of Peripheral Naïve B Cells

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Increased frequencies of autoreactive mature naïve B cells in CD3-deficient and IPEX patients pointed to T cells/regulatory T cells (Tregs) as potential regulators for the peripheral selection of B cells. To assess T cells' role in controlling peripheral autoreactive B cells, we adapted two humanized mouse models. The first model involves engraftment of human hematopoietic stem cells in immunodeficient mice (NSG), which resulted in intact central B cell tolerance but defective peripheral B cell selection. The second model comprises concurrent transplants of human stem cells and autologous fetal thymic grafts in NSG mice (NSG+Thymus), which showed functional central and peripheral B cell tolerance checkpoints. In addition, successful counterselection of peripheral autoreactive B cells in NSG+Thymus mice correlates with functional development of a suppressive Treg compartment, while Tregs from NSG mice showed defective suppression. Furthermore, anti-CD25 injection experiments revealed that peripheral B cell tolerance is compromised in absence of Tregs. Thus, we propose the provocative concept that peripheral autoreactive B cell selection is intimately linked to the central selection of T cells/Tregs. We are currently exploring the mechanism(s) by which Tregs control peripheral B cell tolerance—using unbiased transcriptome analysis and next-generation TCR sequencing of Tregs derived from both mouse models, and development of a novel humanized mouse model in which antigen presentation by B cells is disrupted. Preliminary data from these experiments support our hypothesis that recognition of self-antigens presented on B cells by autoreactive TCRs on Tregs serves as an essential step for the peripheral selection of naïve B cells.

F.81. Characterization of Extra-thymic AIRE-expressing Cells Using Single-cell RNA Sequencing

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Previous work from our lab identified cells that express AIRE outside of the thymus. These extra-thymic AIRE-expressing cells (eTACs) have been found in secondary lymphoid organs in both mice and humans. In two independent models of autoimmune diabetes, they appear to enforce tolerance to self-antigens in the periphery by deleting autoreactive CD8⁺ (killer) T cells and by rendering autoreactive CD4⁺ (helper) T cells anergic or unresponsive to T cell receptor (TCR) stimulation, thereby preventing autoimmune attacks on pancreatic beta islets.

Unfortunately, the characterization of eTACs has been complicated by the fact that they don't have a consistent and uniquely expressed cell surface marker, and they tend to have lower levels of AIRE expression, making cell sorting by

flow cytometry difficult, even with the AIRE-GFP reporter mouse.

We therefore sorted GFP⁺ cells from lymph nodes to obtain a representative “eTAC” population, and sent them for single cell RNA-seq analysis along with known non-eTACs (cDC, B cells etc) as control. Using differential gene expression analysis, and unsupervised clustering we found that eTACs are a heterogeneous population of cells with variable levels of AIRE expression but consistently had high levels of CCR7, ICOSL, and MHC class II. Using lineage tracing and single-cell trajectory models, we are working to clarify if eTAC heterogeneity represents a single population that is undergoing dynamic changes or if AIRE is expressed in multiple immune cell lines.

F.92. Dissecting Human Regulatory T Cell Signaling Using Chimeric Antigen Receptors

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Chimeric antigen receptor (CAR) technology has revolutionized our capacity to create tumor-specific effector T (Teff) cells. CARs direct T cells to target antigens with an extracellular antigen-binding domain and simultaneously deliver TCR signaling and co-stimulation, resulting in potent antigen-specific T cell activation. CAR-Treg engineering is a promising strategy to expediently generate antigen-specific Tregs for treating autoimmune and inflammatory disorders. Yet, there are stark differences in signaling between Tregs and Teff cells. Here, we systematically interrogated signaling in human Tregs and Teff cells using twenty-four CARs with distinct signaling modalities, including CD28 mutants, inhibitory receptors, and TNFR family members. We observed broad differences in CAR T cell activation and expansion between different classes of receptors consistent with their function. Interestingly, some configurations yielded different outcomes in Tregs and Teff cells. CD28 signaling is crucial for Treg development and function. Accordingly, intact CAR-mediated CD28 signaling was indispensable for robust CAR-Treg expansion. In fact, CAR-mediated 4-1BB signaling alone failed to support CAR Treg expansion. Teff cells, in contrast, were only modestly affected by disruptions in CAR-mediated CD28 signaling. Inhibitory receptor expression, a hallmark of Tregs, provokes Teff cell exhaustion. With the exception of PD1, CAR-mediated inhibitory receptor signaling specifically hampered CAR Teff cell activation. CARs incorporating TNFR signaling generally caused delayed activation kinetics in both Tregs and Teff cells. Ongoing *in vivo* experiments gauge the suppressive potencies of these CAR designs. Altogether, our results demonstrate that CAR engineering can uncover differences in signaling between human Tregs and Teff cells, potentially maximizing Treg therapies.

T.39. Immunological Characterization of IgA Nephropathy Patients

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IgA Nephropathy (IgAN) is the leading form of primary glomerulonephritis affecting glomerular mesangium, with proteinuria, hematuria, hypertension and reduction of renal glomerular filtrate. Approximately 40% of cases are related to end-stage renal failure, requiring either dialysis or renal transplantation. The gold-standard technique for IgAN diagnosis is renal biopsy. In the last years, the better knowledge of the disease, has led several authors to describe serum biomarkers that may be useful for diagnosis and prognosis, such as levels of partially degalactosylated IgA1 (Gd-IgA1). Until now, it has not been performed an exhaustive analysis of peripheral leukocyte subpopulations and CD89 expression on monocytes.

A prospective study of 22 patients diagnosed of IgAN by renal biopsy has been performed. By flow cytometry of whole blood, immunophenotype of leukocyte subpopulations and CD89 expression in three monocytes subpopulations has been characterized. Analysis of serum levels of Gd-IgA1 has been performed with commercial kit of ELISA, *Gd-IgA1 Assay kit-IBL*.

The results of this study have shown that those patients with poor renal function and more severe renal biopsy have lower Mean Fluorescence Intensity (MFI) of CD89 on non-classical monocytes. The immunophenotype showed that patients had a higher percentage of activated and effector memory CD4⁺ and CD8⁺ lymphocytes, lower percentages of B transitional lymphocytes and plasmablasts, and higher percentages of NK lymphocytes CD56^{dim}CD16⁺ and myeloid dendritic cells.

In conclusion, this preliminary study shows that MFI of CD89 on non-classical monocytes could be used as a prognostic biomarker of IgAN. In parallel, the immunophenotype maybe useful for IgAN diagnosis.

T.59. Simplified Protocol for Enrichment of Primed Human Th17 and Tc17 Lymphocytes from Peripheral Blood

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Background: Interleukin-17A (IL-17A) is a potent pro-inflammatory cytokine that has been implicated in the pathogenesis of various autoimmune diseases. The production of IL-17A is commonly associated with subsets of CD4⁺ T cells (Th17) and CD8⁺ T cells (Tc17). However, identifying these subsets with intracellular expression of IL-17 or transcription factor RORC limits the study designs involving cell-cell interaction or cellular functions due to compromised cell state. Therefore, identifying surface marker/s that can help in characterizing these cells is very important.

Results: We here demonstrate that both CD4⁺ T cells (Th17) and CD8⁺ T cells (Tc17) cell populations can be identified based on the surface expression of melanoma cell adhesion molecule (MCAM) or CD146. This protocol uses MCAM as a surrogate marker to identify *in vivo* committed human Th17 and Tc17 subsets.

Conclusion: By employing high-speed fluorescence activated cell sorting, we can enrich these IL-17A-producing subsets from human specimen without the need for *in vitro* polarization using exogenous cytokines. These subsets can be investigated following sorting using a variety of methods such as PCR, ELISA, *ex vivo* functional assays and next generation sequencing to gain insights into the role of human Th17 and Tc17 in health and disease.

T.64. Role of Native Mouse Thymus and Lymphopenia-Driven Expansion in the Development of an Autoimmune-like Syndrome in Humanized Mice

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Humanized mice generated by transplantation of human hematopoietic stem cells (HSCs) with/without a human thymus to NSG mice spontaneously develop an autoimmune-like syndrome around 25-35 weeks post-transplantation. Target organs including skin, lungs, spleen, liver, pancreas and intestine are widely infiltrated with effector-memory CD4 and CD8 cells in addition to giant multinucleated phagocytic cells. Using single-cell TCR sequencing, we observed that some T-cell clones are widely expanded and detected in different affected organs with high frequencies. By removing the

native mouse thymus, we showed that human T-cells are necessary for the disease to occur, in mice receiving human HSCs without a human thymus graft. Humanized mice constructed with an intact mouse thymus, which supports a low thymic output, manifested an earlier disease onset (mean 31 weeks) compared to native thymectomized mice with a human thymus (mean 39 weeks). We obtained evidence that lymphopenia-induced proliferation (LIP) of T-cells significantly accelerates disease onset (mean onset 8 weeks post transfer of autologous T cells from humanized mice to T cell-deficient humanized mice). Injection of HSCs right after birth led to a delay in the onset of disease in mice with an intact mouse thymus compared to mice injected in adulthood. In conclusion, LIP and impaired negative selection associated with disorganized native thymic structure facilitate the development of this autoimmune syndrome.

T.75. Foxp3 Domain-Swap Interface is Required to Suppress T Helper Type 2 Transcriptional Program in Regulatory T Cells

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Regulatory T cells (Treg) play a fundamental role in maintaining immune tolerance. These cells are characterized by expression of the master regulator Foxp3, a transcription factor that is crucial for Treg function and homeostasis. Natural occurring mutations in Foxp3 gene have been shown to be responsible for the immunodysregulation polyendocrinopathy enteropathy X-linked (IPEX) syndrome, which is characterized by widespread autoimmune diseases. We recently identified a Foxp3 mutation in a boy that manifested a severe IPEX syndrome. This mutation, resulting in the substitution of methionine for isoleucine at amino acid 370 was determined to be part of a class of Foxp3 mutations that disrupt the dimerization motif in the domain-swap interface of the protein. We have shown that, although dimerization mutants retain DNA binding, allows Treg development and certain Treg characteristics, it inevitably led to an IPEX syndrome in humans and mice. Genomic analysis of M370I-mutant Treg by RNAseq, ChIPseq and HiChIP revealed a de-repression of the T-helper type 2 (Th2) transcriptional program leading to the generation of Th2-like Treg. We have shown that, dimerization mutant proteins bind to multiple locations in the Th2 locus leading to increased intrachromosomal interactions and Th2 cytokines production. As expected, M370I Tregs were unable to suppress Th2 immune responses. Transgenic mice expressing M370I Foxp3 developed autoimmune diseases characterized by Th2 cytokines production and skin and lung inflammation. In conclusion, disruption of the domain swap interface of Foxp3 led to the development of Th2-like Treg which are functionally unable to suppress the development/function of Th2 cells.

T.82. Mice Lacking $\alpha\text{v}\beta 6$ - and $\alpha\text{v}\beta 8$ -integrin Activity Display Impaired Thymic Epithelial Cell Maturation

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The cytokine transforming growth factor- β (TGF- β) is an important pleiotropic regulator of adaptive immunity. The arginine-glycine-aspartate (RGD)-binding integrins $\alpha\text{v}\beta 6$ and $\alpha\text{v}\beta 8$ activate latent TGF $\beta 1$ and TGF $\beta 3$ *in vivo*. Both integrins are expressed by thymic epithelial cells. Here we show that mice selectively lacking $\alpha\text{v}\beta 8$ on medullary thymic epithelial cells (mTECs) (*FoxN1-Cre.Itgb8^{fl/fl}*) display a severe developmental and autoimmune phenotype when treated with an anti- $\alpha\text{v}\beta 6$ integrin blocking antibody. These mice suffer from growth retardation, develop pulmonary inflammation and emphysema, and have an accelerated mortality rate. Importantly, mice with isolated loss of $\alpha\text{v}\beta 8$ or only treated with anti- $\alpha\text{v}\beta 6$ integrin blocking antibody are normal. To determine the mechanisms underlying auto-immunity in these mice, we evaluated thymic T cell and epithelial development. CD4 and CD8 T cell development was normal in experimental mice, but we found a dramatic reduction in the frequency of Aire⁺ mTECs and thymic tuft cells at approximately 20 and 30 days of age. We also found that experimental animals failed to develop iNKT cells in the thymus and that splenic

iNKT numbers were significantly reduced. Taken together, these results suggest that thymic $\alpha\beta6$ and $\alpha\beta8$ -mediated TGF β activation is essential for medullary thymic epithelial maturation and the maintenance of central tolerance.

T.90. The IL-6 Receptor Alpha Chain is Essential for the Biological Function of IL-6

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IL-6 is a pleiotropic cytokine with context-dependent properties. It binds to IL-6 receptor (IL-6R) alpha chain and together with gp130 (glycoprotein 130, a common transducer for IL-6 family cytokines) initiates signaling cascade. Although, often comparable, several mouse models showed discrepancies in immune responses between IL-6 and IL-6R deficient mice. This assumption suggested the presence of an alternative receptor for IL-6. Recently, it was proposed that in addition to the IL-6R, IL-6 can also bind to CD5 and signal via gp130. To test if CD5 can functionally replace the IL-6R, we used a novel mouse strain in which IL-6 can be overexpressed in a Cre-dependent manner. Overexpression of IL-6 using CD11c-cre activator led to a pathogenic phenotype associated with systemic inflammation and mortality within two months of birth. High levels of IL-6 perturbed B and T cells development, both in the primary (bone marrow, thymus) and secondary lymphoid organs (lymph nodes, spleen). Lymphocytes isolated from IL-6 transgenic mice showed impaired Ig production and IL-17A expression. Mice overexpressing IL-6 but lacking of IL-6R were completely protected from IL-6-mediated pathology and fully phenocopied IL-6R deficient mice. Mechanistically, IL-6R deficiency prevented downstream activation of STAT3 in response to IL-6. All together, our data suggest that IL-6R is the only biological relevant receptor of IL-6 in mice and does not appear to be a functionally relevant CD5 ligand.

W.11. Serum Antibody Specificities in Healthy Infants Reveals a Significant Autoreactive Profile

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The antibody specificities of an infant develop in response to infections, environmental exposures, and vaccinations. By adulthood, antibodies that react against self-antigens, often causal to autoimmune diseases, are commonly detected. It remains unknown whether such autoreactive antibodies are present early in infancy. To address this, the serum antibody specificities were screened in a cohort of 100 healthy 1 and 2 year old infants. Analysis revealed that 28% of the infants have moderate to high titered antibodies to diverse array of self-antigens. These numbers are consistent with observed autoantibody positivity in adult population. Ongoing targeted DNA sequencing analysis is assessing the genetic load of known autoimmune risk alleles in autoantibody positive infants. Relationships between the antibody specificities and the genetic risk alleles, assembled for each infant, will be presented. The comparisons will include clinical information, sex, ethnicity, growth records, vaccination status, infectious history, antibiotic and antiviral treatments, disease status and family history. Genomic analysis of known autoimmune risk alleles in infants with autoantibodies may identify those at risk for developing diverse immune system abnormalities, including autoimmune disorders. The information may also support implementation of a new wellness screen to identify autoantibody positive infants who have significant genetic predispositions for disease progression.

W.19. 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 144-gene signature, called the Immune Sex Expression Signature (ISEXS), which is differentially expressed between healthy men and women, between 18-40 years old, in the blood across worldwide populations. We validated ISEXS in 7 additional cohorts of 263 samples. Second, we examined whether ISEXS was driven primarily by autosomal or sex chromosomal genes. We observed that only 25% of ISEXS genes are located on the sex chromosomes. Furthermore, when ISEXS is separated into sex chromosomal ISEXS (XY-ISEXS) and autosomal ISEXS (A-ISEXS), both successfully differentiate male and female samples. This indicates that both autosomal and sex chromosomal genes play significant roles in transcriptomic sex differences. Interestingly, XY-ISEXS separated males and females regardless of age, while A-ISEXS only separated males and females between 18-45 years old and failed to separate individuals between 45-70 years old. As a robust gene signature across populations with a strong autosomal component, ISEXS has applications in understanding why women and men have differential risks of autoimmunity and infection.

W.35. Dynamic Functional Phenotypes of B- and T helper-cells from Autoantibody-positive First Degree Relatives Mark Distinct Stages of Type I Diabetes (T1D) Progression

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Autoreactive B-cells are strongly implicated with effector T cells (Teff) in T1D pathogenesis. An integrated longitudinal approach for understanding the evolution of immune alterations in this process is critical for developing targeted therapies. Previously, we demonstrated functional T- and B-cell phenotypes in established T1D individuals that suggested peripheral tolerance defects. Here, we used natural history samples from the TrialNET Pathway to Prevention Study and Rituximab trial to investigate whether these alterations reflect causative disease mechanisms, promote disease progression, or arise as a consequence of disease. We found early decreased IL-2/pSTAT5 signaling in CD4⁺ Teff from stage 0/stage 1 first degree relatives (FDRs) that persisted into later stages of disease, while Teff resistance to regulation developed with later phenotypes of increased autoantibody (autoAb) number and decreased islet function. Enhanced IL-21/pSTAT3 responses were present in naïve B-cells from stage 1 FDRs that positively correlated with number of autoAbs, and declined by stage 2. Parallel immunophenotyping of the B-cell compartment revealed early expansion of transitional B-cells, which comprise a peripheral tolerance checkpoint, in autoAb⁺ cohorts as compared with autoAb^{neg} FDRs. Further, BCR hyper-responsiveness was present in young stage 1 FDRs, as compared with blunted BCR signaling near or at disease onset. BCR response was improved in new-onset T1D Rituximab trial participants who had relatively sustained C-peptide at 1 year. Our combined data reveal dynamic, stage-dependent T- and B-cell responses in FDRs as they progress to clinical disease, and define novel immune phenotypes of potential clinical relevance for early therapeutic targeting.

W.38. Modeling the Thymic Selection of Human Autoreactive T cells *In Vivo*

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Autoimmune diseases result from a failure to properly eliminate or suppress autoreactive T cells that are randomly generated during thymopoiesis. During thymic development, T cells that encounter self-antigens expressed by the thymic epithelium or brought from the periphery by dendritic cells are normally deleted or converted into regulatory T cells. However, this process does not apply to all tissue antigens and remains poorly understood in humans. To better understand the development and reactivity of human autoreactive T cells, we generated humanized mice whereby hematopoietic stem cells (HSCs) develop in a grafted human thymic tissue, a fraction of which were transduced to express a human autoreactive TCR specific to an HLA-A2-restricted MART1 epitope. Because this epitope is not presented in the thymus, these T cells escape and accumulate in the periphery. When another fraction of HSCs were made to express the cognate peptide, deletion of most tetramer (Tet)⁺ T cells ensued with the remaining circulating T cells showing a memory and anergized phenotype and disappearing over time. All remaining Tet⁺ CD8⁺ thymocytes had upregulated PD-1 and down-regulated CCR7, and no Tet⁺ CD4⁺ T cells were selected as Foxp3⁺ in the presence of the peptide. New constructs have been produced to restrict antigen expression to specific hematopoietic antigen-presenting cell lineages in order to ascertain their contribution to central tolerance and how they differentially impact the phenotype of human autoreactive T cells *in vivo*. This humanized mouse model will prove useful to assess mechanisms of central tolerance and approaches to induce tolerance.

W.39. VISTA.COMP is an Engineered Checkpoint Receptor Agonist that Suppresses T-Cell Mediated Immune Responses.

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VISTA is an immune checkpoint molecule which functions to suppress T-cell activity. The therapeutic potential of activating the VISTA signalling pathway to reduce inflammatory responses remains unknown, largely due to the inability to derive agonistic antibodies towards its unknown counter receptor. A dimeric construct of the IgV domain of VISTA (VISTA-Fc) was shown to suppress the activation of T-cells *in-vitro* however, this effect required its immobilization on a solid surface, suggesting that VISTA-Fc may display limited efficacy as a VISTA-receptor agonist *in-vivo* in the absence of Fc receptor dependent cross-linking. Furthermore, the use of VISTA-Fc *in-vivo* to mechanistically understand the role of VISTA:VISTA-receptor signalling is complicated due to the inherent Fc-mediated effects. We have designed a stable pentameric VISTA construct (VISTA.COMP) by genetically fusing its IgV domain to the pentamerization domain from Cartilage oligomeric matrix protein (COMP). VISTA.COMP is expressed as stable pentamers, and in contrast to VISTA-Fc, does not require immobilization to inhibit the proliferation of CD4⁺ T-cells undergoing anti-CD3 induced activation. Further, VISTA.COMP functions as a potent immunosuppressive agonist *in-vivo*, capable of prolonging the survival of skin allografts in a mouse transplant model, as well as rescuing mice from acute Con-A induced hepatitis. Collectively, our data demonstrates that pentamerization of the VISTA-IgV domain led to the derivation of a checkpoint receptor agonist, with VISTA.COMP representing first agent which targets the putative VISTA-receptor to suppress T-cell immune responses *in-vivo*.

W.49. How Pro-inflammatory Markers and Molecular Mediators May Contribute to Immune Failure and Autoimmunity in Aging

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Aging is characterized by increased low-grade chronic inflammation, a risk factor for morbidity and mortality of elderly individuals and implicated in the pathogenesis of several disabling diseases of the elderly. Cellular senescence is a significant contributor to inflammaging, due to the acquisition of the senescence-associated secretory phenotype (SASP) by immune cells.

We previously demonstrated that markers of the SASP (pro-inflammatory cytokines, inflammatory micro-RNAs, NF- κ B) are highly expressed in human memory B lymphocytes, and especially in the B cell subset called late memory (LM), tissue-like or double negative (DN) (CD19⁺IgD-CD27⁻). This subset is increased in the blood of healthy elderly individuals and also in individuals with obesity, autoimmune diseases and acute or latent viral infections, suggesting that they may expand in the presence of autoantigens or viral antigens. They also contribute to local inflammation through the secretion of pro-inflammatory mediators and this negatively impacts the function of immune cells. We have measured antibody secretion by LM B cells, in addition to cytokines, and found autoimmune specificities such as Malondialdehyde (MDA), a product of lipid peroxidation; we have also identified major signaling processes responsible for their accumulation during aging (AMPK); and ways to manipulate these pathways and enhance B cell responses in the elderly. Our results allow better understanding of the mechanisms through which inflammation induces B cell deficiencies in aging, and also identification of further pathways to be targeted to reduce the negative effects of inflammation and improve humoral immunity in individuals who are living longer and with chronic diseases.

W.61. Low-dose Interleukin-2 Expands and Increase Responsiveness of Circulating Regulatory T Cells in Patients with Autoimmune Hepatitis

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Low-dose interleukin-2 (LDIL-2) selectively expands regulatory T-cells (Tregs) and improves their functional capacity. It has been used in a variety of clinical settings including autoimmune disorders, but not in autoimmune hepatitis (AIH). We conducted immunomonitoring experiments on sequential blood samples from 2 patients not responding to second-line immunosuppression. Both received 6 monthly courses of LDIL-2 (daily subcutaneous of 1million IU for 5 days/cycle). LDIL-2 increased circulating Tregs (CD25⁺FOXP3⁺) significantly after each cycle at day-4, peaking at day-9 (median relative increase of 26%), and returning to baseline at day-28. Detailed immunophenotypic analysis showed increases in proportion of resting (CD45RA⁺FOXP3^{lo}) and effector Tregs (CD45RA⁺FOXP3^{hi}), but not in non-suppressive CD45RA⁺FOXP3^{lo} subset. In contrast, proportion of conventional T-cells (Tcons, CD25⁺FOXP3⁻) reduced significantly following each cycle. The proportion of NK cells was also reduced, although there was significant increase in the CD56^{bright}CD16⁻ NK-subpopulation, but not in the CD56^{dim}CD16⁺ subset. In addition, LDIL-2 increased expression of the proliferation marker, Ki-67, and the activation markers, CD25 and FOXP3, in Tregs but not in Tcons. LDIL-2 also increased the responsiveness of Tregs to IL-2, as measured by expression of intracellular pSTAT5.

There was no significant biochemical response in Patient-1, but Patient-2 achieved biochemical remission after 6-cycles of LDIL-2 therapy (aspartate aminotransferase from 259 to 44IU/ml, immunoglobulin-G from 20.9 to 16.2g/L). Neither patient developed serious adverse events. Interestingly IL-10 and CXCL-10 levels, which are raised in AIH compared to healthy controls, were significantly reduced in Patient-2 at end-of-treatment.

Conclusion:

LDIL-2 expands endogenous circulating Tregs in AIH patients and increases its sensitivity to IL-2.

W.62. Genetically Engineered Tolerogenic Dendritic Cells for Antigen-specific Immunotherapy

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An innovative and challenging approach to control auto-reactive T cells and restore tolerance in autoimmunity is to boost the regulatory arm of the immune system by supplying *ex vivo* generated tolerogenic dendritic cells (tolDC). The development of methods to generate clinical grade cell products allowed their clinical application. Thus far, the safety and feasibility of tolDC-based cell therapy have been demonstrated in proof-of-principle clinical trials. Despite these results several questions remain to be addressed before tolDC-based cell therapies can be used to cure autoimmune diseases, including the maintenance of the tolerogenic cell properties *in vivo*, and the ability to stably present autoAg to inhibit auto-reactive T cells while promoting autoAg-specific Tregs.

We design an innovative approach based on lentiviral vector (LV) technology to generate tolDC that stably express and present autoAg at immature stage or in a microenvironment enriched of anti-inflammatory molecules. We demonstrated that LV-engineered DC (LV-DC) present encoded Ag to autologous T cells and modulate Ag-specific T cell responses *in vitro*. Adoptive transfer of LV-DC while inhibiting Ag-specific T cells, allows the induction of Ag-specific Tregs *in vivo*. We translated the approach to human cells by developing an efficient protocol to engineer with LVs human DC. Human LV-DC modulate autoAg-specific T cells *in vitro*. We are currently validating our approach using primary cells and humanized murine models.

The success of our strategies will help designing a safer tolDC-based cell therapy, abrogating the boosting of autoimmunity, and to stably preserve the tolerogenic properties of *in vivo* transferred DC.

W.67. Regulatory Dendritic Cell-Based Strategies for the Development of Innovative Therapies for Celiac Disease

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The keystone of Celiac Disease (CD) pathogenesis is an altered immune response to gluten in genetically predisposed individuals. At present, the only therapy for CD is a permanent gluten-free-diet (GFD). Compelling evidences indicate a central role for IL-10 in the regulation of gut homeostasis. To better understand the role of regulatory cells in CD progression, we are currently investigating their presence and function in peripheral blood and intestinal mucosa of CD patients at different stage of disease (i.e., potential CD, onset, GFD). Our preliminary data did not highlight major alterations in the frequency of T regulatory cells (either FOXP3-expressing or type-1(Tr1)) and tolerogenic DC (DC-10) in the peripheral blood of patients upon GFD. With the aim of generating tolDC suitable for cell-based therapy of CD, we differentiated *in vitro* monocyte-derived DC enforced to present HLA-class-II restricted Gliadin-derived epitopes in the absence (DC_{LV-Ag}) or presence of tolerogenic molecules (i.e., IL-10 or IDO) by means of lentiviral-vector (LV) transduction. When IL-10-encoding LV was used, the resulting cell population acquired a DC-like phenotype, expressed tolDC associated markers (ILT4), and constitutively produced high amounts of IL-10. DC_{LV-Ag} were able to induce Ag-

specific autologous CD4⁺ T-cell proliferation *in vitro*, while DC co-encoding for IL-10 induced a hypo-proliferative response and promoted the expansion of Ag-specific CD49b⁺LAG-3⁺ Tr1-like cells. We are currently validating our approach using cells from CD patients. Our study will provide new insights into the mechanisms underlying CD progression and response to GFD, and will open new perspectives for the therapy of CD.

W.87. Brugia Malayi Recombinant Cystatin Ameliorates Development of Murine Colitis by Induction of IL-10⁺ T-regulatory Cells and IL-10⁺ B1-cells

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Therapeutic potential of *B. malayi* recombinant cystatin (*rBmaCys*) was evaluated in dextran sulfate sodium (DSS)-induced colitis and streptozotocin (STZ)-induced Type 1 diabetes (T1D) model in mice. To evaluate the therapeutic potential of *rBmaCys* in colitis, mice were treated intraperitoneally with *rBmaCys* (25µg/dose) on days 4, 5 and 6 after DSS administration. Control mice were given PBS or *rBmaCys* alone. Clinical parameters (loss of body weight, blood in feces), gross pathology (shortening of colon length) and histopathological changes were evaluated. Our results show that *rBmaCys* treatment significantly ameliorated the colitis symptoms and reversed the gross and histopathological changes in the colon. We then analyzed the mechanism of *rBmaCys*-induced suppression of inflammation in the DSS-induced colitis. These studies showed that *rBmaCys* treatment decreased the expression of TNF-α, IL-6, IL-17a and Rory in the colon and increased the percentage of IL-10 producing T regulatory cells in the colon and mesenteric lymph nodes. The number of IL-10 producing B1-cells were also increased in the peritoneal cavity, suggesting a central role for IL-10, Treg cells and B1 cells in the *rBmaCys*-induced downregulation of inflammatory responses in DSS-induced colitis. We also evaluated if *rBmaCys* treatment has any effect on reversing the pathology in STZ-induced T1D in mice. In these studies, four doses of *rBmaCys* (50µg/dose) was given intraperitoneally at 7 days interval. Our results indicated that *rBmaCys* treatment had no effect on STZ-induced T1D. In conclusion, our studies demonstrated that *rBmaCys* is a promising therapeutic molecule for treating ulcerative colitis.

W.107. Editing of Effector T Cell Activation Pathways to Model Teff Resistance

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The resistance of T effector cell (Teff) to suppression by regulatory T cells (Treg) is present in multiple autoimmune diseases. Teff resistance has been shown to contribute to the pathogenesis of autoimmunity in a number of murine models. Identifying the mechanisms through which Teff resist suppression by Treg in human disease has been challenging due to variability in suppression assays, and a need for consistent cellular control. In this study, we have modeled Teff resistance by knocking out Cbl-b, a negative regulator of T cell activation in primary human CD4⁺ T cells using gene editing. We designed CRISPR guide RNAs that targeted *CBL-B* and transfected them into CD4⁺ T cells isolated from healthy control subjects. The average cutting rate at the *CBL-B* locus was 60% when transfecting the CRISPR-Cas9 ribonucleoprotein alone and increased to 80% when combining with an ultramer DNA oligonucleotide. Cbl-b protein levels were significantly decreased in Cbl-b edited CD4⁺ T cells compared to mock edited cells. In addition, the Cbl-b edited CD4⁺ T cells were hyper-proliferative upon TCR stimulation and when stimulated with IL-2. Importantly, Cbl-b edited CD4⁺ T cells were resistant to Treg in an *in vitro* suppression assay. Together, these findings highlight the potential of Cbl-b edited CD4⁺ T cells for modeling Teff resistance in human autoimmune disease and the more broadly the power of gene editing of primary T cells to explore disease mechanisms.

Genetics

F.1. Gene Editing in Primary Human T Cells to Assess Autoimmune Associated SNPs Within the Gene *PTPN22*

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Autoimmune diseases arise out of a combination of genetic and environmental factors. GWAS studies have identified genetic risk variants that are associated with multiple autoimmune diseases. One extensively studied risk variant is the R620W coding change within the phosphatase, *PTPN22*, a negative regulator of T cell signaling. This variant is strongly associated with increased risk for multiple autoimmune diseases including T1D, RA, and SLE. Strikingly, despite substantial effort by multiple groups, previous work has led to conflicting data regarding the mechanistic impact of this risk variant. Understanding the functional consequence of this variant and others is critical for an improved understanding of autoimmune pathogenesis and the identification potential pathways for new therapeutic options. Utilizing Crispr/Cas9 Ribonucleoprotein based gene editing, we have disrupted expression of *PTPN22* in primary human CD4⁺ T cells with high efficiency (~80% reduction in protein expression over control locus (CCR5); 2 experiments, 4 human donors). *PTPN22* deficient T cells are hyper-responsive to TCR engagement, demonstrating enhanced calcium flux, increased cytokine secretion, and greater expression of surface activation markers. Furthermore, using this gene editing approach in conjunction with co-delivery of a donor DNA template, we have altered the reading frame of primary T cells through homology-directed-repair, producing R620W risk variant T cells from non-risk donors (~30% of alleles by ddPCR). These edited cells will provide an isogenic human model of the *PTPN22* risk variant, free of developmental and environmental factors, and allow direct assessment of the *PTPN22* R620W risk variant on human T cells.

F.45. RASGRP3 Intronic Variants Affect its Expression, and a Possible Mechanism That Might Contribute to Lupus Risk

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We recently reported that two intronic variants explained RASGRP3-SLE association in Asians [Sun et al. 2016, Nat Genet]. However, the “causal” functional variants and the genetic mechanism(s) by which associated variants contribute to disease are largely unknown. We hypothesized that these intronic variants affect epigenetic regulation and modulate RASGRP3 expression.

We used bioinformatics to define the potential regulatory effects of the candidate variants on gene expression using data on several histone marks and eQTLs. We then used a combination of molecular biological experiments to identify DNA-bound proteins followed by ChIP-qPCR to assess the allele-specific binding. Finally, using EBV-transformed cell lines, RASGRP3 mRNA and protein expressions were compared between risk and non-risk alleles.

We observed significant difference in RASGRP3 transcript and protein levels with increased expression in rs13385731 risk genotype (TT). Luciferase assays demonstrated significant allele-specific enhancer and promoter activities. DNA pulldown, EMSA and Mass-spect suggested bound protein was identified as PARP1 and later confirmed Western blot. We also verified differential allele-specific binding of PARP1 and IRF1 against rs13385731 using ChIP-qPCR. Interestingly, while PARP1 binding affinity is higher with risk (TT) genotype, IRF1 binding shows strong binding affinity with non-risk (CC) genotype of rs13385731.

Our results showed that rs13385731 is an eQTL, and the risk (TT) genotype is associated with increased RASGRP3 expression. Our ChIP-qPCR showed significant allele-specific binding to H3K27Ac, P300, PARP1 and IRF1 proteins, which might alter expression of RASGRP3, contributing to SLE risk. The activity of this variant provides insight into the molecular mechanisms underlying its association with SLE.

T.52. Human Sinonasal Immunoglobulin D Gene Mutations and Clonal Compartmentalization

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Background: Chronic rhinosinusitis (CRS) involves complex interactions between immune system and microbiota. IgD-expressing B cells are targets for respiratory pathogen superantigens, and IgD-secreting plasma cells are uniquely prevalent in the upper respiratory tract, especially in CRS. We characterized immunoglobulin genes encoding variable antigen-binding regions (V_H3) in sinonasal and blood B cells to examine 1) evidence for nonspecific bacterial activation versus antigen-driven selection, and 2) IgD B cell clonotypes shared between mucosal and blood compartments to elucidate sinonasal B cell origins.

Methods: We collected matched blood and sinonasal tissues from 25 human subjects: 21 with CRS and 4 controls. We compared cDNA amplicons of V_H3 genes for IgA, IgD, IgG, and IgM by high-throughput sequencing for mutation frequencies and clonal distributions.

Results: V_H3 gene mutation frequencies for IgD were low (<1%) in blood, but an order of magnitude higher in sinuses, the latter akin to IgA and IgG in both compartments. IgD mutations yielded largely non-conservative replacements in hypervariable CDR1/2 regions, consistent with antigen-driven processes. Unique clonotypes shared by sinus and blood showed high mutation frequencies, represented a substantial proportion of clonotypes in each compartment, and phylogenetic analysis suggested bidirectional migration. We observed no differences between disease and control groups.

Conclusion: The very high frequencies and patterns of mutation and evolving phylogeny of IgD V_H3 genes in sinonasal mucosa appear to derive from antigen-driven processes and selection rather than non-specific, innate immune stimulation. Understanding the origin, antigen-specificity, and function of mucosal IgD antibodies should enhance understanding of sinonasal immunobiology and disease.

T.116. PBMC Fixation and Processing for Chromium Single-Cell RNA Sequencing

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Background: Interest in single-cell transcriptomic analysis is growing rapidly. In almost all reported works, investigators have used live cells which introduces cell stress and hinders complex study designs. Recent studies have indicated that cells fixed by denaturing fixative can be used in single-cell sequencing. But they did not work with most primary cells.

Methods: The methanol-fixation and new processing method was introduced to preserve PBMCs for single-cell RNA sequencing (scRNA-Seq) analysis on 10X Chromium platform.

Results: When methanol fixation protocol was broken up into three steps: fixation, storage and rehydration. We found that PBMC RNA was degraded during rehydration with PBS, not at cell fixation and storage steps. Resuspension but not rehydration in 3X SSC buffer instead of PBS preserved PBMC RNA integrity and prevented RNA leakage. Diluted SSC buffer did not interfere with full-length cDNA synthesis. The methanol-fixed PBMCs resuspended in 3X SSC were successfully implemented into 10X Chromium standard scRNA-seq workflows with no elevated low quality cells and cell doublets. The fixation process did not alter the single-cell transcriptional profiles and gene expression levels. Major subpopulations classified by marker genes could be identified in fixed PBMCs at a similar proportion as in live ones. This new fixation processing protocol was validated in CD8⁺ T cell and several other cell types.

Conclusions: We expect that the cell fixation procedure presented here will allow better and more effective batching schemes and enable complex single cell experimental design.

W.17. Gene Expression Profile of Tolerogenic Dendritic Cells Differentiated with Vitamin D3, Dexamethasone and Rapamycin

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Background: Tolerogenic dendritic cell (tolDC)-based therapies have become promising approaches for the treatment of autoimmune diseases by their potential ability to restore immune tolerance in an antigen-specific manner. There is a broad variety of protocols to generate tolDC *in vitro*, being their differentiation in the presence of vitamin D3 (vitD3-tolDC), dexamethasone (dexa-tolDC) or rapamycin (rapa-tolDC) three of the most frequent. However, the characteristics of these cells are very heterogeneous, thus making the need to find common genetic pathways and biomarkers of high relevance.

Objective: To compare the transcriptomic profile of vitD3-tolDC, dexa-tolDC and rapa-tolDC in order to find common induced pathways and biomarkers.

Methods: Monocyte-derived dendritic cell differentiations of immature (iDC), mature (mDC), vitD3-tolDC, dexa-tolDC and rapa-tolDC from 5 healthy donors were generated, and a microarray analysis was performed (Affymetrix). Results were normalized and filtered, and differentially expressed genes (DEG) were selected. A Gene Set Enrichment Analysis (GSEA) was performed to select common enriched pathways. Statistical analyses were performed using R software.

Results: Common DEG could not be found for the three tolDC, although 14 genes (many of them immune-related) appeared up-regulated in at least one condition. GSEA revealed 11 common protein sets differentially expressed in tolDC. However, all of them were induced for vitD3-tolDC and dexa-tolDC, while down-regulated in rapa-tolDC.

Conclusions: The analysis revealed that, despite not sharing potential common biomarkers, vitD3-tolDC and dexa-tolDC presented similar transcriptomic profiles, suggesting an induction of immune tolerance through common pathways, while rapa-tolDC seem to develop their function through different ones.

W.21. Exploration of SCAMP5 as a Novel Lupus Risk Gene

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Genome-wide association studies [GWAS] in systemic lupus erythematosus [SLE] have identified well over 50 risk loci, confirming a strong genetic component in this disorder. We have previously identified CSK as a potential causal gene on chromosome 15, and at least one GWAS study has been interpreted to confirm this association. Human carriers of a CSK risk haplotype exhibit increased Csk expression in B cells along with enhanced sensitivity to B-cell receptor ligation as well as evidence of altered early B cell development. Unpublished linkage analysis and functional data in mice also

support Csk as a risk locus for SLE related phenotypes, with increased Csk expression on B cells with the risk haplotype. However, a further look at this genetic region in mouse and human identifies *SCAMP5* as a possible additional gene of interest that may regulate the humoral anti-DNA response. *SCAMP5* expression is specifically enriched in plasmacytoid dendritic cells [pDCs] versus other immune cell types. Given the established role for pDCs in animal models of SLE, it is possible that *SCAMP5* variants modulate DNA-directed antibody responses through a pDC-specific trait. In support of this hypothesis, TLR7-stimulated pDCs from Balb/c mice, in which a DNA-mimetic peptide is highly immunogenic, elaborate significantly greater interferon-alpha than do pDCs from DBA/2 mice, in which the same peptide is weakly immunogenic. Current studies are aimed at elucidation of the role of *SCAMP5* in pDCs and SLE pathogenesis in both mouse and human.

W.23. Impact of Genetic Polymorphisms on Human Immune Cell Gene Expression

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While an enormous number of genetic variants have been associated with risk for human diseases, how these variants affect gene expression in various tissues and cell types remains largely unknown. To address this gap as it relates to immune cells, as well as to identify which immune cell types are most susceptible to effects of disease-risk variants, the DICE (Database of Immune Cell Expression, Expression quantitative trait loci (eQTLs) and Epigenomics) project was established. In this report, we describe genetic effects on global gene expression in six human immune cell types and for CD4⁺ and CD8⁺ T lymphocytes, naïve and activated cells were included. Considering all cell types and conditions, we identified *cis*-eQTLs for a total of 9,686 unique genes, which represent 50% and 39% of all protein-coding genes and long non-coding RNAs expressed in these cell types, respectively. Strikingly, the vast majority of these genes showed a strong *cis*-association with genotype only in a single cell type or following *ex vivo* activation, thus highlighting the value of studying homogeneous cell types to identify which cells and genes are most susceptible to the effects of particular genetic variants. We also discovered a large number of associations between ancestry and gene expression and found that biological sex is associated with major differences in immune cell gene expression in a highly cell-specific manner. These datasets will help reveal the effects of disease risk-associated genetic polymorphisms on specific immune cell types providing insights into the mechanism underlying their effects (<http://dice-database.org>).

W.71. iReceptor: A Platform for Querying and Analyzing Antibody/B-cell and T-cell Receptor Repertoire Data across Federated Repositories

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Recent advances in next generation sequencing (NGS) technologies have made it possible to sample the human antibody/B-cell receptor (BcR) and T-cell receptor (TcR) repertoires in tremendous detail. A typical human has ~10⁹ B cells at any given time, with a very large percentage of these cells changing very rapidly over time. It is this rapid adaptation of our immune system over time that makes it so effective at fighting pathogens. NGS technologies allow investigators to sequence the immune receptor genes in essentially all of the B and T cells in an individual, allowing researchers to explore how the immune system adapts. Adaptive Immune Receptor Repertoire sequencing (AIRR-seq) has enormous promise for understanding the immune repertoire dynamics in vaccinology, infectious disease, autoimmunity, and cancer biology.

The iReceptor system is a platform to integrate and analyze these immense data sets by combining: 1) an international network of AIRR-seq data repositories; 2) the ability to federate AIRR-seq data across these distributed repositories; 3)

advanced analytical tools unique to AIRR-seq data; and 4) a scientific gateway that hides the complexity of performing research queries and advanced analyses across these federated data. iReceptor enables data federation across many distributed data sets by defining a web based API that implements the emerging AIRR Community (airr-community.org) data standard. In this presentation, we will demonstrate the iReceptor Scientific Gateway and its ability to federate and explore data across multiple, distributed, AIRR compliant repositories.

Immune monitoring

F.33. MHC Class II Dextramers - A New Tool for Detection of Antigen Specific CD4⁺ T-cells

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The development of MHC multimers is important for the detection, analysis and enumeration of antigen specific T-cells in immunological areas of cancer, virus infections, autoimmunity and immunotherapy. While MHC class I multimers are commonly used for labeling of antigen specific CD8⁺ T-cells, it is less widespread to label antigen specific CD4⁺ T-cells with the use of MHC class II multimers.

The reason for this might be the lesser number of antigen specific CD4⁺ T-cells compared with CD8⁺ T-cells and that the antigen specific CD4⁺ T-cells generally carry a TCR with a lower affinity for its cognate ligand, the MHC-peptide complex, than does the antigen specific CD8⁺ T-cells. Therefore, there is a need for generation of reliable MHC class II Dextramers, that have an increased monomer valency, and thus avidity compared to conventional multimers as tetramers.

Immudex are developing MHC class II Dextramers and have launched the first alleles of HLA class II Dextramers. The MHC class II alleles in development undergo a multitude of tests that ensure their functionality and ability to specifically label antigen specific CD4⁺ T-cells. We will present data on our MHC class II molecules that shows

- 1) their ability to be fully loaded with peptides.
- 2) the peptide binding affinities and characteristics of the MHC class II molecule.
- 3) the functional MHC class II Dextramer labelling of antigen specific CD4⁺ T-cells

F.37. Development of 12⁺ Color Modular Staining Panels with a Common Backbone to Interrogate CD40L-based Activation of Human B Cells, PBMC, Neutrophils and Monocyte-derived Dendritic Cells

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CD40L-CD40 interactions are a vital signal in the activation of multiple cell types, and the CD40L-CD40 axis has been implicated in multiple autoimmune diseases such as systemic lupus erythematosus and multiple sclerosis. To examine the effects of CD40L signaling *in vitro*, four 12⁺ color flow cytometry staining panels were developed (for PBMC/PBL, purified B cells, purified neutrophils, and monocyte-derived dendritic cells), with modular slots for activation markers with specific fluorophores. These panels were developed iteratively, building from a common backbone with consideration for the ability to visualize all cell subsets of interest and to minimize spectral overlap. Cell subset isolation and stimulation was optimized for maximal activation. The activation markers examined were CD40, CD54, CD69, CD70, CD80, CD83, CD86, CD95, and HLA-DR. Healthy control PBMCs, PBLs, B cells, monocytes, monocyte-derived DCs, and neutrophils

were analyzed for *in vitro* response to CD40L stimulation. B cells (both purified and as part of a PBMC or PBL mixture) showed the highest level of activation in response to CD40L, with an increase in CD54, CD69, CD80, CD83, CD86, CD95, and HLA-DR. Monocyte derived DCs showed an increase in CD40, CD54, CD80, CD83, CD86, and HLA-DR. Monocytes within the PBMC fraction showed an increase in CD54, CD83, and CD95. The response to CD40L by neutrophils –either purified or in a PBL fraction – was minimal. Going forward, these panels will allow the interrogation of CD40L-based activation in both healthy controls and diseased individuals.

F.39. Using Indocyanine Green Dye to Image Immune Cells in the Human Retina During Ocular Disease

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It is not currently possible to readily visualise and track immune cells in living human tissues. The eye is a readily accessible and optically transparent organ which reflects the CNS environment. Indocyanine-green (ICG) is an FDA-approved near-infrared emitting angiographic dye, which has been used safely in ophthalmic practice worldwide for decades. In mouse studies we identified that ICG accumulated in myeloid cells which became visible in the retina during experimentally induced inflammation several days after administration.

To determine if human application was possible we commenced a clinical study of 12 patients receiving ICG angiography as part of routine clinical care for a range of ocular diseases including neovascular age-related macular degeneration and uveitis. We extended the typical imaging period beyond the standard 30-minute duration, imaging at 2, 4, 6, 8, 24, 48 hours, 7 and 9 days after intravenous administration using two commercially available platforms (HRA Spectralis and Optos California). We also took peripheral blood samples to assess the presence of ICG labelled cells and activation markers by flow cytometry.

Three patients showed detectable patterns of high signal compatible with immune cells which altered position over subsequent timepoints. Only one patient had detectable ICG signal in peripheral blood, predominantly in granulocytes. We confirmed ICG uptake into endosomes using *in vitro* cultured human macrophages at high dose, so it is likely the standard regime used for angiography is insufficient for robust immune cell labelling and a refined administration dose and formulation may be needed in future work.

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F.60. The CD43-Mediated Signals Differentially Regulate CD4 and CD8 T Lymphocytes Function

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CD43 (sialophorin or leucosialin), is a transmembrane mucin abundantly expressed on almost all hematopoietic cells, except erythrocytes and resting B cells. T cell activation through CD43 alone parallels the TCR signaling pathway, inducing activation of the PKC, MAPK, PI3K/AKT, and PLC γ pathways. When combined with the TCR signals, CD43 signals inhibit the c-Cbl and Cbl-b negative signals, increasing the duration and intensity of the signals of the TCR,

lowering the threshold for activation. These effects are mediated by the highly conserved cytoplasmic domain of CD43 since deletion of the cytoplasmic domain abolishes the co-receptor functions of CD43. We generated transgenic mice expressing a mutant form of the protein lacking the cytoplasmic region (CD43 Δ IC-GFP) under the control of the distal promoter of Lck, thus favoring expression of the transgene in peripheral T lymphocytes. To evaluate the role of CD43 *in vivo*, on an antigen-specific response, we crossed these mice with OT-I or OT-II transgenic mice. Overall, our results indicate that CD43 Δ IC-GFP functions as a dominant negative molecule, dampening antigen-specific T cell response, but that the mechanism through which this happens is not the same for CD4⁺ and CD8⁺ T lymphocytes.

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F.118. Flow Cytometry: Identifying Biomarkers for the Diagnosis of Organ-specific Autoimmune Diseases and Response to Treatment

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In the last years, the significant development in the field of biomedical research has lead to the need to define new biomarkers for diagnosis, prognosis or monitoring of diseases. Multiparametric flow cytometry has been positioned as one of the most useful technologies for monitoring immune-mediated diseases.

For this purpose we designed an exhaustive flow cytometry panel which allows to analyse minor lymphocyte subpopulations in peripheral blood, and validated it in several autoimmune diseases.

- 1) We found changes in peripheral blood lymphocyte compartments of type 1 diabetes patients at onset of the disease.
- 2) In Graves' disease patients, a different pattern of lymphocyte subpopulations was identified in patients clinically stable who maintain the presence of anti-TSH autoantibodies, compared to those without autoantibodies.
- 3) In Multiple sclerosis, we found changes in lymphocyte subpopulations identified in untreated relapsing-remitting patients and progressive forms compared with healthy donors. The influence of immunomodulatory therapies on lymphocyte subpopulations was also analysed in a cross-sectional study.

Analysing the influence of therapies on lymphocyte subpopulations, we identified in a prospective study that Multiple sclerosis patients treated with fingolimod had different patterns of subpopulations, able to discriminate responders versus non-responders to the therapy, in a 12 month follow-up.

In conclusion, characterization of minor lymphocyte subpopulations in peripheral blood, by multiparametric flow cytometry, is a useful tool to identify potential biomarkers for the diagnosis and response to treatment of organ-specific autoimmune diseases, and by extension to other immune-mediated diseases.

T.33. Differential Expression CD16 Epitopes Recognized by Clones 3G8 and B73.1 on Resting and Activated Natural Killer Cells.

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Human CD16 and CD56 are commonly used in combination for the identification of natural killer (NK) cells. Several clones of each are widely available for multiple applications, including IVD diagnostics. However, published data demonstrates that CD16 clones B73.1 and 3G8 recognize different epitopes. Here we show that co-staining with CD56 and both clones of CD16 may detect different frequencies of NK cells, *ex vivo*. In some cases, clone 3G8 yielded a higher frequency of CD56⁺CD16⁺ NK cells in peripheral blood. Because CD16 is known to be shed upon NK cell activation, we evaluated whether B73.1 epitope was specifically shed as a result of NK cell activation. Upon stimulation with PMA and ionomycin or combination of IL-12, IL-15, and IL-18, CD56⁺ NK cells predominantly shed B73.1 whereas 3G8 positive NK cells were still detected with lower signal intensity. Differential expression of 3G8 and B73.1 epitopes on resting and activated NK cells emphasizes the importance of clone selection during assay development, especially in applications such as cells sorting, enrichment/depletion assays and diagnostic assays.

T.41. Peripheral Lymphocyte Subpopulations at Onset of Type 1 Diabetes: Imbalance of Naïve and Memory T Cells

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Introduction: Type 1 diabetes (T1D) is an autoimmune disorder characterized by destruction of pancreatic beta cells resulting in insulin dependency. Changes in T and B cell subpopulations in peripheral blood of T1D patients have been described, but a comprehensive multiparametric flow cytometric analysis is still lacking.

Aim: To identify changes in peripheral blood T- and B- cell compartments in patients at onset of T1D.

Material and methods: CD4⁺ and CD8⁺ T cells (including naïve, central memory, effector memory and terminally differentiated effector (TEMRA), Th17 and Tregs) and B cells subsets (naïve, unswitched memory, switched memory and transitional B cells) were analyzed in peripheral blood of T1D patients at onset (n=26) and healthy donors (HD; n=40) using multiparametric flow cytometry.

Results: A decrease in the percentage of early and late effector memory CD4⁺ and CD8⁺ T cells (TCD4⁺: p=0.001 and p<0.001, TCD8⁺: p=0.046 and p<0.001), TEMRA CD4⁺ and CD8⁺ cells (p=0.003 and p=0.004, respectively) was found. In contrast, the percentage of naïve CD4⁺ T cells (p=0.010), and percentage and absolute counts of naïve CD8⁺ T cells (p<0.001 and p=0.001) were increased in peripheral blood of T1D patients compared with HD. Moreover, an increase in percentage of total B cells and a decrease of transitional B cells was observed in patients compared with HD (p=0.015 and p=0.006, respectively). No changes were found either in Tregs or in Th17 subpopulations.

Conclusion: The observed changes in the percentage and/or absolute number of lymphocyte subpopulations support that effector cells migrate to the pancreas participating in the autoimmune response.

T.43. TotalSeq™, Standardized Oligonucleotide Barcode Antibody Conjugates, and Veri-Cells™ PBMC for Multiplex Immunophenotyping by Single Cell Sequencing

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Single-cell analysis strategies are increasingly employed in studying complex cell populations, such as immune cells responding to infections or tumors, or cell lineage differentiation, etc. The CITE-Seq (Cellular Indexing of Transcriptomes and Epitopes by Sequencing) platform is a recent advance in single-cell analysis, which is based on high-throughput single-cell sequencing (scSeq) and combines measurements of cellular proteins and transcriptomes. This platform will potentially transform how such complex cell populations are analyzed. Published data indicated that combination of oligonucleotide barcode-conjugated antibodies and single-cell sequencing analysis on cell surface marker expression is comparable to multi-color flow cytometry, but provides superior multiplexing capabilities. The availability of standardized oligonucleotide barcode-labeled antibodies as well as control cells can enable reliable comparison of data across longitudinal and multi-site studies. TotalSeq™ is a new product family employing monoclonal antibodies conjugated with unique oligonucleotide barcodes. After assigning a unique oligonucleotide barcode to each of our monoclonal antibodies, we prepared the conjugated reagents. The standardized barcoding system, and ready-to-use antibody conjugates support scSeq based multiplex immunophenotyping. Veri-Cells™ is a proprietary lyophilized cell product that can be used as a control for detection of cell surface and intracellular proteins by flow cytometry. In this study, TotalSeq™ products are validated on the scSeq technology platform, and Veri-Cells™ PBMCs are used as controls vs freshly isolated PBMC in the assays.

T.94. Anti-TNF Therapy in Spondyloarthritis: Prediction of Therapeutic Responses Using Immunological Signatures

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Anti-TNF therapy has proven effective to reduce inflammation and clinical symptoms in several chronic inflammatory diseases. However, not all patients respond to TNF-blockers and it is currently not possible to predict responsiveness of patients to anti-TNF therapy. The goals of this project were to define the impact of anti-TNF therapy on immune responses in patients, and to identify immunological correlates associated with therapeutic responses to TNF-blockers.

Using whole-blood, syringe-based assays to perform *ex vivo* stimulation while preserving physiological cellular interactions (TruCulture assays), we investigated immune responses to microbial challenges and stimuli targeting specific immune pathways before and after anti-TNF therapy in two independent cohorts of axial spondyloarthritis patients.

We observed a highly significant reduction of the secretion of several pro-inflammatory cytokines and chemokines in response to selected stimuli after 3 months of treatment compared to baseline. Quantitative set analysis for gene expression (QuSAGE) of the stimulation cultures revealed that TNF-blockers primarily act by disrupting an autoregulatory loop driven by NFκB. Furthermore, we found that patients with the highest disease activity expressed higher levels of NFκB target genes, IL-1 related genes, and multiple integrin genes following stimulation and we noted a positive correlation between a subset of these transcripts and treatment response.

Our study shows that TruCulture assays are an efficient and robust tool to monitor immune functions in patients and that analyzing immune responses in patients before therapy is a promising strategy to develop biomarkers for disease activity and for prediction of therapeutic responses to TNF-blockers.

T.99. Immune Monitoring of CD34⁺ Stem Cell Derived Natural Killer Cell Therapy (PNK-007) in Phase I Study of Acute Myeloid Leukemia

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Celularity developed a GMP procedure for generating placenta-derived Natural Killer cells (PNK-007) from cord blood CD34⁺ cells with substantial cytotoxic activity against various cancer cell lines. PNK-007 is being evaluated for the treatment of relapsed and/or refractory AML patients following Cy-Flu conditioning in a Phase I trial. In addition to PNK-007 safety assessment, maximum tolerated dose and potential clinical efficacy, we monitored PNK-007 *in vivo* persistence, expansion and phenotypic characterization in context of immune profile and disease state of treated subjects.

Our results showed that circulating PNK-007 cells persisted for 7 to 28 days after infusing $\geq 3 \times 10^6$ cells/kg. Sustained Nkp30, Nkp46, and DNAM-1 expression occurred while CD94 and CD16 increased relative to the pre-infusion product. PNK-007 also expressed CD57 and KIRs unlike the pre-infusion cell product, suggesting further maturation *in vivo*. Upon re-stimulation, persistent PNK-007 cells stained positive for granzyme B, perforin, IFN γ , but not TNF α , indicative of maintained effector functions post infusion.

During 28 days following conditioning, reconstitution of normal myeloid and lymphoid populations was poor in subjects. At day 7, 35 to 65% of T cells were CD4⁺Foxp3⁺ suggestive of regulatory T cells. Elevated PD-1 expression was observed in proliferating CD4⁺ and CD8⁺ T cells. Within the myeloid compartment, a significant portion of cells were MHC II deficient myeloid-derived suppressor cells. Our data highlight limited normal hematopoiesis in conditioned AML patients and the presence of immune-suppressed subpopulations. The results presented here will be used to design further trials in AML, may broadly impact cellular immunotherapy.

T.100. Analysis of Infiltrating B Cell Populations in the Tumor Microenvironment Using Single Cell Transcriptomics

Sarah Taylor, Stéphane C. Boutet, Valeria Giangarrá, Grace X.Y. Zheng, Alvaro M. Barrio, Luz Montesclaros, Josephine Lee, Samuel Marrs, Kevin J. Wu, Paul Ryvkin, Tarjei Mikkelsen and Deanna M. Church
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Understanding the complex interactions between cells in the tumor microenvironment (TME) requires the ability to distinguish each cell type, and is prerequisite to enabling personalized cancer treatments. Analysis of gene expression at a single cell level is required to obtain a high-resolution understanding of the cell populations that contribute to the TME. Here, we use a fully integrated, system for single cell RNA sequencing, to simultaneously profile the transcriptome and immune repertoire of the same cells from primary colorectal cancer (CRC), and non-small cell lung cancer (NSCLC) tumors. Each tumor varied in type and proportion of its cellular components, noticeably in the proportion of TILs. The lymphocyte population in the CRC tumor consisted of both T (3% CD4⁺, 3% CD8⁺) and B cells (5% CD79A⁺), with a cluster of plasma B cells (11% IGH high). Intersection of these data with the repertoire sequencing data identified a clonal expansion, contributing >4% of all B cell clonotypes, suggesting that tumor-specific antibodies were being generated. The NSCLC tumor had a much higher immune cell infiltrate, containing predominantly B cells (30% CD79A⁺ and 8% IGH high plasma B), however, intersection with the repertoire sequencing data indicated very limited clonal expansion. These findings emphasize the importance of combining repertoire and gene expression sequencing data to determine the nature and clonality of an immune response. This technology enables full characterization of tumor heterogeneity and the adaptive immune response to the TME and will serve as a foundation for future research into tumor immunology and immunotherapy.

T.115. All-in-One, Quantitative Immune Repertoire Profiling from Challenging Samples

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Next generation sequencing of the immune repertoire allows detailed, sequence-specific insight into the immune system's adaptive response to environmental challenges. Immune repertoire analysis when applied to bulk RNA typically focuses on one of the receptor chains, such as TCR-beta, TCR-alpha, BCR-IgH, or BCR-IgKL. Here, we present the application of a novel PCR technique, dimer-avoided-multiplex PCR (dam-PCR), to the all-in-one repertoire amplification of BCRs and TCRs in a single, quantitative multiplex reaction. Specifically, we apply this method to the amplification of both FFPE and PBMC RNA using a multiplex primer system covering all TCR and BCR loci including TCR-beta, TCR-alpha, TCR-delta, TCR-gamma, BCR-IgH, and BCR-IgKL, and over 100 additional phenotyping gene-markers. First, we demonstrate the ability of this strategy to cover a synthetic immune repertoire library. Then, we demonstrate the application to both PBMC and FFPE RNA. The ability to inclusively profile immune-specific RNA from FFPE samples provides the opportunity to interrogate historical repertoire data even in situations where sample RNA may be both limited in quantity and degraded in quality. This single reaction method allows for a cost effective, all-inclusive, and quantitative immune-profiling analysis of immune repertoires from a range of sample types, including single cell, PBMC, sorted cells, and FFPE samples.

W.3. Robust Prediction of Clinical Outcomes Using Cytometry Data

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Flow cytometry and mass cytometry are used to diagnose diseases and to predict clinical outcomes. Here, we propose a novel strategy to predict clinical outcomes using cytometry data without cell gating (CytoDx; accessible at github.com/hzc363/CytoDx). CytoDx is able to predict the response to influenza vaccine using heterogeneous datasets, demonstrating that it is not only accurate but also robust to batch effects and cytometry platforms.

W.12. Deep Immunology Model Learns Healthy Patient Status from Cytometry Fingerprint

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In recent years deep learning has seen numerous applications in biomedicine for the diagnosis and prognosis of disease. However, major developments have yet to be seen using machine learning to better understand high-throughput single-cell cytometry data. Published methods usually attempt to learn a specific disease status as differentiated from normal tissue, but these methods require retraining for every disease. Here we describe a convolutional neural network that learns the healthy "normal" status of a patient based solely on publicly available mass cytometry (CyTOF) data, derived from peripheral blood and obtained through the ImmPort database (immport.org). After being trained on healthy patient tissue data at baseline from the first year of a multi-year study, the network is able to recover the patient ID based solely on the cytometry data from a subsequent year. Additionally, because this patient's "cytometry fingerprint" represents healthy status, we are able to interpret deviations from this fingerprint as a proxy for disease status. This method will be useful for subject identification and the preliminary determination of disease status, so long as the disease changes the proportions or states of circulating immune cells.

W.65. Dried Cells as Controls or Standards

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Preserved cells can be used as positive and process controls. In addition, they can be used as standards for instrument setup or standardization, reagent quality control, panel characterization, and in multi-instrument and multicenter longitudinal studies. Both normal and abnormal controls are desired to confirm that assays and reagents are working appropriately. However, effective and stable control cells are difficult to secure for cell-based assays. The challenges of current preserved cells include short shelf life, deteriorated preservation of labile markers, and decreased resolution of dim populations. Current commonly used preservation methods such as cryopreservation/freezing and lyophilization often cause mechanical destruction of cells, resulting in deteriorated performance of cell markers.

Our new drying technology allows us to minimize the nonspecific binding of some problematic markers and maximize the stain resolutions for dim markers. Several types of dried cells can be made using the technology such as leukocytes, peripheral blood mononuclear cells (PBMCs) and cancer cell lines. Upon reconstitution, the dried leukocytes maintain the resolutions of all relevant populations and more than 175 surface markers are consistently expressed. The dried cells have a long shelf life of 12 months at storage conditions of 2°C to 8°C.

Dried cells can be used as controls and standards for flow cytometry and immunology. As a recent advance in genomics and cell therapy, new biological applications of dried cells are expected to emerge in the near future.

W.70. High Prevalence of *S. Pyogenes* Cas9-specific T Cell Sensitization Within the Adult Human Population - A Balanced Effector/Regulatory T Cell Response

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The field of gene therapy has been galvanized by the discovery of the CRISPR/Cas9 system. Immunity against therapeutic gene vectors or gene-modifying cargo nullifies the effect of a possible curative treatment and may pose significant safety issues. Mice treated with CRISPR/Cas9-encoding vectors exhibit humoral and cellular immune responses against the Cas9 protein, that impact the efficacy of treatment and can cause tissue damage. Most applications aim to temporarily express the Cas9 nuclease in or deliver the protein directly into the target cell. Thus, a putative humoral antibody response may be negligible. However, peptide presentation of Cas9-fragments on the surface of gene-edited cells may be recognized by T cells. While a primary T cell response could be easily prevented or delayed, a pre-existing memory would have major impact. Here, we show the presence of a ubiquitous memory/effector T cell response directed towards most popular Cas9 homolog from *S.pyogenes* (SpCas9) within healthy human subjects. We characterized SpCas9-reactive memory/effector T cells (T_{EFF}) within the CD4/CD8 compartments for multi-effector potency and lineage determination. Intriguingly, high frequencies of SpCas9-specific regulatory T cells (T_{REG}) were also detected. The frequency of SpCas9-reactive T_{REG} inversely correlates with the respective T_{EFF} response. SpCas9-specific T_{REG} may be harnessed to ensure the success of SpCas9-mediated gene therapy by combating undesired T_{EFF} response *in vivo*. Furthermore, the Cas9-specific T_{EFF}/T_{REG} balance may have importance in *S.pyogenes*-associated diseases. Our results shed light on the T cell mediated immunity towards SpCas9 and offer a possible solution to overcome the problem of pre-existing immunity.

W.74. A New, Positive Control Peptide Pool with Broad Infectious Antigen and MHC Coverage that may be Useful for Assessing Anti-Infectious T-cell Responsiveness

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Positive stimulation controls are essential for ascertaining functionality of T-cells following activation in assays like ELISPOT/Intracellular Cytokine Staining (ICS). Whereas conventional positive controls often consist of polyclonal stimulators (e.g., PHA or PMA/Ionomycin), peptide-based positive controls provide a more physiological, TCR-mediated signal, better replicating antigens of interest used in such assays. The CEF peptide pool, consisting of 23 class-I-MHC-presented peptides from CMV, EBV, & Influenza A virus, is frequently used as this latter type of positive stimulation control. Because of frequent, widespread exposure to these antigens among humans, most individuals have T-cells responding to one or more peptides in this pool. However, these peptides were originally selected to bind the most frequent HLA alleles in European-Caucasoids but not other ethnic groups, thus the CEF pool can be suboptimal when applied to the latter population. We compiled a significantly extended pool (176 peptides), referred to as the CEFX, that provides increased coverage of infectious agents and HLA-alleles. Unlike the CEF, it also contains peptides stimulating CD4 T-cells. Thus, our new CEFX pool represents a more universal positive control than the original 'CEF' pool. The CEFX pool was successfully tested in various assay formats including ELISPOT, ICS, and ELISA of stimulated T-cell supernatants. Sub-pools of CEFX peptides selected for preferential CD4 or CD8 T-cell stimulation or excluding CMV-derived peptides are also available. In summary, CEFX's broad antigen and HLA coverage offers improved potential for measuring/monitoring general T-cell responsiveness in situations where reduced immunity may be present, e.g., immunodeficiency, immunosuppression, or immunosenescence.

Immunity & infection

F.13. Transcriptional Signatures of Influenza Vaccine Immune Responses in Elderly Individuals

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The goal of this study was to identify transcriptomic signatures associated with humoral immune responses after influenza vaccination in older individuals. We used pre-vaccination (Day 0) and post-vaccination (Days 3 and 28) blood samples from 159 subjects (50-74 yo) following the receipt of influenza A/H1N1 vaccine. Penalized regression modelling was used to identify associations between influenza-specific immune responses (HAI/VNA titers/memory B-cells) and changes in gene expression (mRNA-sequencing). The median age of the subjects was 59.5 (IQR 55.3, 66.3) years. The innate (Day 3-Day 0) genes associated with HAI include a number of uncharacterized genes (*SGCD/ALG10/PABPC4*). The innate genes associated with VNA include genes that play a role in pathogen recognition/activation of innate immunity (*TLR8/ADARB2/ZFP36*). The adaptive (Day 28-Day 0) genes associated with HAI are chemokines/cytokines/receptors (*CCR9/IFNG/IL10RA*). The genes associated with VNA confirmed previous studies demonstrating genes that are mediators of host-immunity, including TNF-ligand *TNFSF11*, cytokines/receptors (*IFNG/IL7/IL27/IL12A*), and IFN-inducible transcription factors (*IRF7,9*). The adaptive (Day 28-Day 3) genes associated with HAI are genes that are involved in the IFN-g production pathway (*FOXP3/TLRs/CEBPG/EB13/IL12A*). The genes associated with VNA are TLR3,7,9, cytokines (*IL1/TGFB2/IL12A*), STAT/tyrosine kinases (*STAT1/STAT3/TYK2*). The Day 28-Day 0 genes associated with B-cell responses are genes involved in cholesterol/lipid/carbohydrate metabolism (*MDV/PMVK/PI4KA/DHRS13*) and cell-signalling (*NF-kB*). Using a systems biology approach, we identified innate/adaptive gene signatures associated with inter-individual variations in humoral responses to influenza vaccine.

The identification of these gene signatures may provide a better understanding of genetic markers of immunity in the elderly, and may assist with the design of better vaccines/adjuvants.

F.18. Role of CD1d Receptor in the Pathology Caused by the Human Respiratory Syncytial Virus and the Human Metapneumovirus

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Human respiratory syncytial virus (hRSV) and human Metapneumovirus (hMPV) cause acute respiratory tract infections in children worldwide. Natural killer T (NKT) cells are unconventional T cell lymphocytes and their TCRs recognize glycolipids bound to the MHC-I-like CD1d molecule. We evaluated the contribution of CD1d and the role of these NKT cells on both hRSV and hMPV infections. A significant decrease of neutrophils and CD103⁺ DCs infiltration to the lungs, and higher levels of IFN- γ were found in hRSV- but not in hMPV-infected CD1d^{-/-} mice as compared to hRSV-infected CD1d^{+/+} mice, being similar to the mock-treated mice. To better understand how NKT cells modulate T cell immunity during these infections, dendritic cells (DCs) were infected with hRSV or hMPV, pulsed with different concentrations of α -GalCer and co-cultured with NKT cells. Interestingly, hRSV- and hMPV-infected DCs led to reduce levels of IL-2 secretion by NKT cells, compared to mock-treated DCs. Our data suggest that CD1d^{-/-} mice are more resistant to hRSV- but not hMPV-infection, thereby NKT cells induced by hRSV may be detrimental for the outcome of the infection. Funding: CONICYT 3160249, FONDECYT 1070352; FONDEF D11E1080; Millennium Institute on Immunology and Immunotherapy (P09/016-F).

F.20. Influenza A Virus (IAV) Infection in Humans Leads to Expansion of Highly Diverse CD8 T Cell Repertoires Cross-reactive with 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 cross-reactive responses would lead to alterations in the IAV-M1₅₈, EBV-BRLF1₁₀₉ and -BMLF1₂₈₀ TCR repertoires from before and during 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 donor. Furthermore, by using next generation sequencing and single-cell analysis of TCR α and TCR β repertoire of tetramer sorted IAV-M1 cells we showed dramatic changes in specific clonotype 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 cross-reactive responses between IAV and the persistent virus, EBV, which leads to permanent changes in TCR repertoires to both of these two viruses (NIH AI049320 and NIH AI109858).

F.30. Human Memory CD8 T Cell Effector Potential is Epigenetically Preserved During *In Vivo* Homeostasis

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Maintenance of memory CD8 T cell quantity and quality through antigen-independent homeostatic proliferation is vital for sustaining long-lived T cell-mediated immunity, yet the underlying mechanisms that preserve memory T cell functions during homeostasis remain largely unexplored. Here we show that preservation of effector-potential among human memory CD8 T cells during *in vitro* and *in vivo* homeostasis is coupled to maintenance of memory-associated DNA methylation programs. Whole-genome bisulfite sequencing of primary human naïve, short-lived effector memory (T_{em}), and longer-lived central memory (T_{cm}) and stem cell memory (T_{scm}) CD8 T cells identified demethylated promoters of effector molecules that are poised for rapid expression among all memory cell subsets. Effector-loci demethylation was heritably preserved during IL-7 and IL-15 mediated *in vitro* cell proliferation. In contrast to the effector-potential, antigen-independent proliferation induced a phenotypic conversion of T_{cm} and T_{scm} memory cells into T_{em} cells that was coupled to increased methylation of the CCR7 locus. Furthermore, *in vivo* proliferation of haploidentical donor memory CD8 T cells in lymphodepleted recipients resulted in a similar preservation of effector-associated methylation programs while enriching for T_{em}-associated programs. These data demonstrate that long-lived human memory CD8 T cells retain the ability to undergo antigen-independent epigenetic reprogramming during their developmental conversion into other memory subsets while at the same time preserving the poised effector state utilized by all memory T cells. Further investigation into upstream signaling events that promote changes in T cell epigenetic states is needed to uncover the role of epigenetics in T cell function during homeostasis.

F.49. Immune Paralysis and Lymphopenia is Associated with Adenosine A1 Receptor Dysfunction

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Most septic patients survive the initial hyper-inflammatory phase of this disease, yet end up in intensive care unit with sepsis-induced immunosuppression. As adenosine is elevated in septic patients, we posit that elevated adenosine levels in sepsis affects the normal development of lymphocytes through down-regulation of A₁R and involved in the induction of immune paralysis. Therefore, cecal ligation and puncture (CLP) was used to induce sepsis in mice. In addition, bone marrow-derived dendritic cells (BMDCs) were cultured and used to examine the effect of adenosine and its receptors (A₁R and A_{2a}R) on IL-15. We found that A₁R mRNA levels were significantly downregulated and A₁R-dependent Gi activity was abolished in T cells of septic mice. In accordance, cAMP was elevated in isolated T cells from CLP-treated animals compared to sham mice. Similar to septic mice, leukopenia was evident in sham A₁R-KO mice, or following treatment with the A₁R antagonist (DPCPX), or after A₁R desensitization. In contrast, A_{2a}R-KO mice were protected from leukopenia following CLP procedure. Septic A₁R-KO mice had reduced IL-15 levels in peritoneal lavage. To conclude, we suggest that sepsis-associated lymphopenia is initiated by A₁R desensitization and that adenosine-mediated inhibition of IL-15 production is part of the mechanism accounting for the delay in leukopenia recovery in severely septic patients. Interference with adenosine signaling may thus be potentially beneficial for leukopenic septic patients. Understanding the mechanism in which immunosuppression occurs may help to understand (and treat) other diseases in which the immune system is suppressed and a similar mechanism is activated.

F.70. Molecular Profiling of Human Regulatory T Cell Subsets Supports a Linear Differentiation Model and Identifies a Novel Regulatory T Cell Population with Stem Cell-like Properties

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Thymic-derived CD4⁺CD25^{high}FoxP3⁺ regulatory T cells (tTREG) represent a unique T cell lineage with the ability for negative regulation of inflammation. Whereas the concept of linear differentiation of immunological memory subsets within pro-inflammatory T cell compartments is well defined, it remains unclear whether tTREG show similar subset plasticity with various sub-populations displaying distinct functional features. The presence of persistently expanding antigen-specific tTREG, protecting against aberrant immune responses, implies the existence of tTREG memory.

However, the lack of definitive markers impedes the phenotypic and functional classification of memory tTREG. The differentiation and memory formation of effector T cells is defined by rigid molecular pathways and lineage regulators for inducing epigenetic changes. In addition, classical cell surface markers may be used to broadly classify T cell subsets of distinct differentiation states.

Here, we applied conventional memory T cell-defining marker profiles of CCR7 and CD45RA expression to tTREG.

Phenotypically, we demarcated naïve-, central memory- and effector memory-like tTREG compartments as functionally distinct subsets within the bulk tTREG population. Intriguingly, we identified most diverse T cell receptor usage in phenotypically naïve-like tTREG, followed by phenotypically memory-like tTREG subsets in a linear pattern. Epigenetic profiling revealed highest DNA methylation in tTREG with early differentiated phenotypes. Furthermore, we could identify a novel stem cell memory-like tTREG subset with superior suppressive function compared to other tTREG subsets.

Positive correlation of conventional T cell and tTREG subsets may demonstrate a synchronized memory formation in the effector and regulatory compartment. Our findings may have direct implication for adoptive tTREG therapy.

F.77. CD161 Promotes Prenatal Immune Suppression of Human IFN γ -producing PLZF⁺ T Cells

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While the fetal immune system exerts a strong program of tolerance *in utero*, the concurrent development of protective T cell immunity necessary for survival after birth points to a remarkable adaptation during human fetal immune development. However, disruption of T cell tolerance in favor of activation leads to fetal inflammation and the termination of pregnancy. As mucosal surfaces are a critical interface in the initiation of inflammation, we examined human T cell immunity in fetal lymphoid and mucosal tissues. We found a fetal-specific population of PLZF⁺ CD4⁺ T cells which accumulated in the small intestine. RNA sequencing revealed that intestinal PLZF⁺ T cells are distinct from either innate or conventional T cells and identified a core gene signature for PLZF⁺ T cells. Additionally, PLZF⁺ T cells possess a unique functional profile defined by Th1-cytokine production and are the predominant source of IFN γ in the human fetal immune system. The presence of fetal T cells with inflammatory potential suggested the existence of natural regulatory mechanisms to prevent inflammation. We found that the majority of intestinal CD4⁺ PLZF⁺ T cells expressed the C-type lectin CD161, ligation of which inhibited IFN γ production in response to T cell receptor activation. Lastly, we discovered that LLT1, the ligand for CD161, is specifically expressed on intestinal macrophages. In sum, this study demonstrates that the origins of protective immunity necessary for survival after birth originate *in utero*, and identifies unique fetal-

specific mechanisms of immune suppression that include the regulation of CD4⁺ PLZF⁺ T cells.

F.80. Pioneering Bacteria in Fetal Meconium Drive Divergent Intestinal T Cell Development

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The uterine environment may not be sterile, because bacteria were detected in the human placenta and meconium. We find that the majority of $\alpha\beta$ T cells in the human fetal intestinal lamina propria (LP) possess a memory phenotype and express the transcription factor PLZF. This led us to hypothesize that bacterial exposure *in utero* regulates intestinal T cell development. In high-quality 16S rRNA sequencing datasets, we found a sparse fetal meconium microbiota dominated either by *Micrococcus* or *Lactobacillus*. Parallel profiling of intestinal LP T cells revealed that *Micrococcus* and *Lactobacillus* presence in meconium was highly associated with the proportion of memory PLZF⁺ T cells and T regulatory cells (Tregs), respectively. *Micrococcus* and *Lactobacillus* were viably isolated from fetal meconium, exposed to sorted fetal splenic antigen presenting cells (APCs), and co-incubated with sorted LP T cells. In contrast to respective ATCC reference strains or unexposed APCs, PLZF⁺ memory T cells exhibited preferential proliferation in response to fetal *Micrococcus* isolate, while *Lactobacillus* isolates specifically increased Treg proportions. *Micrococcus* and *Lactobacillus* drive divergent T cell phenotypes in part by regulation of APC phenotypes. *Micrococcus* induced APC expression of C-type lectin LLT1, which is the natural ligand for CD161, a highly expressed receptor on fetal intestinal PLZF⁺ T cells that inhibits their inflammatory ability. In contrast, *Lactobacillus* induces the expression of CD103 on APCs, which regulates Treg expansion. Thus, pioneering commensals reinforce the program of immunotolerance required for fetal survival, while also priming the T cell memory compartment for bacterial encounter at birth.

F.85. Adjuvant Treatment with a Dialyzable Leukocytes Extract Contributes to Maintain HPV-infected Women Free of Low-grade Cervical Lesions.

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HPV infection, high number of circulating regulatory T cells and low Th1 cytokines *in vivo* production suggests an impaired T cell immunity in patients with pre-cancerous cervical lesions. Patients having low grade cervical lesions diagnosed for the first time were randomly separated in two groups and treated either with surgery (Group A) or with a dialyzable extract of human leucocytes, known as transfer factor (TF, Group B) to investigate if TF may help to restore their T cell immunity balance. Another group of patients with low grade but recurrent cervical lesions was newly treated both with surgery and TF (Group C). CD3⁺ CD4⁺ CD25⁺ levels were higher in the patients than in non-HPV-infected, age-matched, healthy women ($p < 0.05$). Group C had the highest number of CD3⁺ CD4⁺ CD25⁺ and CD3⁺ IL-10⁺ cells before and after treatment. A decreased or absent cervical lesion correlated with a diminished or absent HPV viral load at 1 year of treatment ($r = 0.6$, $p < 0.05$). More importantly, 79 % of patients in group B were free of cervical lesion after 24 months of treatment whereas only 50 % of group C and 38 % of group A patients were also lesion free at that time ($p < 0.05$, B vs A or C). Our data support the value of monitoring levels of CD4⁺ CD25⁺ and CD3⁺ IL-10⁺ peripheral T cells and the administration of TF as adjuvant treatment for HPV-infected women, either having first time or recurrent low grade cervical lesions.

F.91. Antibodies Secretion Promoted by a Recombinant BCG Strain Induces a Protective Immune Response Against Human Respiratory Viruses

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Both the Human Respiratory Syncytial Virus (hRSV) and the Human Metapneumovirus (hMPV) are considered two major etiological agents for acute lower respiratory tract infections (ALRTIs) worldwide. Currently, there are not licensed vaccines for either of those viruses. We have developed two different recombinant *Mycobacterium bovis* Bacillus Calmette-Guérin (BCG) strains as vaccine candidates expressing the hRSV Nucleoprotein (rBCG-N) or the hMPV Phosphoprotein (rBCG-P). These vaccines are able to induce cellular protection against each respective virus. Here, we show that the humoral immune response induced by both recombinant BCG vaccines is able to protect against the viral infection promoting the secretion of specific antibodies against each virus, recognizing various specific proteins through classical linked recognition. Also, we identified that both vaccines promoted an effective antiviral immunity by inducing neutralizing antibody capacity, as well as sera transfer protection *in vivo*. Our results support the notion that the use of a recombinant BCG vaccines could be considered as a new platform against these two major respiratory pathogens.

Key Words: Recombinant BCG, vaccine, respiratory disease, HRSV, HMPV, humoral immune response.

Funding: Millennium Institute of Immunology and Immunotherapy.

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F.102. IL-10 Production by Myeloid Cells is Essential to Keep the Lung Integrity During Infections Caused by the Major Pathogenic Bacteria *Klebsiella Pneumoniae* ST258 and *Streptococcus Pneumoniae*.

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Interleukin-10 (IL-10) is as an anti-inflammatory cytokine that down modulates inflammatory immune response at multiple levels. Several studies have reported the role of IL-10 during bacterial infections. In general, the production of IL-10 down modulates the immune response, impairing bacterial clearance but protecting the host tissue from irreversible injuries. Interestingly, as a consequence of IL-10 production, we can observe an improved or an impaired survival in different infection models. Currently, there are not enough data available to predict whether the production of IL-10 will be beneficial or harmful for the host. In this work, we evaluate the role of IL-10 production in the lungs during an experimental infection with two major pathogenic bacteria: *Streptococcus pneumoniae* D39 strain and Carbapenem-Resistant *Klebsiella pneumoniae*. Despite the most important myeloid cellular source of IL-10 in infected lungs was different between these two pathogens, the absence of IL-10 lead to an increased mortality, accompanied with elevated pro-inflammatory cytokine production, higher lung damage and aberrant neutrophil recruitment or function during the first 48 hpi. Our data demonstrate that during pulmonary infections caused by two different pathogens, the production of IL-10 is essential to favors host survival.

F.103. Epigenetic and Transcriptomic Signatures Associated with Antibiotic-Persistent MRSA Bacteremia

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Methicillin-resistant *Staphylococcus aureus* (MRSA) infection is a major global healthcare problem. Of concern is antibiotic-persistent MRSA bacteremia (APMB), which can cause complicated invasive and metastatic infections such as infective endocarditis and organ abscesses. Moreover, persistent MRSA infections are refractory to antibiotic treatment *in vivo*, even though most such isolates are susceptible *in vitro* to gold-standard agents such as vancomycin. These facts suggest that host-pathogen-antibiotic interactions *in vivo* play crucial roles in the emergence of MRSA persistence. The current study focuses on profiling host signatures associated with APMB clinical outcomes. Biospecimens from thirty-four APMB cases were matched on propensity analysis with those from antibiotic-resolving MRSA bacteremia (ARMB) samples as a training set. Host immune responses were profiled from whole-blood DNA methylome and transcriptome studies using reduced-representative bisulfite sequencing (RRBS) and RNAseq, respectively. Among 0.9 million CpG sites detected by RRBS, 1052 differentially methylated sites (DMS) were identified using a multivariate logistic regression model integrating gender, race and age as covariates. The biological functions of DMS assigned using the GREAT tool were associated with immune responses to inflammation, cytokines, and bacterial infection. Partial least squares regression analysis to determine differentially expressed genes (DEGs) revealed DEGs enriched in APMB include lymphocyte migration, chemotaxis, and T lymphocyte activation. In summary, we identified host immune signatures associated with APMB that may provide predictive indicators applicable to clinical decision-making for improved treatment outcomes.

F.105. Effect of 17 β -estradiol, Progesterone and Prolactin on the Infective Capacity of *Toxoplasma gondii*, Cytokine Modulation and the Expression of Hormonal Receptors on THP-1 Cells

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Toxoplasma gondii is an intracellular protozoan common in pregnancy. There are changes in the concentration of 17 β -estradiol (E2), progesterone (P4) and prolactin (PRL) during pregnancy. Proinflammatory responses reduce the susceptibility of infection, and these may change according to hormonal impairment. Monocytes and macrophages are the main barrier against protozoan, due to their ability to produce cytokines. The aim of this work was to determine the effect of E2, P4 and PRL on the infectivity of *T.gondii*, proinflammatory response modulation, and the expression of hormonal receptors on THP-1 cell stimulated with *T.gondii*. THP-1 cells were infected with *T.gondii* tachyzoites. Stimuli were conducted with PRL, E2 and P4. MTT assays were performed to evaluate cellular viability. Western blot were carried out to evaluate the expression of the hormonal receptors (PRLR, ER α and ER β). Cytokines were measured with a magnetic bead kit. Stimuli with E2 and P4 increased *T.gondii* infection in monocytes, unlike PRL stimulus. E2 decreased the secretion of IL-12 and IL-1 β in infected THP-1 cells, while PRL did not have any effect; however, both hormones increased the production of IL-10. PRL augmented the production of IL-4 and IL-13. In contrast, P4 reduced these cytokines. Our results show that *T.gondii* induces the expression of ER α and ER β and lowers PRLR. The hormones modify the expression of the receptors: P4 decreases PRLR, ER β and increases ER α ; E2 diminishes PRLR; and PRL decreases ER α and ER β expression. The hormones can increase *T.gondii* infection and it could be by an anti-

inflammatory response in THP-1 cells

F.106. CD8 T Cell Memory Frequency and Repertoires to Influenza and Epstein Barr Virus Co-evolve with Increasing Age from Young Adult to Elderly

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CD8⁺ memory T cells are generated during primary infection with intracellular pathogens, such as viruses. These cells play an important role in the protection of the host upon re-infection with the same pathogen. In this study, we compare CD8⁺ memory T cell responses to both influenza A virus (IAV), a recurrent virus that infects millions per year, and Epstein Barr Virus (EBV), a persistent virus which resides in 95% of the population. Using EBV seropositive, HLA-A2⁺, young adult (18-20 years), middle-aged (30-65 years), and elderly (>65 years) donors this study demonstrates that CD8⁺ memory T cell responses to both recurrent and persistent viruses co-evolve as an individual ages. Tetramers/dextramer-staining was used to study both cross-reactive and antigen-specific cells that were present in peripheral blood and proliferated in response to stimulation with immuno-dominant epitopes of IAV and EBV. Interestingly, there was an increase in the frequency of EBV-BMLF1-specific responses, which directly correlated with age. The epitope-specific T cell receptor (TCR) V β repertoire showed that the V β family usage was continuously changing and narrowing for IAV-M158, EBV-BMLF1280, and EBV-BRLF1190 from young adult to elderly donors. There was evidence that the pattern of cross-reactive responses between these two viruses differed between young adult and elderly donors with the narrowing TCR V β repertoire focusing on greater crossreactivity. This study further emphasizes the complexity of human T cell responses to viruses and the need for a better understanding of human T cell responses in order to design successful T cell inducing vaccines.

T.29. Ex Vivo Generation and Single-Cell Analysis of Human Monoclonal Antibodies from Dengue Virus Infected Patients

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Antibodies have been implicated in both protection and pathology of dengue virus infections. However, much of this data is gathered from serum/plasma responses that is a cumulative of historical and ongoing infection. To precisely understand the role of antibodies with respect to the ongoing dengue virus infection, we employed the cutting edge approach of generating of human monoclonal antibodies from individual plasmablasts from peripheral blood of dengue patients that allows us to probe for answers at a single cell level.

This method involves *ex vivo* single cell sorting of plasmablasts from peripheral blood of well-characterized dengue infected patient followed by single cell molecular cloning of immunoglobulin heavy- and light- variable regions into expression vectors containing the defined constant region followed by transient co-transfection of HEK 293A cells with the heavy and light chain expression vectors made from genes arising from the same cell.

Thus far, using this powerful technology, for the first time in India, we have made 73 number of human monoclonals, of which 36 are specific to dengue and 12 neutralize dengue and zika virus at various concentrations. All the neutralizing antibodies are dengue-envelope specific and bind the highly conserved fusion loop of the dengue virus envelope. Together, with the ongoing comprehensive analysis of the B cell repertoire and somatic hypermutations, these studies provide a detailed understanding of the dengue-specific plasmablast cell response at a single cell level and create a platform for testing these antibodies for basic research, diagnostic, prophylactic and as well as therapeutic applications.

T.34. Extended Phenotypic Immunome Characterization (EPIC): A Reference Atlas of the Changing Immune Landscape from Birth to Adulthood

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A developmental immune reference atlas is key to understanding the normal maturation process and identifying disease-associated cell subsets. The lack of a holistic developmental immune normogram is a critical unmet need. We hypothesise that there are mechanistically important, age-related cell subsets changes that shape the immune landscape.

We interrogated the peripheral blood mononuclear cells from 126 healthy individuals with CyTOF with extensive antibody panels embracing the most important cell lineages and their function. Quality control and batch effect correction were performed with outliers excluded before dimensional reduction and clustering (cord blood, newborn to adult) with our analytical pipeline.

Distinct developmental gradients involving multiple cell subsets shaped the maturing immune landscape. The naïve TNF α ⁺ CD4⁺ T cells were enriched in the early childhood period (Pearson correlation coefficient, $r = -0.3669$, $p < 0.001$). In contrast, the memory TNF α ⁺ CD4⁺ T cells increased with age ($r = 0.4224$, $p < 0.0001$). A transitional milestone from naïve to memory TNF α ⁺ CD4⁺ T cells was observed after 2 year old. In the naïve CD8⁺ T cell subset, a transition from IL8 to IFN γ secretion after 12 year old was observed.

Key developmental milestones in the T cell compartment were identified and with the other subsets identified, a holistic description of the developing immune landscape was obtained. This atlas has the dual translational role of defining the stage of immune maturity and distilling the pathological cell subset in diseases. The database and the related pipeline to analyse it will be provided as a free reference.

T.55. The Long Chain Fatty Acid Transporter, MFSD2A, is Essential for Memory CD8⁺ T Cell Formation and Maintenance

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Access to nutrients is critical for an effective T cell immune response to infection. Although transporters for sugars and amino acids have previously been described in the context of the CD8⁺ T cell immune response, the active transport of exogenous esterified fatty acids has remained enigmatic. Here we discovered the long chain fatty acid transporter, Major Facilitator Super Family Domain Containing 2a (MFSD2A), is upregulated on activated CD8⁺ T cells and is essential for their memory cell formation. MFSD2A deficiency resulted in decreased import of long chain fatty acids (LCFAs) esterified to lysophosphatidylcholine (LPC) into activated CD8⁺ T cells but overall resulted in a normal primary effector T cell response. However, loss of MFSD2A led to reduced memory T cell formation and maintenance. MFSD2A deficient memory CD8⁺ T cells showed reduced CD127 and CD62L expression and their cell turnover was significantly impaired in contrast to their wildtype counterparts. Moreover, the secondary response to infection was severely diminished in MFSD2A deficient T cells. Mechanistically, import of LCFAs was required to maintain cell energy requirements and 'fitness'; that when lost resulted in a decreased memory T cell pool and inability to proliferate upon secondary stimulation. Our results show that MFSD2A and LPC may be useful for future small molecule therapy design to generate a more robust T cell response for new vaccines or cancer treatments.

T.63. Phase I Clinical Trial for a Recombinant BCG Expressing the Nucleoprotein of the Human Respiratory Syncytial Virus (hRSV) in Healthy Adults.

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Human respiratory syncytial virus (hRSV) causes lower and upper respiratory tract infections in older adults and young children. Nowadays, no licensed vaccines are available. We have conducted a phase 1 study, double blind and escalated dose to evaluate the safety, tolerability and immunogenicity of *Mycobacterium bovis* BCG vaccine, live attenuated and recombinant for the expression of Nucleoprotein (N) from hRSV (rBCG-N-hRSV) in healthy males aged 18-50 years. Once inclusion criteria were met, volunteers were enrolled in three cohorts in an open and successive cohort design. Each cohort included 6 volunteers vaccinated with the rBCG-N-hRSV and 2 volunteers vaccinated with the standard BCG (BCG-WT) (full dose). Cohort A was vaccinated with 5×10^3 CFU (hundredth of the full dose), cohort B with 5×10^4 CFU (tenth of the full dose) and cohort C with 5×10^5 CFU (full dose) of rBCG-N hRSV. Clinical results indicated that both vaccines, rBCG-N-hRSV and BCG-WT, were safe and well tolerated. Cellular immunogenicity measurements showed that at 14th day after vaccination all volunteers increased the cellular production of IFN- γ and IL-2 upon *in vitro* stimulation with PPD antigen. The cellular response against N protein was increased from the 30th day after vaccination, indicating the induction of specific immune response against antigens included in the vaccine. Further, the amount of specific IgGs against PPD and N in the serum increased at 60th day post-vaccination. In summary, rBCG-N-hRSV showed a good safety profile in healthy adults and induced specific cellular and humoral immune response.

T.80. Tyrosine Phosphorylation of NLRP3 by Src Family Protein Tyrosine Kinase Lyn Suppresses NLRP3 Inflammasome Activation

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The NLRP3 inflammasome is a multi-protein complex that triggers the activation of inflammatory caspase-1 and the maturation of IL-1 β and IL-18 in response to microbes and danger signals in host cells. However, how the NLRP3 inflammasome is regulated is still not fully understood. Here, we show that NLRP3 is tyrosine phosphorylated upon activation of the NLRP3 inflammasome, and that NLRP3 tyrosine phosphorylation correlates with its ubiquitination. Pretreating macrophages with a Src kinase inhibitor abrogates NLRP3 tyrosine phosphorylation and ubiquitination, which leads to enhanced production of IL-1b. Further, we identified Lyn as the protein tyrosine kinase that phosphorylates NLRP3 at Tyr 918, which facilitates its ubiquitination and proteasome-mediated degradation. Consistent with these data, NLRP3 tyrosine phosphorylation and ubiquitination is abrogated in macrophages lacking Lyn, which correlates with heightened IL-1b production. Therefore, our data demonstrate that Lyn-mediated tyrosine phosphorylation of NLRP3 is a prerequisite for its ubiquitination, thus dampening NLRP3 inflammasome activity.

T.84. Serum Level of Soluble Programmed Death Protein-1 as a Predictor of Disease Progression in Patients with Nontuberculous Mycobacterial Lung Disease

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Background: The incidence and importance of nontuberculous mycobacterial lung disease (NTM-LD) have been increasing. Host-pathogen interactions affect the outcomes of NTM-LD. Whether programmed death protein-1 (PD-1), an immune biomarker, is associated with disease progression of NTM-LD is unclear.

Methods: We enrolled 23 untreated patients with NTM-LD from 2016 to 2017, including 11 with *Mycobacterium avium* complex, 8 *M. abscessus* (Mabs), and 4 other species. We measured the serum soluble PD-1 (sPD-1), PD-ligand 1 (sPD-L1) and PD-ligand 2 (sPD-L2) levels at baseline and analyzed their association with disease progression, namely radiographic progression and/or persistent NTM infection exceeding 1 year.

Results: Patients with disease progression of NTM-LD (n=11, 48%) had lower levels of sPD-1 (49.2 ± 17.0 vs. 109.7 ± 70.0 pg/ml, $p=0.011$), sPD-L2 (1458.7 ± 175.6 vs. 1611.8 ± 150.8 pg/ml, $p=0.035$) and more Mabs-infection (55% vs. 17%, $p=0.089$ with a trend) than those without. The 2 groups were similar with respect to age (69 ± 12 years), sex (70% female), acid-fast bacilli (AFB)-positive sputum smear (35%), nodular-bronchiectatic radiographic pattern (78%) and sPD-L1 (9.4 ± 4.3 pg/ml). Patients with Mabs-infection and with AFB-positivity had non-significantly lower levels of sPD-1 than those without, respectively (55.7 ± 30.0 vs. 94.2 ± 67.5 pg/ml, $p=0.143$, and 54.1 ± 28.2 vs. 95.0 ± 67.3 pg/ml, $p=0.118$). The level of sPD-1 was negatively associated with disease progression (per 10-pg/ml increase, odds ratio 0.502 [0.291–0.866], $p=0.013$). Importantly, sPD-1 level remained an independent predictor of disease progression (0.435 [0.207–0.912], $p=0.028$) after adjustment for age, sex, AFB-positivity, Mabs-infection, nodular-bronchiectatic pattern and the level of sPD-L2.

Conclusion: The serum level of sPD-1 at baseline is a potential predictor of disease progression in NTM-LD.

T.89. Mechanosensitivity of Immature Dendritic Cells in Mechanically Tunable Three-Dimensional Matrices

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Appropriate dendritic cell (DC) maturation in response to foreign antigens is key to marshaling an effective adaptive immune response in instances of infection, vaccination, and immune tolerance. However, little is understood about how the surrounding mechanical microenvironments of the DC, which often changes in response to inflammation and other factors, influences maturation. We have designed mechanically tunable 3D cell culture matrices to evaluate DC mechanobiology. The matrix is a biomimetic hydrogel composed of a recombinantly designed elastin-like protein reversibly crosslinked through hydrazone covalent bonds with chemically modified hyaluronic acid. Maturation response of matrix-encapsulated DCs to lipopolysaccharide is evaluated through a combination of confocal microscopy, quantitative PCR, and flow cytometry. These data show the efficiency of DC maturation is affected by a combination of the mechanical properties and the ligand-density of the surrounding matrix, thus suggesting a mechanotransduction mechanism in DCs for controlling the downstream activation of the adaptive immune system. These studies aim improve basic understanding of DC mechanobiology as well as to uncover matrix properties that may improve DC-based therapies ranging from vaccinations to cancer immunotherapies.

T.109. Akt-2 Is a Potential Therapeutic Target for Disseminated Candidiasis

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Opportunistic fungal infections are a leading cause of death for immune-compromised patients and there is pressing need to develop new anti-fungal therapeutic agents because of toxicity and resistance to current anti-fungal drugs. Akt-1

and -2 are the major isoforms of serine/threonine Akt family that play a key role in multiple cellular processes. Although PI3K/Akt pathway has been implicated in the Dectin-2 signaling pathway, the involvement of Akt-1 and Akt-2 isoforms in anti-fungal innate immunity is completely unknown. Here we showed that *Akt2*^{-/-} but not *Akt1*^{-/-} mice are protected from disseminated candidiasis as revealed by improved survival rate and lower fungal burden in the kidneys. At the cellular level, loss of Akt-2 facilitates the recruitment of neutrophils to the spleens and kidneys, and increased ROS expression by the neutrophils in the kidneys and spleen. Our data demonstrate that Akt-2 negatively regulates the neutrophil recruitment, and oxidative burst, suggesting that inhibition of Akt-2 may be a potential therapeutic approach for disseminated candidiasis.

T.110. Differential Signaling Through TLR7 Or TLR8 Determines the Phenotype of Human Monocytes During RNA Viral Infection.

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Despite being a major cell population in blood and one of the major cellular targets of many RNA virus infections in peripheral blood, the molecular consequences of interactions between human monocytes and RNA viruses, and the signaling pathways responsible for the activation of these cells during infection are not well understood. Toll-like receptors (TLR) are a major family of pattern recognition sensors that trigger specific activation pathways in cells from both the innate and adaptive arms of the immune system. There are at least 10 TLRs in humans, from which TLR7 and TLR8 recognize single-stranded RNA. Despite both recognizing the same general ligand, we and others have demonstrated different phenotypic outcomes on cells stimulated through either TLR7 or TLR8. Our laboratory has recently observed fundamental differences in the phenotype and function of monocytes stimulated via either TLR7 or TLR8, in the context of RNA virus infections, and specifically, in terms of type I IFN responses and effector cytokines they produce, as well as differences in cell surface markers. We have defined the molecular mediators that are responsible for these differences in phenotype, performing *ex vivo* experiments with human monocytes isolated from blood and we have shown the relevance of these data in common RNA virus infections, demonstrating that TLR7 and/or TLR8 stimulation by RNA viruses in human monocytes accounts for much of the phenotype the cells acquire upon virus interaction.

T.111. HECT E3 Ubiquitin Ligase Nedd4 is Required for Anti-fungal Innate Immune Response

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Candida albicans is the most common cause of fungal infections in humans, and disseminated candidiasis has become one of the leading causes of hospital-acquired blood stream infections with 45% to 75% mortality rate. The understanding of the host-pathogen interactions and the mechanisms of immune regulation against fungal spread is critical for developing immune-based strategies to combat candidemia. Nedd4 (neuronal precursor cell expressed developmentally down-regulated 4) is a HECT-type E3 ubiquitin ligase which has been shown to positively regulate T cell activation and proliferation. However, the role of Nedd4 in innate immunity is completely unknown. To elucidate the role of Nedd4 in innate immune response against fungal infection, we generated the mice bearing *LoxP*-flanked alleles encoding Nedd4 with *Rosa26-Cre-ER^{T2}* mice to generate *Nedd4^{fl/fl}Rosa26-Cre-ER^{T2}* mice (called '*Nedd4^{CreER}* mice' here). We also generated mice deficient for Nedd4 in dendritic cells (DC) (*Cd11c Cre.Nedd4^{fl/fl}*) or macrophages (*LysM Cre.Nedd4^{fl/fl}*). We found that although Nedd4 might not regulate the signaling via TLRs and Dectin-1, *Rosa26-Cre-ER^{T2}* mice treated with tamoxifen or mice deficient for Nedd4 in the myeloid cell lineages (macrophages and dendritic cells) are highly susceptible to systemic *C. albicans* infection, which correlates with defective pro-inflammatory cytokines in the sera, impaired leukocyte recruitment to the kidneys, defective ROS expression by the granulocytes in the kidneys, and

heightened kidney fungal burden. Therefore, our data suggest that Nedd4 expression in the myeloid cells is crucial for C-type lectin receptor (CLR)-mediated innate immune response against systemic *C. albicans* infection.

W.15. Th17/Regulatory T Cells Balance is Predictive of *Coccidioides* Infection Outcome in Pediatric Patients

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Protective immunity against the fungal pathogen *Coccidioides* requires specific T helper cellular responses. Mouse infection and vaccine studies have defined CD4 T helper (Th)1 and Th17 cells in the resolution of infection and in effective protection. Patients with chronic or disseminated Valley fever demonstrate reduced cellular responses. Peripheral blood and serum were collected from 30 pediatric *Coccidioides* acute-infected patients and 20 healthy controls in the San Joaquin Valley of California. Samples were evaluated by flow cytometry for percentages and total cellularity of innate and adaptive immune populations, and levels of inflammatory and helper cytokines. Clinical and flow data were evaluated according to disease outcome using principal component analysis, high-dimensional flow cytometry analysis tools, chi-square automatic interaction detection, and individual cell population comparisons. We identified several biomarkers during acute infection that distinguish patients that resolve infection from those that develop chronic disease. Significant differences were observed in T regulatory (Treg) and Th17/Treg ratios, but not in Th1 frequencies or total numbers, based on disease outcome. Patients with chronic disease had reduced Th17 and elevated Treg frequencies compared to patients that resolved disease. The inability to clear *Coccidioides* infection may be a result of elevated Treg frequency and functional capacity. Treg frequency at the time of Valley fever diagnosis is indicative of patient disease outcome and may be a diagnostic for calibrating treatment aggressiveness for those patients most likely to develop chronic coccidiomycosis.

W.28. Signal Integration in Immune Regulatory Networks

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The immune system performs two functions concomitantly: protecting against infectious agents and maintaining the body's homeostasis. These two processes require two contradictory operations: strong activation against infected tissues and strong inhibition to prevent reactivity to healthy tissues. An imbalance between the two events can result in autoimmune or inflammatory diseases such as arthritis and inflammatory bowel disease.

The delicate equilibrium between these events is governed by complex interactions between different cell populations, namely effector T-cells (Teff), dendritic cells (DC) and regulatory T-cells (Treg). Studying the interactions and information flow between human immune cells remain an outstanding challenge. For this purpose, we have established 3D collagen gels that support migration of human immune cells. In order to confer specificity to Teff and Treg, we use mRNA electroporation to express specific T-cell receptors in ~80% of polyclonal T-cells. Next, using a library of altered peptide ligands with different affinities, we perform time-lapse microscopy and functional analysis in the 3D system. Using this system we are currently investigating the role of antigen stimulation for Treg suppressive function. Furthermore, we aim at delineating which inhibitory mechanisms are dominant under different homeostatic and inflammatory conditions where we hypothesise that the dynamics of the Teff/DC interactions and the strength of the integrated signals will require different suppressive capacity of Tregs. The experimental data will guide the formulation of a mathematical model, which

will provide a quantitative framework for testing different models for priming of effector cells and shed light into regulatory mechanisms enforced by Tregs.

W.45. The NLRP3 Inflammasome Impairs CD8⁺ T Cell Responses in Murine Viral Hepatitis.

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NLRP3, caspase 1 and IL-1 are known to be required to mount adaptive CD8⁺ T cell responses against IAV. However, in viral hepatitis, IL-1 β production through the NLRP3 inflammasome has been associated to liver inflammation and increased viral load. We have described the intracellular cation channel Tmem176b as a novel inhibitor of the NLRP3 inflammasome. Interestingly, Tmem176b has been associated to HCV clearance in humans. We therefore speculated that Tmem176b might promote viral clearance by inhibiting inflammasome activation. We infected *in vitro* WT and Tmem176b^{-/-} DCs with murine hepatitis virus-A59 (MHV-A59) and studied inflammasome activation. MHV-A59 induced caspase 1 activation and IL-1 β secretion in an NLRP3-dependent manner. Tmem176b^{-/-} DCs showed increased inflammasome activation as compared to WT DCs. In vivo, 7/7 Tmem176b^{-/-} mice died within 5.5 days post-infection whereas 4/6 WT mice survived to infection. Liver viral load was higher in Tmem176b^{-/-} mice. IL-1 β blockade significantly protected Tmem176b^{-/-} mice in a CD8-dependent manner. In agreement with these observations, MHV-A59-infected Tmem176b^{-/-} animals had fewer total and MHV-A59-specific CD8⁺ T cells and decreased *in vivo* CD8-dependent cytotoxicity against MHV-A59 antigens. In complement to these studies MHV-A59 infection of caspase 1/11^{-/-} mice showed improved survival, diminished viral load and augmented total and MHV-A5-specific CD8⁺ T cells as well as increased *in vivo* cytotoxicity against viral antigens. Inflammasome activation led to increased PD-1 expression in total and virus-specific CD8⁺ T cells. Injection of anti-PD1 antibodies significantly improved the survival of MHV-A59-infected Tmem176b^{-/-} mice. Thus, the NLRP3 inflammasome impairs anti-viral CD8 responses through PD-1

W.53. Nutrient Sensing by CD11c⁺ Cells Modulates Host Microbiome to Promote Metabolic Homeostasis

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Alterations in host metabolic state alter gut microbial communities and influence host immune responses. CD11c⁺ cells have a critical role in bridging innate and adaptive immune responses. In the gut, CD11c⁺ cells are located in the lamina propria and play a key role in maintaining gut immune homeostasis. The tuberous sclerosis 1 - mechanistic target of rapamycin complex 1 (Tsc1-mTORC1) pathway is a cell's major nutrient-sensing pathway and determines whether energy-intensive processes, such as nucleotide synthesis and protein translation, should take place. Hypothesis: We thus asked whether perturbing nutrient sensing in CD11c⁺ cells might alter the gut microbiome to ultimately influence host systemic metabolism. Methods: We generated Tsc1^{flox/flox}CD11c^{Cre} mice to delete Tsc1 in all CD11c expressing cells. Tsc1^{flox/flox} ("control; CTRL") and Tsc1^{flox/flox}CD11c^{Cre} ("knockout; KO") mice were housed under thermoneutral (30°C) and thermal stress conditions (22°C), and placed on normal chow diet (NCD) or high-fat diet (HFD). Weight gain, glucose and insulin sensitivity, and food intake were monitored over time, and stool was collected for 16S rRNA sequencing. Results: We found that both thermoneutral and thermally stressed KO mice exhibited reduced weight gain compared to CTRL mice. This decrease in body weight was not due to developmental or growth defects. Analysis of food intake revealed a reduction in food consumption. Our findings reveal an unexpected role for nutrient sensing pathways in CD11c⁺ cells in regulation of gut microbial communities to support host metabolic homeostasis.

W.66. Absence of Sprouty 1 and 2 Enhances CD8⁺ T Cell Memory Development and Function

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Identifying novel pathways that can be targeted for immunotherapeutic strategies to promote robust function and longevity of cytotoxic CD8⁺ T cells has promising potential to combat cancer and chronic viral infections. We identified sprouty 1 and 2 (Spry1/2) molecules that inhibit the TCR signaling pathway, to be key regulators of CD8⁺ T cell metabolism and effector (Teff) and memory development and function. Using systemic infection with LCMV Armstrong, we showed that Spry1/2 KO CD8⁺ T cells expressing the LCMV-specific transgenic TCR (P14) had limited contraction of Teffs and generated significantly more polyfunctional memory cells. Upon intranasal rechallenge with LCMV, Spry1/2 KO memory cells displayed significantly enhanced infiltration into the lungs, suggesting that absence of Spry1/2 can increase memory recall capacity. Additionally, upon adoptive transfer into naïve hosts, Spry1/2 KO memory P14s demonstrated enhanced protection against recombinant *Listeria monocytogenes* expressing gp33. The enhanced functionality of Spry1/2 KO CD8⁺ T cells was associated with metabolic and transcriptional reprogramming. Absence of Spry1/2 in Teffs significantly reduced mTORC1 activity, glucose uptake, p-AKT, p-Foxo1/3a and T-bet while survival was enhanced, which are commensurate with memory accrual. Equally important, Spry1/2 KO OVA-specific (OT-I) cells mounted more vigorous induction of autoimmune diabetes in mice expressing OVA in their pancreatic beta-islets. Collectively, absence of Spry1/2 enhances the cytotoxicity and the number of memory CD8⁺ T cells that have increased recall capacity and protective functionality.

W.77. Molecular Mechanisms Underlying *In Vitro*-rejuvenation of Virus-specific Memory T Cells

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Inhibition of the mechanistic Target of Rapamycin (mTOR) pathway was shown to regulate memory T cell differentiation. We employed Rapamycin, inhibiting mTOR-Complex 1, to arrest memory differentiation during expansion of virus-specific T cells and found enriched proportions of early differentiated central-memory T cells, which is highly attractive for enhanced long-term efficacy of antiviral T cell therapy. To examine the underlying molecular mechanisms we cultured isolated virus-specific T cell memory subsets in the presence or absence of Rapamycin, thereby analyzing specific effects on distinct subsets. Furthermore, we analyzed apoptosis, performed metabolic and epigenetic profiling, as well as RNA and TCR sequencing.

Our findings imply Rapamycin-enhanced rejuvenation of late-differentiated human virus-specific T cells and arrested further differentiation of early-differentiated virus-specific T cells *in vitro*. In addition, Rapamycin provoked increased cell survival and decreased sensitivity to apoptosis induction. Accordingly, anti-apoptotic Bcl-2 protein expression increased upon treatment with Rapamycin. Whole genome RNA sequencing revealed distinct cellular processes influenced by Rapamycin in virus-specific T cells. Of note, Rapamycin increased the clonal diversity of expanded T cells indicating less clonal loss during *in vitro*-expansion.

Our study illustrates the plasticity of virus-specific T cell memory subsets and suggests distinct molecular mechanisms underlying memory T cell differentiation. Our pre-clinical data imply that Rapamycin-treated virus-specific T cell lines possess optimal features for adoptive antiviral T cell therapy with regard to longevity, clonal diversity and anti-viral effector function. We were already able to generate GMP-compatible Rapamycin-treated antiviral T cell products from immunosuppressed patients, which illustrates the feasibility of clinical translation.

W.91. Novel Adjuvant Systems Elicit Unique and Protective Cellular and Humoral Immune Responses in Q Fever Challenge Model Using a Polyvalent *C. Burnetii* Vaccine

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Coxiella burnetii is the causative agent of Q-fever. This disease manifests with flu-like symptoms, but can progress into a fatal chronic infection resulting in endocarditis or neurological manifestations up to 20 years following the initial infection. *C. burnetii* is a Category B bioterrorism pathogen due to its low infectious dose and hearty nature. Despite these facts, the sole vaccine available is only licensed in Australia due to significant safety concerns, which necessitates the development of a less immunogenic yet efficacious vaccine. Previous unsuccessful attempts to develop a protective Q-fever vaccine have identified single immunogenic antigens. Our research group has developed unique, polyvalent anti-*C. burnetii* vaccine candidates that take advantage of two distinct technologies: 1) a protein microarray to identify sero-reactive *C. burnetii* proteins and 2) novel adjuvanting systems. We have used the protein microarray to identify several highly immunogenic *C. burnetii* proteins from sera of previously infected humans and combined these with our novel adjuvants, which include polymers and conjugated Toll-like receptor agonists. We have previously shown that our adjuvants elicit antigen specific B and T cell responses and successfully increase antibody scope and diversity compared to non-adjuvanted vaccine candidates, suggesting downstream changes in immune signaling and adaptive immune activation. We have tested these *C. burnetii* vaccine candidates *in vitro* and *in vivo* for safety, immunogenicity and ability to protect animals in a *C. burnetii* aerosol challenge. Our studies suggest that our novel polyvalent *C. burnetii* vaccine could be an effective and safer alternative to Q-Vax®.

W.94. Macrophages Contribute to Protective Memory in Recurrent Staphylococcus Aureus Skin and Invasive Infection

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Staphylococcus aureus is the leading cause of skin and skin structure infection (SSSI), a primary portal of entry for dissemination. Our prior studies discovered a role for protective innate memory against recurrent MRSA SSSI. Here, dynamics and mechanisms of immune memory were explored. Recurrent SSSI was induced in wild-type (WT) and molecular/cellular responses compared at days 2 vs. 7 in various tissues. Priming by prior infection yielded smaller skin lesions, with reduced MRSA burden at day 7 but not day 2 versus naïve controls. Moreover, priming was protective against localized and disseminated infection by day 7. Cytokine and cellular signatures in SSSI differed at day 2 vs. 7, and were distinct in skin versus blood or spleen. Cytokines associated with protection in SSSI included IL-17, IL-6, and MIG in wild-type mice, whereas increased IP-10 correlated with protection from invasion. Cellular signatures of protection included increased M1 macrophages accumulating in abscesses whereas increased dendritic cells were found in lymph nodes. Furthermore, primed bone-marrow derived macrophages exerted greater opsonophagocytic killing of MRSA *in vitro*, and their adoptive transfer into naïve skin confirmed protective efficacy *in vivo*. Together, results in this model indicate protective immunity in recurrent *S. aureus* infection is temporally and spatially targeted, and involves memory conferred, in-part, by macrophages. Given challenges of antibiotic resistance, these insights support novel vaccines and

targeted immunotherapeutic strategies to address MRSA infections.

W.96. Riding the Perfect Storm. Severe Rash Resulting from Simultaneous Infection with Influenza B Virus, Erythrovirus B19 and Influenza B Immunization

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This is the case of a 39-year-old female with a widespread pruritic erythematous rash, no history of allergies, taking oseltamivir due to exposure to influenza B virus (IBV) against which, she had been immunized.

Initially the rash was attributed to oseltamivir, therefore it was suspended, and the patient treated with antihistamines, however, it fast progressed to a severe, intensely pruriginous erythroderma, weakly responsive to high doses of antihistamines and systemic steroids, with dyspnea and myasthenia. Auscultation and imaging revealed clear lungs. Testing for viral and bacterial pathogens confirmed IBV infection.

A literature search for IBV related rashes yielded a report on a cohort of seven children whose rash was mostly mild and localized with one exception: the only child immunized against influenza B virus developed symptoms closely resembling the patient's. This information raised the hypothesis that the current rash could be a vaccine-induced type III hypersensitivity reaction against IBV, meaning it should disappear once the infection ended. Dyspnea and myasthenia were deemed a byproduct of the high doses of steroids used. Treatment then focused on symptom control. Within 2 days, the rash improved, and 2 days later, the patient was discharged.

After her discharge, test results showed normal levels of circulating immunocomplexes and a positive IgM for erythrovirus B19 (EBV) which challenged our hypothesis, however, by no means this presentation is typical for EBV which, together with the previous description on the child, leads to the conclusion that the rash was indeed a byproduct of the immunization for IBV

W.97. Human Tonsil Cultures as a Model to Study Adaptive Immune Responses *In Vitro*

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Predicting vaccine efficacy has thus far proved challenging, as several pathogens have resisted the classical approach of attenuation or dissociation that has led to many effective vaccines. Identifying and screening candidate formulations is a tedious and time-consuming process; animal models, even including non-human primates, have been unreliable in predicting protection in humans. As *in vitro* organoid systems have become more sophisticated, we decided to take advantage of the widespread availability of tonsils to develop an *in vitro* system that might replicate an adaptive immune response to a vaccine. We have developed tonsil organoids that maintain the cellular composition of and partially recapitulate the structure of germinal centers. These organoids are able to respond to model antigens (live attenuated influenza vaccine (LAIV) and RSV surface proteins) and mount robust humoral responses, with microgram quantities of specific antibody detected in culture supernatants. We also observe B cell differentiation and maintenance of T follicular helper phenotypes. Using cell sorting experiments, we have determined which cells types are necessary for a successful humoral response to LAIV. We have also identified CDR3 motifs from LAIV-stimulated cultures that are known to bind influenza by BCR heavy chain sequencing. We detected IgM responses under certain conditions in response to HIV proteins using a high throughput adjuvant and cytokine screening process. This platform provides many opportunities to understand and manipulate the cellular interactions that occur during an adaptive immune response, and will enable translational applications such as high throughput vaccine design and adjuvant testing.

W.99. Estrogen Receptor Alpha (ER α) Antagonist Mediates Therapeutic Benefit Against Urinary Tract Infection (UTI) by Boosting TNF α Production in Bladder

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Differential susceptibility to UTI in premenopausal and postmenopausal women has established estrogen as an important etiological factor. Our *in vitro* and ER α knock-out mice studies have shown that estrogen mediates protective immune responses via ER α against Dr *E. coli* induced experimental UTI. However, our recent studies utilizing ER α antagonist, methyl-piperidino-pyrazole (MPP), treatment in mice caused bacterial clearance in bladder ($P < 0.05$) but not in kidney. Although, TNF α is an important cytokine for mucosal immunity, its role in the urinary tract under homeostasis or infection is poorly understood. *We hypothesized that MPP pretreatment in mice boosts TNF α production in bladder at homeostasis, inducing better bacterial clearance during UTI.*

C3H/HeJ mice received subcutaneous MPP injections (7 days) and were divided into uninfected and infected (experimental UTI) groups. Mice were terminated at 2 and 6 days post-infection. TNF α protein levels in bladder and kidney sections were determined in both groups by immunohistochemistry and quantitated using ImageJ software. We observed that MPP pre-treatment upregulated TNF α levels in uninfected bladders but significantly reduced TNF α ($P < 0.05$) in infected bladders, that correlated with reduced bacterial load in the bladder at 6 days. In the kidney, MPP pre-treatment induced no changes in TNF α levels at homeostasis. However, during UTI, TNF α levels (protein and mRNA) were suppressed at both time points in MPP group explaining the compromised bacterial clearance. Our results suggest that boosting TNF α production in bladder by MPP or similar acting agents will serve as novel therapeutic strategy to clear reservoirs of infection inhibiting recurrence of UTI.

W.101. Fc γ -Receptor Binding and Fc-Mediated Antibody Effector Functions in a Human HIV-1 Vaccine Efficacy Trial

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Identification of immune correlates of HIV-1 risk from vaccine efficacy trials provides insights into protective immune responses for benchmarking vaccine candidates. In the most recent, completed HIV-1 vaccine efficacy trial, HIV Vaccine Trials Network (HVTN) 505, there was a virus sieve effect, and polyfunctional Envelope-specific CD8⁺ T cells correlated with decreased HIV-1 risk. Evaluation of antibody effector functions and humoral correlates of risk are important for understanding the breadth of immunity, and to improve upon this regimen toward an efficacious vaccine.

We measured vaccine elicited HIV-1 IgG, IgA, Fc receptor (Fc γ R2A, Fc γ R3A) tetramer binding, and antibody-dependent

cell-mediated phagocytosis (ADCP) by validated or standardized flow cytometric bead-based assays. An analysis of serum antibody responses in 25 primary endpoint vaccine HIV-infected cases and 125 covariate and vaccine-frequency matched vaccine controls was performed. We measured FcγR and ADCP against HIV envelop on 40 HIV-uninfected vaccinees and 10 placebos, assessing immunogenicity of antibody effector functions.

Envelope-specific IgG responses significantly correlated with decreased HIV-1 risk ($p < 0.05$, $q < 0.1$). Interaction of IgG responses and Env-specific CD8⁺ T-cell polyfunctionality score were significantly associated with HIV-1 risk after adjustment for multiple comparisons. Antibody effector functions of ADCP, FcγR2a and FcγR3a binding were elicited in 92% ($n=37/40$ following quality control exclusions), 100% ($n=40/40$), and 97% ($n=36/37$) of vaccinees, respectively. The magnitude and broad dynamic range of these responses are suitable for immune correlates testing.

Identifying antibody Fc effector functions elucidates potential mechanisms underpinning the binding antibody correlates of HIV-1 risk, and may reveal important immune mechanisms necessary for a successful HIV-1 vaccine.

W.102. Immune Response to Influenza Vaccine in Sick Cell Disease Patients

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Sickle cell disease (SCD) patients develop particularly serious complications after contact with the influenza virus and annual vaccination against the influenza virus is strongly recommended for these patients. Recent studies suggest that some immune parameters differ according to the therapeutic regimen of SCD patients (OH-urea (HU) or chronic transfusions (CT)). In this work we investigated the immune status of SCD patients after vaccination by exploring their ability to establish a humoral and cellular response. We also aimed to correlate them with their treatment.

A total of 98 subjects were collected to participate to the study among which 28 healthy donors and 70 SCD patients. All subjects received the quadrivalent influenza vaccine (season 2016-2017) and samples were collected at 0, 1, 3 and 6 months post-vaccination.

The anti-influenza antibody assay showed that SCD patients had a significantly lower seroconversion capacity than the control group. However, most of them had sufficient levels to protect them against the influenza virus. Furthermore, SCD patients presented lower levels of memory B cells and memory CD8⁺ T cells, as confirmed by a functional assay.

Based on a recently proposed model to explain the influence of transfusions on immunity, we evaluated the expression of heme oxygenase-1 (HO-1) and the proportion of regulatory T cells (Treg) among the populations included in the study. The increased expression of HO-1 observed in CT patients was consistent with their particularly high Treg cells level and their lower ability to produce antibodies.

W.103. Lymphatic Filariasis Infection is Reemerging in India Emphasizing the Need for a Prophylactic Vaccine for Mass Control

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Lymphatic filariasis (LF) is a mosquito-transmitted tropical parasitic infection that currently affects 120 million people around the world and another 856 million people are at risk of acquiring the infection. Mass Drug Administration (MDA) spearheaded by the World Health Organization is currently the only strategy to control this infection in endemic areas. Several endemic regions in India has been receiving MDA for the past 17 years. To assess the impact of MDA and to determine the status of LF infection, we performed an epidemiological survey of several communities within the southern states of India. Our studies suggested reemergence of the infection in nearly all of the communities we surveyed. These communities received several rounds of MDA in the past 17 years. After informed consent, night blood smears were randomly collected from 1,100 subjects and screened for the presence of LF microfilariae. Our results showed 5.56% infection in the communities and the majority of these infections were new. We then trapped mosquitoes from these regions, collected the DNA and analyzed for the presence of *Wuchereria bancrofti* L3 Ssp1 gene by PCR. Mosquitoes from nearly all the communities were found positive for LF infection, suggesting active disease transmission. Analysis of the sera samples from all the subjects for the protective antibodies against LF showed that only 5.5% $\pm$ 2.5% subjects were potentially immune to the infection. These findings suggest an urgent need for a more stringent control strategy such as an effective vaccination to achieve interruption of transmission and elimination of LF.

W.104. Early Cytokine Signatures Discriminate Persistent from Resolving MRSA Bacteremia

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Staphylococcus aureus bacteremia is a frequent, life-threatening bloodstream infection often caused by methicillin-resistant strains (MRSA). Up to 30% of patients fail to clear infection with gold-standard anti-MRSA antibiotics, although isolates from such patients may be “susceptible” *in vitro*. The aim of this study is to profile human immune signatures unique to Antibiotic-Persistent MRSA Bacteremia (APMB). We measured 38 cytokines and chemokines in the plasma of 34 pairs of APMB vs resolving (ARMB) cases, matched by age, sex, presence of device, type 1 diabetes, and hemodialysis, sampled at the time of diagnosis of bacteremia. Hierarchical clustering segregated APMB from ARMB patients, suggesting that MRSA evokes distinct cytokine signatures during initial stages of infection in the two groups. Th2-polarized and anti-inflammatory cytokines correlated with a subset of APMB patients, while a cluster of pro-inflammatory cytokines/chemokines attracting monocytes, T cells, neutrophils, eosinophils and endothelial cells correlated with ARMB patients. These data suggest that persistence may be shaped early in the course of infection by a failure of the patient to mount a protective type of pro-inflammatory response. This could be due to an intrinsically dysfunctional host immunity, or to immune subversion by MRSA. A combination of 11 cytokines classified patients as APMB or ARMB with an AUC of 88.58%. In a preliminary analysis on a second cohort of 40 matched patient pairs, we were able to validate the classification performance of a subset of the cytokines. These findings suggest that plasma cytokine signature(s) may enable predictive algorithm(s) of persistent bacteremia.

W.106. Pharmacological Induction of Heme Oxygenase-1 Hinders Herpes Simplex Virus Replication by Interfering with the Nuclear Accumulation of Viral Capsids after Infection

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Heme oxygenase-1 (HO-1) is an inducible enzyme that degrades iron-containing heme into ferrous iron (Fe²⁺), carbon monoxide (CO) and biliverdin. Expression of this enzyme is induced upon numerous stresses, such as ultraviolet radiation, hyperthermia, hypoxia, as well as reactive oxygen species (ROS), among others. HO-1 activity can be pharmacologically induced with cobalt protoporphyrin (CoPP) and inhibited with tin protoporphyrin (SnPP). Importantly, the products of HO-1 are cytoprotective and furthermore have been shown to have antiviral properties against multiple viruses, such as, influenza, hepatitis B, hepatitis C, the human immunodeficiency virus (HIV) and Ebola virus. Because of these reported antiviral effects, we assessed the effects of HO-1 activity on herpes simplex virus type infection and replication. Importantly, we found that induction of HO-1 activity with CoPP significantly decreased infection by reducing virus plaque formation, as well as the expression of virus-encoded genes in infected cells. Treatment with CoPP did not affect virus binding to the cell surface, nor entry into the cytoplasm, but rather virus capsid migration to the nucleus. Additionally, we found that carbon monoxide, one of the products of HO-1 which can be delivered with a CO-releasing molecule, such as CORM-2 repeated some of the anti-HSV effects elicited by CoPP. These findings indicate that HO-1 activity interferes with the HSV replication cycle and that its antiviral effects can be recapitulated by CO.

W.112. Soluble Protein Biomarkers in Late-Onset Neonatal Sepsis: Potential Value in Diagnosis

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The availability of a panel of protein biomarkers that can differentiate sepsis from Systemic Inflammatory Response Syndrome (SIRS) earlier may cause a significant impact on the morbidity and mortality of neonatal sepsis. The purpose is to describe the protein expression of selected biomarkers in newborns (NBs) with clinical (SIRS) and culture-proven late-onset sepsis on the day of diagnosis. To date, blood samples were collected from 10 culture-proven septic NBs and 6 NBs with SIRS compared to 10 controls. Soluble protein biomarkers IL-1 β , IL-6, IL-8, IL-10, TNF- α , IL-12, IL-27, IL-33, MBL and hepcidin were evaluated by ELISA; Lipoproteins were determined by gel filtration chromatography and enzymatic-colorimetric method using Labtest kits. Hematological and laboratorial data from sepsis and SIRS groups were characterized by higher neutrophil count, immature/total neutrophil ratios, CRP and lactate than control group. IL-6 and IL-8 were higher in sepsis and SIRS groups than in the control group. IL-10, TNF, IL-12, hepcidin and IL-33 showed no differences among the groups, although IL-33 showed a tendency to lower levels in the sepsis group. IL-27 concentrations were similar in the SIRS and control groups and both showed higher concentrations than in the sepsis group. MBL was equivalent in the sepsis and SIRS groups and higher when both were compared to the controls. Of the lipoproteins tested, only HDL was lower in sepsis and SIRS groups when compared to control group. The differences in MBL, IL-27 and HDL levels indicate that these proteins can be used as early biomarkers of late neonatal sepsis.

W.114. Respiratory Syncytial Virus Glycoproteins Activate Primary Human Dendritic Cells

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Background: RSV is an important pathogen of children, but reinfection occurs throughout life. Inability to induce protective immunity despite repeated infection suggests dendritic cells (DCs) may not be appropriately activated during RSV infection, limiting generation of memory responses. DC-RSV interactions may also be inadequate in susceptible infants, resulting in severe disease. These studies evaluate the impact of RSV virions and proteins on DC activation. Understanding RSV-DC interactions and subsequent lymphocyte differentiation will facilitate design of vaccines and therapies that avoid disease-enhancing responses and increase immunogenicity.

Methods: Primary human DCs were separated from elutriated monocytes, then exposed to purified RSV attachment (G) glycoprotein, or inoculated with recombinant GFP-expressing RSV or the same virus lacking RSV G, the small hydrophobic (SH) protein, or both RSV G and SH. Infection and CD83, CD86, and CD209 expression were monitored by flow cytometry. DC-produced cytokines were measured by ELISA.

Results: RSV infection or purified RSV G induced maturation of myeloid (mDC) and plasmacytoid (pDC) subsets. Recombinant RSVs lacking G infected fewer DCs. Deficiency of only G or only SH induced more RANTES and MIP-1 α/β relative to complete RSV. Additionally, RSV lacking only G enhanced CD83 expression, while RSV lacking only SH increased CD86 and IFN- α . Conversely, complete RSV and RSV missing both G and SH activated DCs similarly.

Conclusions: Individual RSV proteins differentially affected DC activation. Purified RSV G activated primary DCs. However, RSV G and SH appear to cross-regulate each other, with the activating potential of each suppressed in the presence of the other.

Immunodeficiency: primary or acquired

F.12. Tumor Suppressor BAP1 is Essential for Thymic Development and Proliferative Responses of T Lymphocytes

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BAP1 is a ubiquitously expressed nuclear deubiquitinating enzyme studied mostly for its tumor suppressor function. Loss-of-function mutations of BAP1 have been identified in a variety of solid tumor types, and are strongly linked to metastasis and poor prognosis.

Surprisingly, we found that tamoxifen-induced BAP1 deletion in adult mice resulted in severe thymic atrophy and complete loss of the T cell lineage. B cell development was also abrogated suggesting a broader function for BAP1 in maintaining the lymphoid, but not the myeloid lineage. BAP1 deficiency resulted in a block at the DN3 stage prior to the pre-T cell receptor checkpoint. Peripheral T cells in CD4.Cre-driven T cell-conditional BAP1 KO mice exhibited a defect in homeostatic and antigen-driven expansion, showed strongly reduced production of Th1 and Th17 cytokines, and were completely protected from T cell-mediated autoimmunity.

Deletion of BAP1 resulted in suppression of E2F target genes and defects in cell cycle progression, which was dependent on the catalytic activity of BAP1. Similar to the role of the BAP1 homolog Calypso in *Drosophila*, loss of BAP1 led to strongly increased global mono-ubiquitination of histone H2A at lysine 119 (H2AK119ub) throughout the T cell lineage, in particular in immature thymocytes. Histone PTM profiling by MS also identified diminished histone H3S10 phosphorylation, a hallmark of mitosis, further demonstrating a crucial role for BAP1 in regulating G2/M transition in T

lymphocytes. Our findings uncover a non-redundant epigenetic function for BAP1 in regulating and maintaining proliferative capacity within the lymphoid lineage.

F.22. A Combined Immunodeficiency with Severe Inflammation and Allergy Caused by ARPC1B Deficiency

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We report the genetics, clinical manifestations, natural history and immune-hematological findings in 12 patients with actin related protein C1B (ARPC1B) deficiency as a novel primary immunodeficiency. ARPC1B is one of the seven subunit of the ARP2/3 complex, which regulates actin polymerization. Although consanguinity was not revealed by clinical history in five families, all cases carried homozygous mutations in *ARPC1B*. The mutations resulted in loss of protein expression in all but three pedigrees, carrying missense and splice site mutations. Nonetheless, all mutations resulted in aberrant actin polymerization in hematopoietic cells. Early gastrointestinal bleeding was a common finding (7/12), whereas bacterial (10/12) and viral (8/12) infections, including warts (2/12), molluscum (4/12) and CMV infections (3/12), together with growth failure (8/12) and vasculitis (7/12) became predominant features later in life. The majority of children suffered from allergy (7/12), including extensive eczema and high IgA and IgE levels. Thrombocytopenia was present in 6/12 patients, while extensive immunophenotyping showed several abnormalities such as B cell lymphocytosis and abnormalities in T and NK lymphocyte subsets. In-vitro lymphocyte proliferation in response to PHA stimulation and response to vaccination were normal in respectively 6/6 and 9/10 patients whose data were available. In conclusion, our cohort delineates the spectrum of clinical, hematological and immunological manifestations in subjects with ARPC1B deficiency, that involve platelet abnormalities, defects of innate and adaptive immunity with susceptibility to bacterial and viral infections, autoimmunity, inflammation and growth failure. The disease appears progressive in most cases and challenging to manage clinically with standard immunosuppressors.

F.36. Flow Cytometry as a Screening Tool of Primary Immunodeficiency Diseases

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Flow cytometry(FCM) is applicable for immunophenotyping and functional assays in patients who suspected Primary Immunodeficiency Diseases(PIDs). We retrospectively reviewed the single-center experience of FCM application for the diagnosis of PIDs.

Of total 56 patients who have met the ESID criteria as definite or probable PID, 23 cases had specific abnormal findings at FCM and confirmed by genetic tests. There were nearly absent CD19⁺ B cells and markedly decreased or absent intracellular BTK expression in 7 and 3 patients, respectively and 6 of them had mutations in *BTK*. Of 5 patients who had almost absent CD3⁺ T cells and markedly decreased 16/56⁺ NK cells, thus suspected for severe combined immunodeficiency(SCID), 4 patients had mutations in *IL2RG*. Among 13 patients who diagnosed chronic granulomatous diseases(CGD), neutrophil respiratory burst activity was almost absent in 11 patients having *CYBB* mutations but

preserved in two patients having mutations in *CYBA* and *NCF2*. Three patients had absent CD18 expression and two of them had mutations on *ITGB2*, which is compatible to leukocyte adhesion deficiency(LAD). Twelve patients with variable diagnoses who could have been easily screened by FCM were not subjected to the appropriate FCM tests. Particularly, subset analysis including naïve and memory T/B profiles has been used for monitoring Sirolimus therapy in two patients who had mutation in *PIK3CD*. Conclusively, flow cytometry would be a practical and reasonable screening tool for patients who need rapid diagnosis of PID such as agammaglobulinemia, SCID, CGD and LAD.

F.46. The Thymus in the Chromosome 22q11.2 Deletion Syndrome (22q11DS)

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80% of patients with 22q11DS have T lymphopenia, affecting both effector and regulatory T cells, and a concomitant increased risk of viral infections, loss of immunologic memory after immunization, and dysregulation, especially atopy and autoimmunity, e.g., immune cytopenias. Unlike Severe Combined Immune Deficiency (SCID), the primary defect in 22q11.2DS is not in the hematopoietic precursors, but instead is in microenvironmental thymic epithelial cells (TEC). We have analyzed 22q11.2DS thymocytes by FACS, CyTOF, and RNA-Seq to determine whether thymopoietic defects are global or specific to certain thymocyte differentiation stages. Total thymic cellularity from both 22q11.2DS and unaffected patients each varied over a one-half log range. In 22q11DS, there is a relative decrease in the frequency of CD3⁺ CD4⁺ CD8⁺ (DP) intermediate thymocytes, relative to the frequency of either CD3⁺ CD4⁺ CD8⁻ (SP4) or CD3⁺ CD8⁺ CD4⁻ (SP8) cells. CyTOF analyses demonstrated multiple maturational defects occurring between immature CD3- CD4- CD8- (TN) and DP stages, including quantitative defects and failure to up-regulate CD5 and CD7 expression, consistent with loss of either T cell receptor (TCR) repertoire formation, positive selection, or both. Since development of TN and DP thymocytes occurs under the inductive influence of cortical TEC (cTEC), the primary thymic defect in 22q11DS is most likely specific to the cTEC compartment. Comparison of normal and 22q11DS TEC is underway and will be presented. The implications of this work for both understanding of 22q11.2DS immune pathophysiology and the development of strategies for TEC regeneration will be discussed.

F.68. Antibody Profiling Identifies a Strong and Wide Spread Auto-Immune Response in Idiopathic CD4 Lymphopenic Patients

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Patients with Idiopathic CD4 Lymphocytopenia (ICL) have low CD4 T-cell numbers, accompanied sometimes by low CD8 T-cell numbers, making them susceptible to opportunistic infections. The etiology is unknown and probably caused by multifactorial inheritance since there is no familial linkage. In spite of their immune deficiency (or perhaps because of it), 32% of ICL patients have clinically defined auto-immune disease (AID) with the presence of clinical auto-antibodies. Here we globally profile the extent and target distribution of the auto-antibody response to better characterize ICL and its pathogenic process. We hybridized 15 ICL patients and 10 healthy control (HC) sera to a human proteomic array and found that all 15 patients contained IgG auto-antibodies against at least 100 proteins, while none of the HC samples had any. Among the patients, there was big variability on both number and identity of targets. Additionally, we identified groups of protein targets (co-expression modules) against which the auto-antibody levels show coherent variations across subjects. Consistent with the proteomic analysis, flow cytometry analysis using sera from 70 patients revealed that 70% of ICL patients had either IgG or IgM antibodies specific against lymphocyte membrane proteins. Functionally, we found that some of these auto-antibodies induced either CDC or ADCC. Based on these results, we propose, that:1) the broad spectrum of auto-antibodies in ICL patients might be contributing to ICL pathogenicity, and 2) such extensive

auto-antibody responses might be present in other primary immunodeficiencies, where AID has also been described

F.82. Using Transcriptional Profiling to Understand Early Human T Cell Development

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Unlike their adult counterparts, fetal naïve CD4⁺ T cells are predisposed to become regulatory cells. These differences seem to be cell-intrinsic, as transplanted fetal and adult hematopoietic stem cells (HSCs) give rise to naïve T cells with corresponding developmentally associated phenotypes in humanized mice. Transcriptional profiling of fetal or adult HSC-derived naïve T cells revealed divergent gene signatures consistent with previously shown profiles generated from *ex vivo* fetal and adult naïve T cells. This suggests that distinct populations of stem cells give rise to fetal-like and adult-like immune cells, and a transition occurs that allows for the switch from a fetal-like to adult-like immune system. Profiling of human cord blood naïve T cells revealed their gene signatures showed inter-individual variability in the progression from fetal-like to adult-like transcriptional profiles. We hypothesized that the inter-individual variability in transition from fetal to adult transcriptional profile comes from incomplete replacement of fetal stem cell by adult stem cells, and we performed single-cell RNA-sequencing on sorted fetal, adult, and cord blood naïve T cells to answer this question. We found that most fetal and adult naïve T cells do cluster separately based on gene expression, and some cord blood naïve T cells cluster with their fetal and adult counterparts. However, there is also a separate cluster of cord blood T cells, suggesting that there may be a gradual transition whereby the same originally fetal stem cells gradually produce progeny with a more and more adult-like phenotype.

F.83. Role of Rho Kinases In B Cell Development

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Cytoskeletal rearrangement is a critical component to hematopoietic stem cell development and differentiation into mature leukocyte populations. A number of chemokines and biolipids have been shown to regulate these events via binding to G protein coupled receptors. Many of these receptors couple to the G_{12/13} family of G proteins to regulate actin reorganization via the RhoA small GTPase. The Rho-kinases, Rock1 and Rock2, are activated downstream of RhoA to induce cytoskeletal changes that regulate numerous cellular processes, including contraction, polarity, proliferation, motility, and viability. A role for RhoA in regulating hematopoiesis and subsequent leukocyte development has been established. However, a similar role for the Rock1 and Rock2 has not been demonstrated. As global deletion of either kinase causes embryonic lethality, we used conditional gene targeting to ablate either the *Rock1* or *Rock2* allele in adult mice. *Rock1*^{flox/flox} or *Rock2*^{flox/flox} mice were crossed to the Rosa26-Cre ERT2 line to globally delete the floxed alleles. In contrast to deletion of RhoA, conditional deletion of either *Rock1* or *Rock2* was well-tolerated, with no aberrant effects on hematopoiesis. These results suggest functional redundancy between Rock1 and Rock2 in regulating RhoA activity in hematopoiesis. However, we observed a significant decrease in marginal zone B cell number in the spleen of mice lacking Rock1. We also observed a follicular B cell defect in the mice lacking Rock2. These results are consistent with the known roles of GPCRs in B cell migration/positioning, and suggest there are isoform specific functions of these kinases.

F.97. Importance of Individual Titration and Monitoring *In Vitro* of Ruxolitinib Treatment of patients with STAT1 GOF Mutations

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Gain-of-function (GOF) mutations in the signal transducer and activator of transcription (STAT) genes can lead to hyperactivation of the JAK-STAT signaling pathways, causing immunodeficiencies and autoimmune manifestations. STAT1 GOF patients have a heterogeneous and complex phenotype primarily presenting with chronic mucocutaneous candidiasis and autoimmunity. Additionally, affected patients have altered CD4⁺ T helper (Th) subsets with impaired Th17 polarization, decreased production of IL-17A, and an exaggerated Th1 response. Here, we present a case of a patient with a novel GOF mutation in the STAT1 gene (c.1154C>A p.T385M) who suffers from recurrent pneumonias, chronic candida esophagitis, oral ulcers, and disseminated molluscum lesions. Since ruxolitinib (a JAK1/2 inhibitor) has been a promising treatment option for some patients with STAT1 GOF, we investigated whether this therapy would be beneficial for our patient, in particular as a bridge to hematopoietic stem cell transplantation.

We used phospho-flow cytometry to assess the effect of ruxolitinib *in vitro* on phosphorylation of STAT1 in IFN- α activated PBMCs and observed a dose-dependent inhibition of the patient's interferon-induced STAT1-tyrosine-phosphorylation to approximate that of normal controls. Standard ruxolitinib dosing caused decrease in clinical inflammatory manifestations, but T-cell STAT1 hyperphosphorylation persisted. The ruxolitinib dose is being slowly escalated with continued clinical improvement and no toxicity, but correction of STAT1 hyperphosphorylation has not yet been observed. Because each patient's mutation is different, ruxolitinib therapy will require individual dose titration and monitoring should include clinical efficacy, side effects, and biochemical normalization in patients with STAT1 GOF mutations and other interferonopathies.

F.104. Mechanisms of Thymic Hypoplasia in Mouse Models of 22q11.2-DiGeorge Syndrome

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Patients with 22q11.2 deletion syndrome have multi-system disorders, including thymic hypoplasia, cardiac anomalies, and hypoparathyroidism of varying penetrance and severity. Most patients have a deletion of 2.5-3 Mb on chromosome 22q11.2, affecting protein coding genes, and noncoding genes such as microRNAs and long noncoding RNAs. 50%~70% have some degree of T cell lymphopenia due to a thymic hypoplasia (DiGeorge syndrome). The thymic hypoplasia and rare aplasia are linked to stromal tissue abnormalities. This includes thymic epithelial cells (TEC), mesenchymal cells, fibroblasts, and endothelial populations. We are characterizing the abnormalities in TEC-mesenchyme-endothelial interactions in mouse model of 22q11.2 deletion syndrome (Df1/+ and Tbx1/neo). Comparative transcriptome analyses of hypoplastic and normal-sized thymic lobes from embryos revealed a unique mesenchymal cells mRNA expression signature, including reduced levels of the PDGFR α . Crossing PDGFR α haploinsufficient mice with the Df1/+ model will reveal whether the hypoplasia is influenced by levels of this receptor. Mesenchymal cell characterizations of fetal thymii from the different mouse models of 22q11.2 is being used to identify which genes are causal to the hypoplasia of the thymus. The experiments will uncover how *Tbx1* (encoded on 22q11.2) influences pharyngeal pouch mesenchymal-TEC development. The results from the study will likely lead to novel approaches for thymus reconstitution in various clinical settings.

T.53. Aberrant Innate-Like Lymphocytes Causing Atopy and Immune Dysregulation in a Patient with a Novel BCL11B Variant Revealed by Mass Cytometry (CyTOF)

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Background: *B cell CLL/lymphoma 11B (BCL11B)* is a zinc finger protein transcription factor with a multitude of regulatory functions, including T cell lineage commitment, development, differentiation, survival, and function. Importantly, it specifies the identity and function of innate-like lymphocytes including $\gamma\delta$ T cells, innate lymphoid cells (ILCs), and invariant natural killer T cells (iNKT). However, little is known about its function in the human immune system. Here, we describe a patient with immune dysregulation associated with a novel p.C826Y *BCL11B* variant.

Methods: Expression of a panel of 30 markers was examined by CyTOF on peripheral blood mononuclear cells isolated from the patient, her father, and multiple healthy age-matched controls. Data was analyzed using the viSNE and SPADE algorithms.

Results: We have identified the second described case of immune dysregulation caused by a *de novo* heterozygous damaging variant of *BCL11B* (p.C826Y). This young girl presented with intellectual disability, microcephaly, severe atopy, eczema, alopecia totalis, and brittle nails. Clinical immunophenotyping revealed that the patient possessed abnormal rare innate-like lymphocyte populations (iNKT, DN T cells). Using CyTOF, we found that the patient had severely compromised numbers of the unique CD161⁺CD127⁺ $\gamma\delta$ T cell subset, thus potentially implicating the p.C826Y variant in $\gamma\delta$ T cell development and function.

Conclusions: The identification of decreased CD161⁺CD127⁺ $\gamma\delta$ T cells in a patient with a p.C826Y variant of *BCL11B* suggests that *BCL11B* is important for human $\gamma\delta$ T cell development and provides novel insights into the roles of both *BCL11B* and $\gamma\delta$ T cells in regulating atopy and autoimmunity.

T.58. Lentiviral-mediated FOXP3 Gene Transfer to Convert CD4⁺ T Cells to Treg Cells: a Suitable Method for Immunotherapy

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FOXP3 is an essential transcription factor for the regulatory T cell (Treg) function and therefore is a key regulator for the tolerance maintenance. Mutations in FOXP3 gene result in dysfunction of FOXP3⁺ Treg and a severe autoimmune disease known as Immune dysregulation, Polyendocrinopathy, Enteropathy, X-linked (IPEX) syndrome. Current therapy for IPEX syndrome are limited to immunosuppression and hematopoietic stem cell transplantation, however, both have side effects and limited efficacy.

We investigated lentiviral FOXP3 gene transfer (LV-FOXP3) as therapeutic approaches for IPEX syndrome. We have previously shown that LV-FOXP3 successfully converts IPEX patients-derived CD4⁺ effector T cells into Treg-like cells (CD4^{LV-FOXP3} T cells) (Passerini, Sci Transl Med, 2013). These results clearly demonstrated the potential clinical benefit of CD4^{LV-FOXP3} T cells.

To improve the safety and efficacy of this approach and to make it suitable for large scale GMP generation of CD4^{LV-FOXP3} T cells, lentiviral vector was optimized and we assessed the phenotype and function of CD4^{LV-FOXP3} T cells both *in vitro* and *in vivo*. CD4^{LV-FOXP3} T cells have stable expression of FOXP3 and suppressive function *in vivo* using different humanized mice models. CD4^{LV-FOXP3} T cells can significantly extend the survival of GVHD model mice, both in autologous and allogenic conditions.

In conclusion, we could optimize the production of CD4^{LV-FOXP3} T cells and confirm their suppressive function both *in vitro* and *in vivo*. Our data is suggesting that cell therapy with CD4^{LV-FOXP3} T cells is a promising treatment for IPEX syndrome and autoimmunity of different origin.

T.91. Patients with Novel Compound Heterozygous Mutations in Forkhead Box N1 (FOXN1) have a Severe Immunodeficiency Without Alopecia or Nail Dystrophy

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Patients with mutations in the *Forkhead Box N1* (*FOXN1*) transcription factor are born with a severe T-cell lymphopenia in conjunction with alopecia and nail dystrophy (OMIM # 600838). The T-cell lymphopenia results from the impaired development and/or function of the thymic epithelial cells (TECs) due to the loss of Foxn1 protein expression. TECs are essential regulators of positive and negative selection of developing thymocytes. We report on 3 independent patients with compound heterozygous mutations in *FOXN1*. All 3 patients had a severe T cell lymphopenia that was linked to a thymic hypoplasia/aplasia. However, each affected child had normal hair growth and nail bed formation. This demonstrates that the compound heterozygous mutations in *FOXN1* result in an atypical clinical presentation. To determine how the different mutations impact murine *Foxn1* function, transcriptional reporter assays and protein expression studies were undertaken. Only one of the mutations affected the transcriptional activity of murine Foxn1, with Western blot analyses indicating that this mutation caused production of a truncated protein. CRISPR/Cas9 technologies were used to create mouse lines with compound heterozygous mutations in *Foxn1*. The mice are currently being intercrossed. The impact of the compound heterozygous *Foxn1* mutations on T cell development in the thymus will be presented. Findings from this study may provide further understandings of how thymic epithelial transcription factors regulate T cell development.

W.18. A Novel Btk Mutation Manifesting as Late-onset X-linked Agammaglobulinemia

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Introduction: X-linked agammaglobulinemia (XLA) is a primary immunodeficiency typically diagnosed in childhood that is characterized by absent B cells secondary to *Btk* gene mutation. Here we present an unusual case of a 60-year-old male with history of frequent sinusitis/pneumonia and family history of recurrent pneumonias in male relatives, evaluation confirmed a diagnosis of XLA with a novel mutation.

Methods: Case report and literature review.

Results: The patient reported annual episodes of pneumonia during childhood, which improved during early adulthood, but became more frequent during his 6th decade. His absolute lymphocyte count was normal. Immunoglobulin levels were reduced. Vaccine responses to diphtheria and pneumococcus were reduced, but tetanus was normal. B cells were virtually undetectable. Btk protein flow showed normal Btk expression on monocytes. Genetic testing was positive for a novel missense mutation in exon 14 of the *Btk* gene (p.Gly414Trp) possibly abrogating function. Literature review reveals 18 cases of "atypical" XLA diagnosed after the 2nd decade have been reported. Reported IgG levels vary between markedly decreased to near-normal levels. All cases exhibited absent to significantly decreased CD19⁺ B cells. Wide phenotypic variability may occur between family members with a common genetic mutation.

Conclusion: XLA may be diagnosed later in life. Males with a diagnosis of common variable immunodeficiency or presenting with recurrent infection and hypogammaglobulinemia should be evaluated for lymphocyte subsets, with a diagnosis of XLA considered in the presence of low to absent CD19⁺ B cells.

W.25. The Mechanisms of Immune System Dysregulation in Common Variable Immunodeficiency - The Role of T Helper Cells

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Background: Common Variable Immunodeficiency (CVID) is characterized by impaired immunoglobulin production and immune system dysregulation. The changes in B-cell compartment are well described. On the contrary, the role of T-cells (including T helper (Th) cells) has not been entirely revealed yet.

Methods: Upon isolation the first portion of PBMC was stimulated with ionomycin and PMA. We measured expression and production of activation markers (CD69, CD154, HLA-DR), chemokine receptors (CXCR3, CRTH2, CCR6), transcription factors (T-Bet, GATA-3, ROR-gamma) and intracellular cytokine (IFN-gamma, IL-5, IL-17). In the second one Th-cell subpopulations were determined (using expression of CD3, CD4, CD8, CD45RO, CCR7).

Results: Together 21 patients and 14 matched healthy controls were included into the study. We revealed significant differences in expression of chemokine receptors, transcription factors and in production of intracellular cytokines favouring Th1-response and in Th-cell development with reduction of naïve and expansion of mature forms. Moreover, CVID patients had higher expression of HLADR and limited response to stimulation with reduced increase of CD69 expression.

Conclusions: The dysregulation of immune system in CVID patients occurs in B as well as in T-cell compartment. We revealed disturbed development of Th-cells with increased number of mature forms together with signs of chronic stimulation and exhaustion of immune system. There is also skewing towards Th1-response.

Immuno-dermatology

F.14. Immunomodulation and Regeneration Effect of Biosynthetic Dressing Loaded with Silver Nanoparticles and TNFa Inhibitor in a Non-traumatic Wounds Experimental Model

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The chronic wounds are stopped in the inflammatory phase, thus preventing the regeneration of the same. Silver-like nanoparticles have antibacterial properties, these have been used in a wide range of biomedical applications due to their selective toxicity in microorganisms and low immunogenicity. In addition, the use of immunomodulators contributes to inflammatory regulation to promote healing and regeneration. Aim. Formulate a biosynthetic dressing loaded with silver nanoparticles and a TNFa inhibitor that promotes tissue regeneration and bacterial inhibition in an experimental model of chronic wounds. Methodology. Chitosan gel was formulated. Silver nanoparticles were synthesized and their size was corroborated by SEM, TEM and IR. Dissolution time tests of the pharmaceutical form were carried out according to the Mexican pharmacopoeia. A bacterial inhibition test was performed in solid culture media type antibiogram. An experimental model of chronic wounds was established. Tissue histologies were performed. The genes of TNFa, TGFb1, MMP8, IL-1 and IL-17 were measured. Results. Chitosan with 90% purity was obtained. Subsequently, the chitosan was solubilized until it became semi-solid. Silver nanoparticles of 30 nm were synthesized by chemical synthesis. A formula was standardized with previous compounds and a TNFa inhibitor. In the solubility tests, formula was kept releasing drug for 5 days at different pH. Bacterial inhibition was achieved by visualizing agar bacteria inhibition. The inflammation was decreased histologically. As well as a decrease in the expression of proinflammatory cytokines was observed in the treated group. Healing of the wound was achieved before 15 days compared to the negative control.

F.41. MIF Gene Polymorphisms are not a Genetic Risk Factor for NSV Susceptibility in a Western Mexican Population

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Vitiligo is a depigmenting disorder of the skin, characterized by achromic macules caused by a selective loss of melanocytes. Its etiopathogenesis is unclear, but it has been associated with autoimmune processes. Macrophage migration inhibitory factor (MIF) is an immunoregulatory cytokine associated with autoimmune inflammatory diseases. Two polymorphisms identified in the promoter region has been associated with increased plasma levels of MIF and with an increased risk to develop autoimmune diseases.

In this case-control study, we investigated the association of *MIF* promoter polymorphisms with NSV and MIF serum levels in Western Mexican population. Genotyping of the -794CATT₅₋₈ and -173 G>C *MIF* polymorphisms was performed by PCR and PCR-RFLP respectively in 94 NSV patients and 103 healthy subjects. MIF serum levels were determined by ELISA.

For STR -794 CATT₅₋₈ *MIF* a significant increase of MIF (genotypes without the risk allele: 5.57 vs. genotypes with allele risk: 4.40 ng/mL; pC *MIF* a slight increase of MIF (GG: 4.86 vs. GC+CC: 4.57 ng/mL; p=0.09), this difference was not significant. The distribution of STR -794 CATT₅₋₈ and SNP -173 G>C *MIF* genotypes and allele frequencies in NSV patients did not differ from that in healthy subjects (p > 0.05). Moreover, there was no association according to a genetic model with susceptibility to NSV. In conclusion, we suggest that *MIF* gene polymorphisms are not a genetic risk factor for NSV susceptibility in a Western Mexican population.

F.48. Multidimensional Approaches to Define the Risk and Immunopathogenesis of Stevens-Johnson Syndrome/Toxic Epidermal Necrolysis Associated with Nevirapine in South Africa

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Nevirapine use in antiretroviral therapy is limited by adverse reactions including Stevens-Johnson Syndrome/toxic epidermal necrolysis (SJS/TEN). SJS/TEN is a human leukocyte antigen (HLA) class I restricted, CD8⁺ T-cell dependent hypersensitivity, which presents as a blistering rash that can lead to life-threatening epidermal necrosis. To study the disease immunopathogenesis, we accrued a biobank of samples including PBMCs, plasma, saliva, blister fluid and skin from >80 nevirapine-exposed HIV⁺ patients that included mother-child nevirapine-exposed pairs where only one relative developed SJS/TEN. HLA, killer-cell immunoglobulin-like receptor (KIR), endoplasmic reticulum aminopeptidase (ERAP) and CYP2B6 typing was performed on all subjects. 100% of SJS/TEN cases (n=19) carried HLA-C*04:01 compared to 26.3% of unrelated individuals tolerating nevirapine (n=10/38) (p<0.00001). CYP2B6 genotyping revealed that hypersensitive patients had slower nevirapine metabolizing phenotypes than tolerant controls. Formalin-fixed, paraffin-embedded skin was stained with antibodies against SJS/TEN biomarker granulysin as well as general T cell (CD3, CD4, CD8), skin homing (CCR4, CLA), natural killer cell (CD56), regulatory T cell (FoxP3), and tissue-resident (CD103) markers. These slides were analyzed blindly by a dermatopathologist and in SlidePath's Digital Image Hub. Single cell TCR sequencing (sc-TCRseq) paired with whole transcriptome sc-RNAseq was performed on blister fluid and skin T cells from acutely ill nevirapine SJS/TEN patients. HLA-C*04:01 carriage in 100% of those with nevirapine SJS/TEN and 26.3% of tolerant controls suggests that this allele is necessary but not sufficient for SJS/TEN development. Results from the immunohistochemistry and sc-TCRseq will provide insights into why not all nevirapine-exposed patients carrying an HLA risk allele develop a reaction.

F.76. IgA Nephropathy Presenting with Chronic Urticaria

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Intro: IgA nephropathy is a common cause of glomerulonephritis worldwide. It has been noted to have other immunologic associations as does chronic urticaria, another common disorder. To our knowledge, this is the first case of IgA nephropathy presenting with chronic urticaria.

Case presentation: A 47-year-old male with IgA nephropathy, notable for a kidney transplant 14 years ago secondary to worsening kidney disease, presented to allergy clinic with daily hives for seven months. He noted pruritic papules throughout his body that occurred at night and resolved with Benadryl. He recalled having similar symptoms in his mid-twenties around the time that he was diagnosed with IgA nephropathy and needed a kidney transplant as a result. He was then without hives until this most recent presentation. On further evaluation, he was found to have normal liver function, ANA, complement, thyroid function, and negative food and environmental allergy testing. He was concurrently found to have worsening hematuria and proteinuria. Given the timing of symptom onset and negative work up, a clinical association between urticaria and IgA nephropathy may exist.

Conclusion: Although chronic urticaria is not well understood, it has been suggested that autoantibodies or another autoimmune dysfunction mediates the disorder. Additionally, though no previous cases of IgA nephropathy and urticaria have been reported, hereditary angioedema and IgA nephropathy have been linked in a previous case report and may demonstrate an underlying common mechanism. Possible pathogenesis mechanisms could include complement consumption versus autoantibody formation and would warrant further investigation.

T.3. SEB Stimulation Induces Functional Pathogenic Features in Th17 Cells from Psoriasis Patients

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Introduction. Psoriasis is an inflammatory skin disease mediated by Th17 cells. There are two types of Th17 cells, conventional and pathogenic. Throat infections, increased presence and superantigens of *Staphylococcus aureus* such as enterotoxin-B (SEB) in lesional skin have been associated with psoriasis onset and severity. However, the phenotype and function of Th17 cells in the presence of SEB remains unknown in psoriasis patients.

Aim. To evaluate the conventional and pathogenic Th17 transcriptional phenotypes and their functional features in skin biopsies from psoriasis patients after SEB stimulation.

Material and methods. We included fifty psoriasis patients and obtained biopsies from lesional and non-lesional skin and peripheral blood. We analyzed the Th17 cells phenotype and cytokine production through flow cytometry and confocal microscopy.

Results. We identified both Th17 cell phenotypes according to ROR γ t, Runx1 and T-bet expression in skin biopsies. Remarkably after SEB stimulation, the conventional Th17 cells were decreased and pathogenic Th17 cells increased in lesional skin. *In situ*, some CD4⁺ lymphocytes simultaneously express IL-17 and IFN- γ . After SEB activation the pathogenic phenotype was intensified in Th17 cells due to the increased expression of T-bet, acquiring functional features such as IFN- γ production and a reduction of IL-17 expression. SEB treatment promotes a pathogenic environment revealed by low quantities of IL-17 and IL-9, but high levels of IFN- γ .

Conclusion. The Th17 pathogenic phenotype and functional features are intensified by SEB stimulation. These findings

may be relevant in the physiopathology of psoriasis. Blocking the acquisition of pathogenic features could be promising in psoriasis treatment.

T.15. Tc17/MAIT Cells in Psoriatic Arthritis: Regulatory Role of the IL-23 and its Receptor System

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The mucosal-associated invariant T (MAIT) cells recently have been implicated in autoimmune diseases. Their gut origin and lineage towards IL-23/IL-17 cytokine signatures make them relevant for spondyloarthritis. Here we have explored functional significance of the MAIT cell in psoriatic arthritis (PsA). PBMC and synovial fluid mononuclear cells (SFMC) from age/sex matched active untreated PsA (n=10) and osteoarthritis (OA) (n=10) patients were studied. Sorted, activated CD3⁺ T cells were cultured. In HiD-FACS studies CD3⁺VαTCR⁺CD161^{high} cells were identified as MAIT cells. In PsA and OA, CD3⁺IL-17⁺ MAIT cells were identified in PBMC (<1%). SFMC of PsA patients had a significant high numbers of MAIT cells compared to OA (3.5%±0.8 vs 0.61% ± 0.1; p<.01); about 40% of these MAIT cells secreted IL-17A and predominantly they were CD8⁺ T cells (> 90%). We noticed IL-23R expression was found to be significantly higher in SFMC of PsA compared to OA (22.1 % ± 1.2 vs 2%±1.2; p<.001). rIL-23 induced proliferation and inhibited apoptosis of these IL-17⁺ MAIT cells. IL-23R expression was further confirmed by immunoblot and RT-PCR studies in sorted CD3⁺VαTCR⁺ cells. Conclusion: 1. MHC class-1 association and enrichment of Tc17/MAIT cells in SFMC in PsA could be of immense pathological significance and needs further evaluation. 2. Identification of functionally active IL-23R on MAIT cells brings a new dimension: (i) these cells are a part of the IL-23/IL-17 cytokine network, (ii) that once these cells migrates to joint tissue IL-23 can independently regulate these critical Tc17 CD8⁺ MAIT cells.

T.71. Unexpected Cutaneous Effects in IL31-deficient Animals

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T-helper 2 (Th2) cells are important mediators of atopic skin inflammation. In addition to the canonical Th2 cytokines (interleukin-4, -5, and -13), atopic skin also produces interleukin-31 (IL-31), an IL-6 family cytokine associated with itch. IL31 transgenic animals develop spontaneous dermatitis and scratching behaviors akin to atopic dermatitis. Its receptor heterodimer, IL31RA/OSMR, is expressed on myeloid lineage cells in addition to TRPV1⁺ afferent sensory nerves. We generated IL31-deficient animals in order to better define the contributions of IL-31 to cutaneous inflammation. We hypothesized that IL31-deficient mice would display reduced susceptibility to Th2-mediated inflammation and scratching behavior in mouse models of AD-like dermatitis. We further hypothesized that IL-31 might be capable of promoting dermatitis independent of its pruritogenic effects on afferent sensory nerves. We observe model-dependent alterations in T-cell cytokine production and overall skin inflammation in our IL31-deficient animals.

W.57. Assessing a Potential Role of Stem Cell Memory T Cells as Cancer-initiating Cells in Sézary Syndrome

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Sézary syndrome is a rare, leukemic and aggressive form of Cutaneous T-cell Lymphomas. We previously proved the specificity of KIR3DL2 as a surface marker of malignant T-cells and reported an unexpected heterogeneity of circulating

malignant cells displaying multiple phenotypic features from “naïve” to central memory T-cells, whereas skin Sézary cells displayed more mature phenotypes. Some circulant malignant cells had markers consistent with stem-cell memory T-lymphocytes (TSCM). This subset is described in healthy subjects (HS) and in adult T-cell leukemias, as possessing self-renewal and multipotency capacities. Such heterogeneity may imply a malignant T cell plasticity, with TSCM as potential cancer-initiating cells. Our study is aimed at characterizing the diversity and the dynamic relationships of KIR3DL2⁺ T-cells in the blood and the skin as well as the “benign” part of KIR3DL2⁺ T-cells. Based on genes discriminating naïve/memory CD4⁺ subsets in HS, molecular signatures from sorted KIR3DL2⁺TSCM and CD4⁺TSCM from HS showed similar patterns, that were distinct from KIR3DL2⁺TCM and CD4⁺TCM. However, the analysis of cytokine and costimulatory molecules pathways from each subset revealed significant differences between HS and patients, especially for IL-2, IL-7, TIGIT, CD70 and PD-1 pathways. Some of them are also affected in “benign” KIR3DL2-CD4⁺ T-cells from patients, underlying qualitative immune disorders of these cells. Extended phenotypic analyses are ongoing to complement our data.

Our results will allow us to exploit the potential stemness of Sézary TSCM and assess the ability of these malignant TSCM cells to sustain themselves and differentiate into TCM and TEM.

Immunology of the eye

F.55. Macrophage Activity and Survival are Regulated by Exosomes from Retinal Pigment Epithelial Cells

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Our research studies the molecules released by retinal pigment epithelial cells (RPE) that promote health of the retina, and ocular immune privilege. In this study, we examined the possibility that RPE exosomes are the source of the apoptotic signal. Posterior eyecups were dissected from eyes of C57BL/6 mice with or without autoimmune uveitis. The RPE eyecups were submerged in serum-free media in wells of a 96-well culture plate. The conditioned media (CM) was removed 24 hours later. Also, CM was collected from cultures of established ARPE-19 cell monolayers in serum free media. From the conditioned media, exosomes were isolated using precipitation methods. The isolated exosomes were added to cultures of resting and LPS activated macrophages. The macrophage cultures were assayed for Nitric Oxide (NO), IL-1 β , TNF- α , and for apoptosis. The isolated exosomes were assayed by immunoblot for pro-apoptotic FasL, and by PCR for miR204 and miR155, which are microRNA that can influence both apoptotic, and inflammatory activity in macrophages. The exosomes from the RPE eyecups of healthy and uveitic eyes, but not from cultured ARPE-19 cells induced apoptosis in macrophages. The exosomes from only the RPE eyecups had membrane FasL, and the pro-apoptotic mature-miR204. In contrast, the anti-apoptotic, pro-inflammatory miR155 was found only in the exosomes from uveitic RPE eyecups, but as pre-miRNA. The exosomes from the ARPE-19 cultures suppressed NO, and IL-1 β , but not TNF- α production by activated macrophages. The results demonstrate that RPE exosomes carry proteins and miRNA that can regulate macrophage activity within the ocular microenvironment.

T.4. An Abnormal Mucosal Immune Response to an Ocular Surface Commensal Precipitates Inflammation in a Model of Muckle-Wells Syndrome

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We recently demonstrated that the ocular surface, which is profoundly antibacterial, nevertheless harbors a resident commensal flora. *Corynebacterium mastitidis* (*C. mast*), a commensal which also colonizes humans, tunes mucosal immunity at the ocular surface by eliciting production of IL-17 from conjunctival $\gamma\delta$ T cells, and thereby confers resistance to pathogenic fungal and bacterial infection (St. Leger et al, Immunity 2017). Muckle-Wells Syndrome (MWS) is one of

several human autoinflammatory diseases known as Cryopyrin Associated Periodic Syndromes (CAPS), in which a gain-of-function mutation of the NLRP3 inflammasome gene *CIAS1* results in inflammation of the skin, joints and conjunctiva. Using a gene knock-in mouse model that partially recapitulates MWS, we show that, in this immunologically abnormal host, the commensal *C. mast* behaves as a pathobiont and induces ocular inflammation. Mechanistic studies suggested that the NLRP3 inflammasome acts extrinsically on, and intrinsically within, conjunctival $\gamma\delta$ T cells, to enhance production of IL-1 and IL-17, leading to tissue pathology. We propose that an abnormal immune responses to normal environmental stimuli, such as commensal bacteria, may underlie the recurrent conjunctivitis seen in patients with MWS and similar autoinflammatory disorders.

T.10. A New Model of Autoimmune Retinopathy Demonstrates the Critical Importance of IL-10 to Retinal Protection

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Purpose: Autoimmune retinopathy (AIR) is a distinct form of retinal degeneration involving poorly understood breakdowns in ocular immune privilege. AIR has been associated with other autoimmune conditions, other forms of retinal dystrophy, or immune responses toward cancers that express retinal antigens. Our laboratory has found that AIR patients have a skewed T_H cell response toward recoverin that results in a high ratio of IFN γ to IL-10. This study was initiated to test the hypothesis that IL-10 plays a major role in inhibiting retinal degeneration caused by T_H1 responses against recoverin.

Methods: C57BL6 (B6) and IL-10 deficient B6 mice were immunized with recoverin using standard T_H1 - inducing conditions. Retinal imaging was performed at 3 week intervals to determine the extent of retinal damage. At sacrifice, infiltration of cells into the eye and the humoral and cellular immune responses toward recoverin were measured. An immune dominant peptide of recoverin (AG-16) was identified and used for immunization that was further tracked using AG-16 peptide/I-A^b tetramer.

Results: Retinal degeneration was inducible in both B6 and IL-10 deficient B6 mice with recoverin. Disease onset was significantly accelerated and more severe in the absence of IL-10. B6 mice had a more classical AIR presentation, while IL-10 deficient mice showed evidence of uveitis. An array of lymphoid and myeloid cells infiltrated the eyes in both strains. Peptide immunization produced disease in IL-10 deficient mice which included infiltration of tetramer-specific T_H cells.

Conclusion: This new mouse model will allow for extensive further study of the pathogenesis of AIR.

T.56. Association Study Between HLA Genes and Climatic Droplet Keratopathy (CDK) in a Cohort from the Patagonian Region of Argentina

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Climatic Droplet Keratopathy (CDK) is an acquired degenerative disease predominantly affecting males over 40 years old. It results in progressive corneal opacities usually affecting both eyes. CDK is multifactorial and its etiology remains unknown. Recent findings are consistent with CDK pathology being driven by oxidative stress and inflammation factors. Contribution of immunogenetic factors to the etiopathology of CDK remains still understudied. The goal of this study was to investigate the association between HLA class I (-A, -B, -C) and HLA class II (-DPA1, -DPB1, -DQA1, -DRB1, -DRB3, -DRB4, -DRB5) genes, which were phased genotyped by a NGS-based method, in a cohort of 20 individuals diagnosed with grade 2 of CDK (16 males and 4 females) and 20 healthy controls (not presenting any pathology in the anterior segment of the eye: 17 males and 3 females) from El Cuy Department in Argentine Patagonia. Frequencies

between genotypes for all HLA loci showed no deviation from Hardy-Weinberg equilibrium in cases or controls. The frequencies of all HLA alleles were calculated and no significant differences (two-tailed Fisher's exact test) were found between the two groups studied, except for the protective allele *HLA-B*51:01* ($P = 0.0284$; OR = 0.101; 95%CI = 0.012-0.856) and for the risk allele *HLA-DRB3*01:01* ($P = 0.0329$; OR = 9.000; 95%CI = 1.060-76.427). Our initial analyses suggest a possible influence of certain HLA genes on susceptibility and resistance respectively to CDK in this studied cohort although further studies are necessary for a complete elucidation of this data obtained in this study.

T.65. The Induction of Specific Systemic Tolerance to Encephalitogenic Antigens via the Ocular Route or Adoptive Transfer of Cells

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Antigens introduced into the anterior chamber (AC) of the eye generate a form of specific systemic tolerance that has been named termed AC-associated immune deviation (ACAID). Experimental autoimmune encephalomyelitis (EAE) is an animal model of multiple sclerosis (MS) and other demyelinating conditions. We used the encephalitogenic antigens myelin oligodendrocyte glycoprotein (MOG35-55) or myelin basic protein (MBP) to induce specific tolerance and hypothesized that injection of MOG35-55/MBP induces antigen-specific tolerance. Injection of antigens was carried out via the ocular route. We also adoptively transferred *in vitro*-generated MOG35-55-specific/MBP-specific ACAID antigen presenting cells (APCs). Finally, we tested for tolerance following the adoptive transfer of MOG35-55-specific/MBP-specific ACAID T regulatory cells (Tregs). Delayed-type hypersensitivity (DTH) responses were assayed and were found to be impaired. The functional local adoptive transfer (LAT) assays revealed regulatory functions for MOG35-55-specific/MBP-specific Tregs. The induction of specific tolerance following ocular or adoptive transfer of cells could be utilized in the development of specific treatments for MS.

W.24. Clinical Effect of IRT5 Probiotics on Dry Eyes

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Purpose: To investigate clinical effects IRT5 probiotics on dry eyes

Methods: 12-weeks-old NOD.B10.H2^b mice were used as an autoimmune dry eye model. Probiotic IRT5 contain 5 commensal strains. Either IRT5 probiotics or phosphate buffered saline (PBS) were administered orally for 3 weeks. Clinical manifestations were evaluated with phenol red thread test and corneal dye staining with 3% lissamine green B. The changes of T cells were evaluated in drainage lymph nodes using fluorescence-activated cell sorting. The feces before and after treatment with IRT5 or PBS were collected and analyzed for microorganisms.

Results: Ocular staining score was significantly decreased in IRT5 treated mice compared with the score of both pretreatment status ($p=0.0083$) and PBS administered mice ($p=0.0005$). Phenol red thread test also showed a significant increase of tear secretion in IRT5 treated mice after 3 weeks treatment ($p=0.0039$). In the submandibular lymph node, the percentage of CD4⁺ T cells and IFN γ -secreting CD8 T cells were significantly decreased after treatment ($p=0.0317$ and 0.0362), but regulatory T (Treg) cells were significantly increased after treatment ($p=0.0317$). In the mesenteric lymph node, the percentage of Th17 cells decreased ($p=0.0317$). In fecal analysis, microbial diversity was increased, and the butyrate producing strain, *Lachnospiraceae*, was more abundant in the IRT5 treated group.

Conclusions: This suggests that the administration of IRT5 probiotics may improve the clinical manifestations of ocular dryness through immune regulation by changes in composition of gut microbiota.

W.88. Therapeutic Recovery of Retinal Pigment Epithelial Cell Suppression of Macrophage Phagocytic Activity by Alpha-Melanocyte Stimulating Hormone is not Through the Melanocortin 5 Receptor

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The therapeutic application of the neuropeptide alpha-melanocyte stimulating hormone (α -MSH) suppresses experimental autoimmune uveitis (EAU) in mice. This suppression is mediated in part by the expression of melanocortin 5 receptor (MC5r) on immune cells. In MC5r^(-/-) mice, α -MSH-therapy suppresses EAU, but does not generate protective regulatory immunity. To further differentiate the role of the MC5r from other melanocortin receptors in α -MSH-therapy, we assayed whether MC5r is required for RPE suppression of phagocytic activity in macrophages.

EAU was induced in wildtype and MC5r^(-/-) C57BL/6 mice by immunization with adjuvant. Some of the wildtype mice were treated with α -MSH or MC5r-agonist (PXXXX) when EAU scores reached maximum. Mouse eyes collected when the EAU scores reached zero for the α -MSH treated mice were dissected to make RPE-eyecups, and cultured in serum-free medium for 24 hrs. The conditioned medium (CM) was collected, and used to treat peritoneal macrophages. The treated macrophages were fed opsonized pH-rodo-bioparticles, incubated for 24 hours, and bioparticle fluorescence intensity was measured as an indicator of phagolysosome activation.

Phagolysosome activation was suppressed in macrophages treated with CM from healthy eyecups, but not with CM of eyecups from wildtype, MC5r^(-/-), and MC5r-agonist treated EAU mouse eyes. The CM of RPE-eyecups from EAU mice treated with α -MSH recovered suppression of phagolysosome activation. While the role of MC5r in recovery from EAU is important for induction of regulatory immunity, it must be through other melanocortin receptors involved in inducing RPE regulation of macrophage activity.

Immuno-oncology

F.4. Augmentation of a Novel Adenoviral Vaccine Strategy by Checkpoint Inhibitors

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The profoundly immunosuppressive tumor microenvironment, the lack of 'danger' signals, and the need to break tolerance without causing autoimmunity are all considerations when designing an anti-cancer vaccine. Immune checkpoint blockade (ICB) including programmed death 1 (PD1) and cytotoxic T-lymphocyte antigen 4 (CTLA-4) monoclonal antibodies have revolutionized cancer treatment as a whole, including the potential for a successful cancer vaccine. Human epidermal growth factor receptor 2 (HER2) is an oncogene that is overexpressed in 20-25% of breast cancers and has been successfully targeted with anti-HER2 antibody combinations. However, even the most potent anti-HER2 therapy is accompanied by a high rate of recurrence and resistance. Given the success of combination therapy using antibodies against different epitopes of HER2, we hypothesized that a HER2 vaccine approach could broaden the immune repertoire and reduce rates of resistance and recurrence. We developed an implantable and a mammary specific spontaneous tumor model driven by an oncogenic isoform of HER2 (HER2 Δ 16). Our vaccine platform in these models, in combination with two recently approved checkpoint inhibitors anti-CTLA-4 and anti-PD-1, greatly enhanced the HER2-specific immune response as well as the anti-tumor effect seen post vaccination, with many tumors exhibiting complete regression. We have further shown that vaccination against HER2 Δ 16 can prevent spontaneous tumor

formation and work is ongoing to test therapeutic vaccine strategies in combination with ICB. Future studies are focused on determining the mechanism of regression and evaluating the impact on resistance by combining this novel therapeutic platform with current standard of care HER2 targeted therapies.

F.6. ConvertibleCAR T-Cells Provide Dose Control of Activity and Targeting Flexibility

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Current chimeric antigen receptor (CAR) cellular therapies, while remarkably efficacious, have a number of limitations that impact their efficacy and perseverance in the clinical setting including commitment to a single antigenic target, utilization of non-human components, and lack of dose control. We have developed the *convertibleCAR*TM platform to function as a flexible and controllable universal system to address these limitations. *convertibleCAR* T-cells possess an inert NKG2D receptor that is activated only when an exclusive “privileged partnering” interaction is generated with an orthogonal human ligand fused to an antigen-targeting antibody (MicAbodyTM). Studies in NSG mice exploring efficacy against Raji tumors have demonstrated Rituximab-based MicAbody dose-dependent control of tumor mass. Furthermore, we have demonstrated that targeting can be switched or multiplexed without modifying the *convertibleCAR* T-cell enabling the development of a single autologous *convertibleCAR* cell for all targets in a single donor patient or one allogeneic *convertibleCAR* cell for all patients and all targets.

F.8. Boosting Anti-tumor Response by Conferring Cysteine Autonomy to T Cells

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Impressive results have been recently obtained in patients with cancer by engineering T cells with anti-tumor T cell receptors (TCRs) or chimeric antigen receptors (CARs) upon adoptive cell transfer (ACT). Most of the attention is currently focused on tumor antigen specificity, precision, expression/signaling tuning and safety of the infused T cells. However, additional therapeutic weapons will be needed to reach long-term remissions, especially in solid cancers where the tumor microenvironment can exert strong suppressive effects on T cells. Beyond CTLA-4 and PD-1 blockade, multiple other targets likely remain to be discovered and could be overcome by original T cell engineering. Cysteine deprivation within the tumor environment may represent a major inhibitory mechanism favored by tumor cells and/or suppressive myeloid cells. Cysteine (along with its oxidized dimeric form, cystine) is the least abundant of the amino acids in the extracellular space and is normally mainly provided to T cells by dendritic cells. In this work, we explored the therapeutic potential of engineering genes of the transsulfuration pathway in T cells with the aim to confer cysteine production autonomy. We addressed this hypothesis in a preclinical mouse model of antigen-specific antitumor ACT. Our preliminary results show that this strategy can significantly enhance the control of tumor growth and highlight the potential importance of this metabolic checkpoint.

F.21. Engineering Antigen-Specific T Cells from Human Pluripotent Stem Cells

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Immunotherapy has rapidly emerged as a promising treatment for many different types of cancer. Ongoing clinical trials continue to validate the efficacy of T cell-based cancer immunotherapy, such as chimeric antigen receptor (CAR) T cells, as a valid therapeutic approach. As such, new sources of both autologous and “off the shelf” T cells available in large quantities would be invaluable to widespread application of these treatments. Human induced pluripotent stem cells (iPSCs) represent a potentially inexhaustible source of clinically useful cell types. Previous studies have demonstrated the feasibility of generating T cells from iPSCs, but yields have been low due to the inefficiency of lymphoid differentiation. We report a novel cell engineering platform capable of generating large numbers of T cells from iPSCs using a combination of transcription factors and chromatin modifiers. Repression of the Polycomb group protein EZH1 uniquely enhances lymphoid differentiation from human iPSCs. An average batch of 10⁴ hPSC-derived blood progenitors yields 10⁸ T cells after differentiation, representing a platform that can be further optimized for clinical translation. Coupled with genome editing and CAR technologies, iPSC-derived CAR T cells undergo antigen-mediated activation and expansion, release effector cytokines and mediate antigen-specific tumor killing. Future work is aimed at improving antigen-specificity using non-viral integration methods in iPSC-derived T cells. Together, this work highlights the combined power of CRISPR/Cas9 genome editing, CAR technology and human pluripotent stem cells to generate antigen-specific T cells *de novo*, which will greatly contribute to the widespread use of T cells for cancer immunotherapy.

F.23. Using Synthetic Biotic™ Medicines to Activate Innate and Adaptive Immunity and Drive Antitumor Immune Response

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T-cell priming and blockade of immunosuppression play critical roles in generating an efficacious antitumor immune response. Recent studies show that activation of the stimulator of interferon genes (STING) pathway plays a critical role in initiating an antitumor immune response through activation of antigen presenting cells (APCs) while metabolites derived from tryptophan metabolism by indoleamine 2,3 dioxygenase (IDO), such as kynurenine, have been shown to drive the immune-suppressive tumor microenvironment. At Synlogic we are combining synthetic biology with probiotic bacteria to develop “Synthetic Biotic Medicines” capable of manipulating multiple immunological pathways relevant in cancer and autoimmunity and present here the development of engineered strains targeting the STING and Kynurenine pathways. Using synthetic biology we generated bacterial strains capable of producing the STING agonist cyclic-di-AMP (SYN-STING) or metabolizing kynurenine (SYN-Kyn). In *in vitro* assays, SYN-STING generated high levels of cyclic-di-AMP and triggered expression of inflammatory cytokines when co-cultured with APCs, while SYN-Kyn actively depleted test media containing high levels kynurenine. In syngeneic tumor-bearing mice intratumoral administration of SYN-STING resulted in an early rise of innate cytokines which later shifted towards molecules indicative of an effector-T cell response, whereas injection of SYN-Kyn led to significant decreases in tumor kynurenine levels. Finally, administration of SYN-STING or SYN-Kyn in combination with checkpoint inhibition led to significant anti-tumor effects in tumor-bearing mice. Taken together, these results demonstrate that using synthetic biology to engineer bacteria is a viable approach to deliver profound efficacy in experimental models of cancer, supporting further development of Synthetic Biotic Medicines for cancer-immunotherapy.

F.86. Activation of Endogenous Anergic Self-specific CD8⁺ T cells by Polymeric Nanoparticles for Enhanced Cancer Immunotherapy

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While the human immune system is often able to protect the body from infectious pathogens, it has multiple mechanisms to inhibit mounting an immune system against what it perceives as “self” and thus often fails to eliminate cancer cells since they seem to have the characteristics of “self”. Recently, our lab found that self-peptide MHC-specific CD8⁺ T cells in the blood of healthy humans were present in frequencies similar to those specific for non-self antigens, but these cells are resistant to activation and/or expansion. We hypothesized that tumor infiltrating T cells are often in an anergized state but that they could be activated with the appropriate innate immunity signals in addition to antigen-MHC exposure. In order to activate these cells, however, a large dose of immunological stimulating agents needs to be injected into the body. Those drugs often have life-threatening side effects. To address this issue, we have developed a poly(lactide-co-glycolide) nanoparticle (PLGA NP) based immunostimulatory platform, providing sustained immune stimulation in the tumor microenvironment to continuously activate anergic self-specific CD8⁺ T cells with minimal systemic toxicity. The result demonstrated the developed NPs have elicited potent immune response with markedly increased self-specific effector CD8⁺ T cells in the tumor tissue, resulting in significantly delayed tumor growth in mice bearing melanoma. We further analyze the TCR sequences and phenotypic characteristics of CD8⁺ T cells harvested from tumor after NP stimulation at single cell level. The results we obtained are crucial for identifying potentially important melanoma-associated TCRs and subsequent discovering their antigens.

F.95. PD-1 Blockade Markedly Enhances Immunity to Prostate Tumors by Adoptively Transferred Human V γ 2V δ 2 T Cells in an NSG Mouse Model

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Human $\gamma\delta$ T cells expressing V γ 2V δ 2 TCRs monitor foreign- and self-prenyl pyrophosphate metabolites in isoprenoid biosynthesis to mediate immunity to microbes and tumors. Bisphosphonate treatment of tumors results in increases in isopentenyl pyrophosphate that is sensed by butyrophilin 3A1. This allows V γ 2V δ 2 cells to recognize and kill tumors independent of MHC expression or the number of mutations. In clinical trials, adoptive immunotherapy with V γ 2V δ 2 cells has few side effects but has only resulted in a few partial and complete remissions. To improve effectiveness, we have now tested the combination of PD-1 blockade with V γ 2V δ 2 cells and the bisphosphonate, pamidronate. V γ 2V δ 2 cells were found to express PD-1, CTLA-4, LAG-3, and TIM-3 inhibitory receptors during the 14 day *ex vivo* expansion. Moreover, tumor expression of PD-L1 was increased by co-culture with activated V γ 2V δ 2 cells or by exogenous IFN- γ . To assess the effect of PD-1 blockade, immunodeficient NSG mice were inoculated with human PC-3 prostate cancer cells that naturally express PD-L1. After 13 days, the mice were treated with pamidronate followed 1 day later by purified V γ 2V δ 2 cells combined with either an anti-PD-1 or a control IgG₁ mAb. This regimen was repeated for 5 weeks. PD-1 mAb treatment markedly enhanced V γ 2V δ 2 T cell immunity to PC-3 tumors, reducing tumor volume nearly to zero after 5 weeks. These results demonstrate that PD-1 blockade can enhance the effectiveness of immunotherapy with $\gamma\delta$ T cells in treating prostate tumors in a preclinical model and suggests that this combination should be tested in patients.

F.96. Tmem176b is a Checkpoint in IL-1 β -dependent Tumor Immunity

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Tmem176b is an emerging immunoregulatory non-selective cation channel highly expressed in macrophages and DCs. We speculated that Tmem176b may promote tumor progression. In agreement with this hypothesis, we found that in a cohort of colon cancer patients (n=90), Tmem176b expression in the tumor infiltrate is significantly associated to

diminished survival. Given this result, we were interested in determining the role of Tmem176b in cancer progression. We found that Tmem176b^{-/-} mice had delayed tumor growth and better survival than WT mice when injected with two different transplantable cancer cell lines. Tumors from Tmem176b^{-/-} were heavily infiltrated by tumor specific and total CD8⁺ T cells. CTLs depletion in Tmem176b^{-/-} mice led to tumor development similar to the one observed in WT mice. In the tumor-draining lymph node (TDLN), CD4⁺Rorγt⁺ T cells were increased in Tmem176b^{-/-} mice as compared to WT ones. IL-1β is known as critical cytokine to differentiate anti-tumoral Th17 cell. Active caspase-1 was increased in the TDLN from Tmem176b^{-/-} mice as compared to WT. Moreover, Tmem176b^{-/-} BMDCs showed significant higher IL-1β secretion upon different NLRP3 inflammasome activators than the WT ones. This was dependent on caspase-1 activation, K⁺ efflux and cytosolic Ca²⁺ release. Strikingly, *in vivo* IL-1β blockade in tumor-bearing Tmem176b^{-/-} mice led to tumor development similar to the one observed in WT mice. In conclusion, Tmem176b impairs anti-tumoral immune responses by controlling IL-1β-dependent tumor immunity. Tmem176b may represent a new biomarker for cancer prognosis. We identified a pharmacologic Tmem176b inhibitor, which triggers tumor rejection depending on Tmem176b, caspase-1 and CD8⁺T cells.

F.108. Single-cell Polyfunctionality of CD4⁺ T-cells Shows Promise as a Predictor of Overall Survival of Pancreatic Cancer Patients Treated with GVAX Vaccine

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The therapeutic GVAX vaccine boosts the body's immune system T-cells to fight pancreatic cancer. To identify clinical correlates of polyfunctional response of T-cells that produce 2⁺ cytokines per single cell to GVAX vaccine, we employed 32-plex single-cell cytokine profiling platform to evaluate T-cell polyfunctionality in patients with pancreatic cancer who had GVAX vaccination.

CD4⁺ T-cells were isolated with anti-CD4 microbeads from pre- and post-vaccination PBMCs and stimulated with anti-CD3/CD28 at 37°C, 5% CO₂ for 24 hours. After stimulation, cells were loaded into a single-cell IsoCode chip containing ~12000 microchambers, each pre-patterned with a complete copy of a 32-plex antibody array. Protein secretions from ~1000 individual CD4⁺ T-cells were analyzed after 16-hour-on-chip incubation. Polyfunctional profile was assessed by the 5 functional groups: effector (Granzyme B, IFN-γ, MIP-1α, Perforin, TNF-α, TNF-β), stimulatory (GM-CSF, IL-2, IL-5, IL-7, IL-8, IL-9, IL-12, IL-15, IL-21), regulatory (IL-4, IL-10, IL-13, IL-22, TGF-β1, sCD40L, sCD137), inflammatory (IL-1b, IL-6, IL-17A, IL-17F, MCP-1, MCP-4), and chemoattractive (CCL-11, IP-10, MIP-1β, RANTES).

The single-cell analysis demonstrates a marked upregulation of polyfunctional CD4⁺ T-cells across 5 patients after GVAX vaccination compared to pre-vaccinated CD4⁺ T-cells. The enhanced polyfunctional strength index (PSI) of CD4⁺ T-cells by GVAX was predominated by antitumor-associated effector proteins including Granzyme B, IFN-γ, MIP-1α, Perforin and TNF-α, mixed with small amounts of MIP-1β, sCD137 secretions. Most importantly, post- versus pre-vaccination fold-change of PSI was significantly associated with patient overall survival (P = 0.001), indicating a potential of PSI in predicting GVAX vaccine efficacy and outcomes of patients with pancreatic cancer.

F.109. Chemotherapy Potentiates Immunotherapeutic Efficacy of Recombinant Oncolytic Poliovirus

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Oncolytic viruses are replication-competent viruses that can selectively infect and lyse cancer cells while stimulating potent antitumor immunity. PVSRIPO is a highly-attenuated, recombinant polio:rhinovirus chimera that has demonstrated promising and robust clinical and radiographic responses in phase I clinical trials for recurrent glioblastoma (GBM). An unexpected discovery during the phase I trial was the achievement of complete and durable responses in patients who were treated with single-dose chemotherapy months after tumor progression following PVSRIPO infusion. This study

aims to elucidate the therapeutic mechanism of combined PVSRIPO and single-dose lymphodepletive chemotherapy using syngeneic mouse tumor models. We hypothesize that post-PVSRIPO lymphodepletive chemotherapy elicits an 'immunologic reset'; that unmasks antitumor immune responses initially generated by PVSRIPO. To test this hypothesis, we implanted CT2A murine GBM or TRAMP-C2 prostate cancer cells subcutaneously on the right flank of mice, followed by intratumoral PVSRIPO and intraperitoneal temozolomide injections. Combination treatment resulted in decreased tumor growth and increased overall survival compared to PVSRIPO alone. Early flow cytometry data suggest that the addition of lymphodepletive chemotherapy may induce CD8⁺ effector T cell infiltration of the tumor and increase the intratumoral CD8⁺/regulatory T cell ratio. Ongoing experiments utilizing temozolomide-resistant cell lines will serve to better highlight the role of chemotherapy in restoring antitumor immunity by minimizing the direct cytotoxic effects of chemotherapy on the tumor. This study has important implications not only for guiding future GBM treatments, but also for the inclusion of chemotherapy in other immunotherapeutic approaches.

T.5. Induction of T-allo10, an Alloantigen Specific Type 1 Regulatory T Cell Product Suitable for Cellular Therapy in Hematopoietic Stem Cell Transplantation for Hematologic Malignancies

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Hematologic malignancies are fast-growing and most common cancer in children. Current treatments for these patients include chemotherapy and allogeneic hematopoietic stem cell transplantation (HSCT). HSCT reconstitutes new blood tissue and supplies allogeneic T cells which can kill leukemic cells. The main side effect of HSCT is Graft-versus-Host disease (GvHD), which results in significant morbidity and increased non-relapse mortality.

Our approach to prevent GvHD without affecting the beneficial anti-leukemic effect of donor T cells is adoptive transfer of Type 1 regulatory T (Tr1) cells (Roncarolo et al., Immunol Rev., 2011). We developed a novel and highly reproducible method to produce a donor-derived T cell product, T-allo10, enriched of host specific Tr1 cells. T-allo10 cells are obtained from CD4⁺ T cells stimulated with host tolerogenic dendritic cells (DC-10), in the presence of IL-10. T-allo10 cells contain a consistent proportion of CD49b and LAG3 positive cells (average 13.4%±2.4), identified as co-expressed on Tr1 cells (Gagliani et al., Nature Medicine, 2013). T-allo10 cells present alloantigen-specific anergy (91.7%±2.4), and suppress alloantigen-specific CD4⁺ T cell response (62.1%±12.3).

This approach for *in vitro* generation of T-allo10 cells has been scaled up in Good Manufacturing Practice (GMP) conditions at Stanford LCGM facility, and T-allo10 cells are currently used in a phase I trial in pediatric patients transplanted with mismatched related or unrelated HSC for hematologic malignancies. T-allo10 cells treated patients will be monitored for engraftment and immune reconstitution. Different strategies to trace the infused Tr1 cells *in vivo* are under investigation.

T.26. Resistance to PD1 Blockade in the Absence of Metalloprotease-Mediated LAG3 Shedding

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Inhibitory receptors prevent exacerbated T cell activation, yet can be hijacked by tumors for protection from immune attack. LAG3, regulated by ADAM10/17-mediated cell surface cleavage, co-expresses with PD1 defining functionally

exhausted T cells. Our previous studies have shown that non-cleavable LAG3 (LAG3.NC) mutant T cells exhibit reduced cytokine production and proliferation *in vitro* prompting us to examine whether LAG3 shedding is necessary to mount an anti-tumor immune response *in vivo*. To investigate this, a conditional knock-in mouse was generated resulting in LAG3.NC restricted to all T cells (CD4^{Cre}) or specifically CD8⁺ (E8I^{Cre.GFP}), CD4⁺ (ThPOK^{CreERT2}) or CD4⁺ Foxp3⁺ Treg populations (Foxp3^{CreERT2}).

LAG3.NC-CD4^{Cre} and LAG3.NC-ThPOK^{CreERT2} mice resist anti-PD1 therapy and succumb to MC38 tumor growth, compared to ~40% of controls and LAG3.NC-E8I^{Cre.GFP} mice becoming tumor-free. This phenotype is effector CD4⁺ T cell-mediated, and not by Tregs, as LAG3.NC-Foxp3^{CreERT2} mice clear tumors. Intrinsically, CD4⁺Foxp3⁻ TIL isolated from LAG3.NC-CD4^{Cre} mice show reduced proliferation and IFN- γ /TNF- α compared with controls. CD8⁺ TIL also show reduced cytokine release, extrinsically affecting CD8⁺ pmel-1 T cells in an adoptive transfer model into B16-gp100-bearing LAG3.NC-CD4^{Cre} hosts. Selective ADAM10 inhibition *in vivo*, also reduces IFN- γ /TNF- α proposing that metalloproteinase-mediated LAG3 cleavage limits an antitumor immune response.

Overall, these data suggest that failure of LAG3.NC-CD4^{Cre}/ThPOK^{CreERT2}, but not LAG3.NC-E8I^{Cre}/Foxp3^{CreERT2}, mice to clear MC38 tumors suggests that LAG3 shedding on CD4⁺Foxp3⁻ T cells is necessary for CD8⁺ TIL to mediate an effective anti-tumor immune response. Understanding the role of LAG3 shedding on T cells is clinically relevant as it may impact patient responsiveness to anti-PD1 immunotherapy.

T.38. ESCAPE TCR Sequencing: Combining TCR Sequencing with Protein Based Cellular Phenotyping in Immune Profiling

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T cell receptor (TCR) sequencing has become an important tool in immuno-oncology research and has led to the development of new cancer treatments. However, standard TCR sequencing does not provide information about the cell type expressing a given TCR, resulting in a loss of key information for understanding the immune response to disease. Here we present data using Proteona's ESCAPE™ TCR sequencing assay, which combines TCR sequencing with protein based cellular profiling to give a comprehensive picture of the T cell repertoire present in a sample. Using ESCAPE™ TCR sequencing, we profile T cells from cancer patients and healthy controls to identify changes in the T cell and TCR repertoire correlated to the presence of different cancers. Ongoing studies using ESCAPE™ TCR sequencing will track changes in T cell/TCR profiles over time and across treatments to identify additional therapeutic targets and develop new diagnostics for immuno-oncology treatments.

T.62. Epigenetic Reprogramming of Intratumoral Regulatory T Cells Enhances Cancer Immunity

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Regulatory T cells (Tregs) are critical for maintaining immune homeostasis, but their presence in tumor tissues impairs anti-tumor immunity and portends poor prognoses in cancer patients. Methods are needed to selectively and safely target the Tregs that prevent anti-tumor immunity without instigating adverse autoimmune toxicities. Here we reveal a mechanism to selectively target and reprogram the function of tumor-infiltrating Tregs by exploiting their dependency for the histone H3K27 methyltransferase Enhancer of Zeste Homolog 2 (EZH2) in tumors. We found that EZH2 expression and activity is increased in Tregs infiltrating both human and murine cancers. We show that genetic disruption of EZH2

activity in Tregs drives the destabilization of Foxp3 expression specifically in intratumoral Tregs. This leads both to a selective depletion of intratumoral Tregs and their acquisition of pro-inflammatory functions, which dramatically remodels the tumor microenvironment by enhancing the recruitment and function of CD8⁺ and CD4⁺ effector T cells that can eliminate tumors. Importantly, pharmacologic inhibition of EZH2 also leads to improved CD8⁺ T cell responses within tumors that impede tumor progression and are associated with reduced FOXP3 stability in Tregs. Finally, by comparing Treg depletion in the absence or presence of Ezh2-deficient Tregs, we show that the presence of Ezh2-deficient Tregs blocked tumor progression more potently than Treg depletion alone and without the devastating autoimmunity that ensued upon complete Treg depletion. Thus, our study reveals a novel strategy to target Tregs in cancer that mitigates autoimmunity by epigenetically reprogramming their function in tumors to enhance anti-cancer immunity.

T.74. Dissecting the Cellular Mechanisms of Disease Pathology Caused by Gain-of-function Mutations in PIK3CD

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Gain of function (GOF) mutations in *PIK3CD*, encoding the p110 δ subunit of PI3-kinase, cause a primary immunodeficiency characterized by recurrent respiratory infections, lymphoproliferation, increased susceptibility to herpes virus (EBV, CMV, VZV) infection, poor Ab responses to polysaccharide Ags, altered serum immunoglobulin levels and B-cell lymphoma. Interestingly, many of these patients also suffer from a range of autoimmune conditions. However, it is unclear what the cell intrinsic effects of GOF in PI3K are in different immune cell populations and how this results in the immune pathology observed in these patients. To determine the cell intrinsic effects of PI3K GOF in T cells and B cells we generated a mouse model using CRISPR/Cas9 to introduce the most common disease-causing mutation (E1021K) into *Pik3cd*. The development, activation and differentiation of T and B cells was assessed in these mice. We found that PI3K GOF resulted in dysregulation of T and B cells at many stages. We observed splenomegaly in the PI3K GOF mice, which could be attributed to increases in both T and B cells. We also observed multiple defects in B cell development and differentiation including isotype switching. Similarly PI3K overactivation also affected the activation and differentiation of T cells resulting in changes such as altered cytokine production and Tfh generation. This demonstrates that PI3K overactivation results in cell intrinsic defects in both T and B cells. These in turn help to explain the clinical manifestations including poor humoral responses, lymphoproliferation and autoimmunity.

T.92. Reprogramming Human T Cell Function and TCR Specificity With Non-viral Genome Targeting

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Several decades of work have aimed to genetically reprogram T cells for therapeutic purposes, but these approaches have relied on recombinant viral vectors, which do not target transgenes to specific genomic sites. Genome editing brought the promise of specific and efficient insertion of large transgenes into target cells through homology-directed repair (HDR), but to date in human T cells this still requires viral transduction. Here, we developed a non-viral, CRISPR-Cas9 genome targeting system that permits the rapid and efficient insertion of individual or multiplexed large (>1 kilobase) DNA sequences at specific sites in the genomes of primary human T cells while preserving cell viability and function. We successfully tested the potential therapeutic use of this approach in two settings. First, we corrected a pathogenic IL2RA mutation in primary T cells from multiple family members with monogenic autoimmune disease and demonstrated enhanced signalling function. We further corrected the mutation specifically in the patient's abnormal regulatory T cells. Second, we replaced the endogenous T cell receptor (TCR) locus with a new TCR redirecting T cells to a cancer antigen. The resulting TCR-engineered T cells specifically recognized the tumour antigen, with concomitant cytokine release and tumour cell killing. Non-virally engineered T cells with a user defined TCR specificity could be produced at clinical scales of hundreds of millions of cells. Taken together, these studies provide preclinical evidence that non-viral genome targeting will enable rapid and flexible experimental manipulation and therapeutic engineering of

primary human immune cells.

T.93. Discovery Screen Platform for Co-modulators Of T Cell Effector Function Against Tumors

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T cell checkpoint therapy aims to enhance T cell responses during priming and/or within tumor tissue, but the key drivers of efficacy in broad patient populations has remained elusive. Here we first sought to determine whether responsiveness to anti-PD1 therapy in established syngeneic murine tumors depends on recruitment of T cells from lymphoid organs during treatment, or if PD1 blockade on T cells already within tumors is sufficient to drive efficacy. We compared anti-PD1 efficacy on established MC38 tumors when FTY720 (blocks lymphocyte egress from lymphoid organs) or vehicle control was administered from the start of treatment. We found that anti-PD1 efficacy did not require additional T cells migrating from lymphoid organs, suggesting that PD1 blockade can act directly on T cells that were already present within tumors. We next examined the functionality of tumor-infiltrating T cells during anti-PD1 treatment and found that cytokine production is enhanced in a population of PD1^{intermediate} T cells, but not on PD1^{high} T cells. Together, this suggests that PD1 checkpoint therapy is driven by amelioration of a fraction of PD1⁺ tumor-infiltrated T cells, and that additional pathways within established tumors may need to be co-targeted to drive optimal efficacy. We then developed an *in vitro* discovery screen platform to identify pathways that co-modulate human T cell effector function against tumor cells under conditions that drive high levels of PD1 on T cells. We are currently screening genes of interest that are expressed in patient tumor tissues and highly associated with a T cell-inflamed signature.

T.95. Skewed CD4 and CD8 T Cell Differentiation in Pancreatic Cancer Patients

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To understand potential immune system alterations in newly diagnosed, untreated, pancreatic cancer patients and provide a foundation for immunotherapy, we profiled PBMC from pancreatic ductal adenocarcinoma (PDA) patients and age matched healthy controls using high dimensional cytometry by time-of-flight (CyTOF) analysis. We developed two immune profiling panels: a *broad* profiling panel that includes 45 phenotypic markers that together permit the identification and enumeration of the main innate and adaptive immune cell subsets in humans, and a *deep* profiling panel that includes 45 features focusing on T cell phenotype and biology. We report a 2-fold increase in CD14-positive CD16-negative monocytes (P=0.02) in pancreatic cancer patients compared to age-matched controls. Additionally, we observe skewed T cell differentiation in pancreatic cancer patients, with both CD4 and CD8 T cells biased towards more CD45RA-negative CD27-positive CCR7-positive central memory cells (P=0.003 and P=0.01 respectively) and less CD45RA-negative CD27-positive CCR7-negative effector memory cells (P=0.005 and 0.02 respectively) than age-matched controls. We further interrogated alterations of T cell differentiation in both CD4 and CD8 T cell compartments using high dimensional approaches, and report clusters of T cells uniquely observed in pancreatic cancer patients. We are now investigating the mechanisms underlying these observations. Our goal is to understand the nature of the skewing and how any changes in baseline immune health of the T cell compartment related to disease progression and/or response to therapy. These studies should provide a foundation for improving therapy in pancreatic cancer patients.

T.98. Genetic Modification Potentiates the Anti-Tumor Activity of Human Cord Blood Progenitor (CD34⁺) Derived NK Cells

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Celularity is developing a proprietary allogeneic NK cell (PNK) product, derived from placenta/cord blood CD34⁺ progenitors. In developing the genetically modified PNK platform, a study that augmented the anti-tumor function of PNK cells by simultaneous knockout (KO) of CBLB (negative regulator of NK cytotoxicity) and TGFβ receptor II (TGFB2, tumor microenvironment) genes using CRISPR/Cas9 was conducted. High efficiency of editing (>80%) was achieved for both genes which did not affect expansion and differentiation of CD34⁺ cells into PNK double KO (PNK-DKCT) cells. The results showed that CBLB KO in PNK cells (PNK-CBLB KO) led to 2-4-fold increased cytotoxicity and cytokine secretion against a range of cancer cell lines and primary tumor cells. However, these enhanced functions from PNK-CBLB-KO cells were still sensitive to TGFβ-mediated suppression. TGFB2 KO rendered the ability of PNK cells (PNK-TGFB2 KO) to maintain high expression of NK activating receptors and resist the inhibition of cytotoxicity after TGFβ exposure. Furthermore, PNK-DKCT cells were insensitive to exogenous TGFβ and exhibited 2-4-fold improved cytotoxicity. Subsequently, we compared the anti-tumor activity of PNK-DKCT to controls in an *in vivo* AML model, where conditioned NSG mice were engrafted with HL60-GFP-luc cells 3 days prior to PNK infusion. All PNK groups exhibited increased survival compared to vehicle. In PNK-CBLB KO and PNK-DKCT groups, the tumor burden was similar but significantly reduced compared to Cas9 control or PNK-TGFB2 KO. Future studies will address the benefit of PNK-DKCT in TGFβ secreting tumor-bearing animal models to pave the way for genetically modified NK cell therapy.

T.102. CXCR3 Determines Migratory and Expansion Fate of Memory Stem T Cells (T_{SCM})

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CD8⁺ CXCR3^{high} memory stem T cells (T_{SCM}) expressing stem cell-like properties have the ability to elicit a long-lasting anti-tumor response. However, how T_{SCM} cells execute effector T cell functionality by homing to the tumor site and how the antigen-specific expansion of T_{SCM} cells in the lymphoid compartment is controlled, remains unclear. We found CXCR3 as the highest expressed chemokine receptor on T_{SCM} cells by means of a thorough marker search on all CD8 T cell subsets. Functionally we show, that CXCR3 ligands (MIG, IP-10, I-TAC) differentially regulate the effector function and migration of T_{SCM} cells under antigenic re-challenge conditions. Whereas MIG and IP-10 induce a strong migratory response, I-TAC is a main driver for the expansion of antigen-specific T_{SCM} cells. Mechanistically, CXCR3 ligation of T_{SCM} cells induced a significant down-regulation of CXCR3 that we identified as an alternative splicing variant CXCR3-alt that solely binds I-TAC. We accessed lymph nodes of patients with urothelial carcinoma and found significantly higher frequencies of T_{SCM} cells accompanied by higher levels of MIG and IP-10 compared to the peripheral blood. Analogously to the lymphoid compartment, we found MIG and IP-10 as the main inflammatory stimuli in the tumor tissue to potentially attract tumor-specific T_{SCM} cells. Combining these in-vitro and in-vivo findings, we conclude that CXCR3 is a major regulator for T_{SCM} cells to enter and proliferate in the lymph node and its ligand I-TAC may be an attractive therapeutic stimulus to enhance the expansion of antigen-specific T_{SCM} cells in the tumor site.

T.104. Measurement of Fc-mediated ADCC and CDC Activity of anti-TNF α and anti-VEGF Therapeutic Antibodies using Reporter-based Bioassays and Engineered TNF α ⁺ and VEGF⁺ Target Cells

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Fc effector functions are critical to the efficacy of therapeutic antibodies. Measurement of ADCC and CDC during drug development is not only important for antibodies that harness ADCC and/or CDC as their primary mechanism of action (e.g. rituximab, trastuzumab), but also for antibodies designed to target soluble ligands such as TNF α and VEGF. We previously reported the development of functional bioassays used to measure ADCC and ADCP mediated through Fc γ RI, Fc γ RIIa and Fc γ RIIIa. These reporter bioassays exhibit the specificity, accuracy, precision, and robustness necessary for qualification according to ICH guidelines and have been used extensively to measure the potency of antibody-based biologics that target cell surface immune receptors. Here, we sought to evaluate ADCC and CDC activities of therapeutic antibodies designed to target and block TNF α and VEGF. To measure ADCC activity of these blocking antibodies, we developed engineered target cells that express either membrane-bound TNF α or VEGF. When used as target cells with reporter-based effector cells expressing a relevant Fc γ R, ADCC activity of adalimumab (anti-TNF α) and bevacizumab (anti-VEGF) was detected in a specific and dose-dependent manner. When used in a luminescence-based CDC assay, the target cells elicited an appropriate Fc γ R-mediated response. The assay signals demonstrated IgG isotype specificity in both ADCC and CDC assays. The combined use of cell-based reporter bioassays with target cells engineered to express membrane-bound soluble ligands can provide a simple, specific, and quantitative platform to measure Fc-mediated effector functions of therapeutic antibodies targeting soluble ligands.

T.105. Improved T Cell Activation Bioassays to Advance the Development of Bispecific Antibodies and Engineered T Cell Immunotherapies

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In recent years, a variety of immunotherapy strategies aimed at inducing, strengthening or engineering T cell responses have emerged as promising approaches for the treatment of diseases such as cancer and autoimmunity. Current methods used to measure TCR-mediated T cell proliferation and cytokine production rely on primary PBMCs as a source of T cells, which must be stimulated via co-culture with APCs or anti-TCR/CD3 antibodies. These assays are laborious and highly variable due to their reliance on donor primary cells, complex assay protocols and unqualified assay reagents. As a result, these assays are difficult to establish in quality-controlled drug development settings.

To overcome this barrier, we developed two reporter-based bioluminescent T cell activation bioassays that can be used for the development of bispecific antibodies and engineered T cell immunotherapies. The assays consist of Jurkat T cells genetically engineered to express luciferase downstream of either NFAT or IL-2 response elements. The T cell activation bioassays reflect the mechanisms of action of biologics designed to induce TCR and/or CD28-mediated T cell activation, as demonstrated using anti-CD3 and/or anti-CD28 antibodies as well as blinatumomab, a bispecific antibody that simultaneously binds CD3 expressed on T cells and CD19 expressed on malignant B cells. The bioassays are pre-qualified according to ICH guidelines and show assay specificity, precision, accuracy and linearity required for routine use in potency and stability studies. Finally, our data illustrate the use of reporter-based T cell activation bioassays for characterizing and measuring the activity of engineered chimeric antigen receptor T cells.

T.106. Quantitative Cell-based Bioassays to Advance Immunotherapy Programs Targeting Immune Checkpoint Receptors

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The human immune system is comprised of a complex network of immune checkpoint receptors that are promising new immunotherapy targets for the treatment of a variety of cancers and autoimmune-mediated disorders. Immunotherapies designed to block co-inhibitory receptors (e.g. PD-1, CTLA-4) are showing unprecedented efficacy in the treatment of cancer. However, not all patients and tumor types respond to this approach. This has resulted in broadening of immunotherapy research programs to target additional co-inhibitory (e.g. LAG-3, TIM-3) and co-stimulatory (e.g. GITR, 4-1BB, OX40, CD40) receptors individually and in combination.

A major challenge in the development of biologics is access to quantitative and reproducible functional bioassays. Existing methods rely on primary cells and measurement of complex functional endpoints. These assays are cumbersome, highly variable and fail to yield data quality required for drug development in a quality-controlled environment. To address this need, we have developed a suite of cell-based functional bioassays to interrogate modulation of immune checkpoint receptors individually (e.g. PD-1, LAG-3, TIM-3, GITR, 4-1BB) and in combination (e.g. PD-1+CTLA-4, PD-1+LAG-3). These assays consist of stable cell lines that express luciferase reporters driven by response elements under the precise control of mechanistically relevant intracellular signals. Thus, the bioassays reflect mechanisms of action for the drug candidates designed for each immune checkpoint receptor and demonstrate high specificity, sensitivity and reproducibility. In summary, these reporter-based bioassays can serve as powerful tools in immunotherapy drug development for antibody screening, potency testing and stability studies.

T.107. Reproducible MOA-Reflecting Reporter-Based Bioassays to Enable Drug Development of Biosimilars and Biobetters

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Cytokines and growth factors are secreted by a wide variety of cells including fibroblasts, endothelial and stromal cells, whose role it is to regulate surrounding cells in an autocrine, paracrine or endocrine fashion. Immunocytokines represent a promising class of activators of the immune system, with the potential to be used alone or in combination with other therapeutic modalities. Many are currently FDA approved therapy agents (e.g. IFN, IL-2 and Epo), while others are targets for approved biologic drugs. Examples of cytokine blocking agents include basiliximab (IL-2R), tocilizumab and sarilumab (IL-6R), siltuximab (IL-6), ustekinumab and its biosimilars (IL-12/IL-23 p40), secukinumab (IL-17A), bevasizumab (VEGF), and denosumab (RANKL). Many more are in development and trials as biosimilars and biobetters. IL-2 and IL-15 are still clinically important cytokines as researchers look to improve potency, patient tolerance and response by developing new molecules with sustained and targeted activities.

We have developed MOA-based luciferase reporter bioassays which can be used for the quantitation of a variety of cytokines and growth factors including IL-2, IL-6, IL-12, IL-15, IL-17, IL-23, VEGF and RANKL. The bioassay format is based on thaw-and use cells, eliminating the need to establish and pre-culture cytokine responsive cell lines which provides the benefits of convenience, reproducibility, and transferability. We demonstrate these assays measure cytokine response and inhibition with blocking drugs or potency changes from stressed samples. In summary, these reporter-based bioassays provides valuable tools for the development, stability testing, and potency determination in the manufacture of cytokine biosimilars and biobetters.

T.117. Forcing Melanoma Cells to be Stressed Stimulates Apoptotic and Immunogenic Responses

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Here we investigate the impact of an ER stress-inducing agent on the cell cycle and immunological dynamics within a melanoma tumour microenvironment. We have demonstrated in multiple melanoma cell lines that targeting the endoplasmic reticulum with Bortezomib (26S proteasome inhibitor) promotes cell cycle arrest and induces G₂-phase-dependent Noxa-mediated apoptosis of metastatic melanoma cells *in vitro* (Beaumont et al. 2016, *J Dermatol Res*).

The combination of ER stress and apoptosis in melanoma cells raised the question whether Bortezomib could induce immunogenic cell death (ICD) and is the cell cycle involved in this induction? Induction of ICD by Bortezomib has been demonstrated in other cancers but not yet in melanoma. Indeed, we observe upregulation of some ICD markers such as surface expression of calreticulin, HSP70, HSP90, and the secretion of HMGB1 and ATP, in Bortezomib-treated melanoma cells. To visualise ER stress in real time, we transduced our cells with the F-XBP1ΔDBD-venus reporter construct and indeed low doses of bortezomib trigger a stress response and resulted in apoptosis. Further, as bortezomib has the suggested armory to instill an ICD response, we use bortezomib as a model to determine if ICD inducing drugs can further enhance the efficacy of currently approved melanoma therapies. Preliminary *In vivo* data suggest that there is an immune response in immunocompetent mice vaccinated with dead/dying treated cells. Taken together, our data indicate that bortezomib is a good strategy worth investigating to improve melanoma therapy by a more efficacious apoptotic response and enhanced through ICD induction.

W.14. ESCAPE RNA Sequencing: Combining Protein and Nucleic Acid Measurements to Profile Immune Cells

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Cancer-immune cell interactions are at the forefront of today's immuno-oncology revolution. However, in studying the body's immune response to tumors, researchers are generally limited to studying the state of immune cells via either protein expression (flow or mass cytometry) or gene expression (RNA sequencing or qPCR). Each type of measurement requires different equipment and workflows and data from the two methods are not easily combined. While both approaches are necessary to understand immuno-oncology, neither approach alone is sufficient to provide a holistic view of the immune response to cancer that will be necessary to develop new therapies and diagnostics.

We have used Proteona's Enhanced Single Cell Analysis with Protein Expression (ESCAPE) RNA sequencing platform to measure both protein and gene expression in thousands of single peripheral blood mononuclear cells (PBMCs) from both cancer patients and healthy controls. We used the protein information to phenotype the cells and the gene expression to identify subsets of cells reacting differently to their environment. Protein expression patterns were benchmarked against mass cytometry showing that ESCAPE RNA-Seq provides an accurate representation of cell types present.

We find that Proteona's ESCAPE RNA-Seq reagents are compatible across multiple single cell RNA sequencing platforms, making them ideal reagents for any lab studying single cell biology. Ongoing studies using ESCAPE RNA sequencing will provide us even greater insight into the cancer immune response, how it evolves over time and changes with treatment.

W.58. Mutations of Forkhead Box P3 and Their Impact on Hepatocellular Carcinoma

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Increasing evidence showing that transcription factor forkhead box P3 (FOXP3), a critical molecule for regulatory T cells, can express not only in lymphocytes but also in cancer cells. However the status of FOXP3 in hepatocellular carcinoma (HCC) remain unclear. In this study, we used HCC tissue samples and cell lines to explore the status and function of FOXP3 in HCC. The results were confirmed in the mouse tumor model. The result showed that mutations in forkhead (FKH) domain of FOXP3 mRNA occurred in 19 of 60 (30%) HCC tumor tissues but none of the adjacent non-tumorous tissues. Many point mutations resulted in amino acid substitutions, which could subsequently cause changes of FOXP3 subcellular localization and further attenuated transcriptional activity and xenograft tumor-suppressing capability of FOXP3 in mice. Our results have suggested for the first time that mutations generated in FOXP3 transcription may play remarkable roles in HCC development.

W.64. The Interaction of ILT3/LILRB4 Receptor with its Ligand CD166/ALCAM Inhibits Tumor Cell Growth

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Inhibition of immune receptors and/or of their targets by antibodies or recombinant proteins may provide important tools for immunotherapy of cancer, autoimmune diseases and transplant rejection. The family of Immunoglobulin-like transcript (ILT) or leukocyte immunoglobulin-like receptor (LILR) and their ligands have recently come into focus as potential immune checkpoints for cancer therapy. We have previously demonstrated that both membrane and soluble ILT3/LILRB4 inhibits the activation and function of T helper cells, while inducing the differentiation of CD8⁺ T suppressor cells. More recently, we found that the ligand of ILT3/LILRB4 is CD166/Alcam molecule. Surface plasmon resonance (SPR) studies indicated that ILT3.Fc:CD166 interaction has a K_d value of $\sim 0.9 \mu\text{M}$, which is within the range of many immune ligand and receptor interactions. *In vitro* studies demonstrated that binding of ILT3.Fc to CD166 expressed by acute T cell leukemia, cutaneous T cell lymphoma, Burkitt lymphoma and osteosarcoma cell lines inhibits their proliferation. Cell cycle arrest is accompanied by inhibition of p70S6K signaling pathway. Knockout of CD166 from tumor cells abrogated ILT3.Fc binding and its tumor-inhibitory effect. Cutaneous T cell lymphoma transplanted in NOD.Cg-Prkdc Il-2rg/SzJ (NSG) mice treated with ILT3.Fc showed a significantly slower rate of growth compared to that of tumors from control mice treated with human IgG. The *in vitro* and *in vivo* inhibitory effect of ILT3.Fc is probably attributable to the blockade of homophilic CD166-CD166 interaction between tumor cells. We postulate that ILT3.Fc is of therapeutic value for preventing GVHD and inducing tumor regression in leukemic transplant recipients.

W.72. In Vitro Generated Tr1 Cells Eliminate Primary Pediatric Acute Myeloid Leukemia

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Acute myeloid leukemia (AML) is the second most common pediatric leukemia, affecting almost 1,000 US children annually, and accounts for over 50% of pediatric leukemia deaths. Optimal treatment for refractory AML is hematopoietic stem cell transplantation (HSCT), which unfortunately often leads to Graft vs Host Disease (GvHD), wherein T cells from the graft attack host tissues. GvHD is managed with immunosuppressive drugs, which increases the susceptibility to infections and impairs the Graft vs Leukemia effect (GvL) of the graft, increasing the risk for relapse.

T regulatory Type 1 cells (Tr1) are a regulatory T cell subtype that promotes antigen-specific tolerance. Tr1 cells mainly regulate immune responses by secreting high levels of IL-10. Tr1 cells can also eliminate myeloid cells through a granzyme-mediated, antigen-independent lysis. Tr1 cells immunotherapy for AML could thus specifically induce

tolerance to the host tissues to prevent the GvHD, while promoting GvL by direct killing of myeloid leukemia cells.

Lentiviral transduction of the IL-10 gene into CD4⁺ T cells converts them to Tr1 cells. These cells have a characteristic Tr1 cytokine profile, are anergic, and kill myeloid cell lines. We have screened 15 primary pediatric AML samples for sensitivity to Tr1 killing. AML samples fell into three categories: resistant, sensitive, or partially sensitive to killing. AML samples with an MLL gene rearrangement, which is a poor prognostic marker, were also susceptible to killing by Tr1 cells. This data suggests that Tr1 immunotherapy may provide a potent anti-leukemic effect in difficult-to-treat AML subtypes, in addition to protecting against GvHD.

W.76. Tumor-resident Memory CD8⁺ T Cells are Exhausted And Less Cytotoxic Despite Epigenetically Committed in Bladder Cancer

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Background: High amount of tumor infiltrating lymphocytes (TIL) within the tumor correlates with better prognosis in most solid cancers. TIL is dominated by tissue-resident memory CD8⁺ T cells (T_{RM}) subset, reported to possess cytotoxic features. Here, we aimed to profile the T_{RM} in tumor in regards to cytotoxic potential and exhaustion.

Materials and methods: 12 patients diagnosed with urinary bladder cancer (UBC) were recruited. T_{RM} cells from PBMC and TIL were isolated for FACS and DNA methylation analysis of perforin locus (-1053bp upstream TSS). T_{RM} was determined by CD103 expression.

Results: The fraction of T_{RM} in TIL was significantly higher compared to PBMC (mean PBMC = 2.5% vs TIL = 49%) (p<0.0001). Moreover, the DNA methylation percentage of perforin locus in TIL-T_{RM} (34%) was lower than PBMC-T_{RM} (61%) (p<0.01), indicating commitment of T_{RM} to be cytotoxic in the tumor. However, the T_{RM} cells did not show a potent cytotoxic capacity, marked by no significant differences on granzyme B, perforin, and T-bet expressing T_{RM} cells between TIL and PBMC. In addition, TIL-T_{RM} cells expressed significantly higher exhaustion marker, PD-1 compared to PBMC-T_{RM} (p<0.0001).

Conclusions: We discovered that T_{RM} cells in bladder cancer tumor were less cytotoxic despite being committed to be, and these cells were exhausted.

W.79. NLRP3 Inflammasome Promotes IL-17-mediated Tumor Immunity while Increasing IDO1 in the Tumor Microenvironment

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We have recently shown that Tmem176b is an immunoregulatory non-selective cation channel highly expressed in macrophages and DCs. In those cells, Tmem176b inhibits activation of the NLRP3 inflammasome. We found that Tmem176b^{-/-} mice had delayed tumor growth and better survival than WT mice when injected with transplantable cancer cells. In the tumor-draining lymph node (TDLN), CD4⁺Rorγt⁺T cells were increased in Tmem176b^{-/-} mice as compared to WT ones. In vivo IL-17 blockade in tumor-bearing Tmem176b^{-/-} mice led to tumor development similar to the one observed in WT mice, showing a role of Th17 cells in the anti-tumor immunity developed in these mice. We also found that caspase-1 activation was higher in TDLN from Tmem176b^{-/-} mice comparing to the WT ones. IL-1β is known as critical cytokine to differentiate anti-tumoral Th17 cell. In vivo IL-1β blockade in tumor-bearing Tmem176b^{-/-} mice led to a reduction of CD4⁺Rorγt⁺ T cells at the TDLN. On the other hand, it was recently shown that IL-1β induced the

expression of indoleamine 2, 3-dioxygenase (IDO1), highly known for its regulatory function. We found that there was a higher expression of IDO1 in the tumor microenvironment of Tmem176b^{-/-} mice comparing to WT mice. In conclusion, NLRP3 inflammasome activation at the TDLN promotes IL-17 mediated anti-tumor immunity and, at the same time, it promotes a counterregulatory mechanism mediated by IDO1. Therefore, the activation of NLRP3 inflammasome and IDO1 inhibition may have synergistic effects in tumor rejection.

W.81. Control of Lymphocytic Leukemia Through Regulation of TRAF1 Protein Degradation

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Chronic lymphocytic leukemia (CLL) is the most common adult leukemia in North America. The signaling adaptor tumor necrosis receptor (TNFR)-associated factor-1 (TRAF1) is overexpressed in refractory CLL, and in almost half of all B cell cancers. TRAF1 is important in linking a subset of TNFR family members, including CD40 in B cells to NF- κ B and MAPK activation. Phosphorylation of TRAF1 by the protein kinase PKN1 is essential for TRAF1 protein stability. Knockdown of PKN1 in Raji cells leads to TRAF1 protein degradation. TRAF1 or PKN1 knockdown in B lymphoma also reduces constitutive survival signaling as evidenced by lower pNF- κ B, pAkt, pS6 and pErk. First generation PKN1 inhibitors have been identified with selectivity for PKN1 over PKC θ . Three of the top inhibitors were tested on primary patient CLL samples grown on OP9 stromal lines expressing CD40L. Seven of 9 patient samples tested so far showed reduction of TRAF1 levels after overnight incubation with inhibitors, with concomitant decrease in signaling intermediates, pS6 and pErk. As B cell cancers rely on both constitutive BCR signaling as well as survival signals through TNFRs such as CD40, the ability to reduce TRAF1 in CLL through PKN1 inhibition could be useful in conjunction with drugs that target BCR signaling.

W.108. Single-cell Highly Multiplexed Proteomics Identifies Novel Polyfunctional Human CD8⁺ T-cell Signatures Induced by a Nanoparticle-Based Melanoma Vaccine in Human Immune System Mice

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Polyfunctional T cells (co-secretion of 2⁺ proteins per single cell) are an important functional attribute of a quality human T-cell immune response to antigen. Assessment of human T-cell polyfunctional responses to tumor vaccines in humanized mice, which are reconstituted with human immune system (HIS), provides a potentially valuable translational tool for tumor vaccine candidate screening and efficacy determination pre-clinically. We utilized 32-plex single-cell functional proteomics platform to profile human CD8⁺ T-cells in HIS mice induced by a nanoparticle-based melanoma vaccine (NP). HIS mice were vaccinated with different formulations of NP or vehicle. Splenic human CD8⁺ T-cells were isolated, stimulated with anti-human CD3/CD28 at 37°C, 5% CO₂ for 24 hours and loaded into a single-cell IsoCode Chip, pre-patterned with a 32-plex antibody ELISA array per cellular microchamber. Protein secretions were analyzed from ~ 1000 single human CD8⁺ T-cells after 16-hour-on-chip incubation. The single-cell analysis demonstrates sensitive detection of robust upregulation of polyfunctional human CD8⁺ T-cell subsets in NP-vaccinated HIS mice compared to controls. The enhanced polyfunctional strength index of the human CD8⁺ T-cells by the vaccine was driven by antitumor-associated proteins including granzyme B, IFN- γ , MIP-1 α , perforin and TNF- α . Additionally, novel single-cell visualizations revealed distinct polyfunctional cell signatures with these combinatorial human cytokine secretions that drove human CD8⁺ T-cell response by the NP. Polyfunctional human CD8⁺ T-cell responses sensitively demonstrated successful induction of the tumor vaccine in HIS mice, which may serve as a translational tool for more accurate evaluation of tumor vaccine efficacy in a pre-clinical setting.

Inflammatory bowel diseases

F.50. Mice Overexpressing MKL1 in Macrophages are Susceptible to DSS-induced Colitis

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Background and Aims: Mice deficient in the megakaryoblastic leukaemia 1 (*Mkl1*) gene are protected from dextran sulphate sodium (DSS)-induced colitis, implying that *Mkl1* plays a pathological role in inflammatory bowel disease (IBD). However, how *Mkl1* contributes to the development of colitis remains unknown. It was found that the expression of *Mkl1* is higher in the colonic lamina propria macrophages (LPMac) of DSS-treated mice than in those of control mice. In this study, we established a transgenic mouse line that overexpresses human *MKL1* (MKL1-Tg) specifically in cells of the monocyte/macrophage lineage, in order to investigate the potential role of macrophage *MKL1* in the pathogenesis of colitis.

Methods and Results: MKL1-Tg mice displayed spontaneous colon shortening and rectal prolapse. Furthermore, MKL1-Tg mice had higher susceptibility to DSS-induced colitis. Flow cytometric and quantitative RT-PCR analyses revealed that, in MKL1-Tg mice compared to littermate controls, the proportion of LPMac was decreased and had an altered inflammatory phenotype indicative of impaired anti-inflammatory properties, whereas bone marrow-derived macrophages from MKL1-Tg mice skewed towards M1 polarisation. Of note, overexpression of MKL1 impacts transcriptional activities of NF- κ B p65 and PPAR γ .

Conclusions: Together, our observations indicated that MKL1 crucially contributes to the development of colitis via the regulation of the function of macrophages, suggesting that it may be a potential therapeutic target for the prevention of IBD.

F.64. Effects of Short-term Dietary Changes on Immune System and Beyond

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In western countries, both obese and conscious eaters regularly expose themselves to unhealthy diet during Christmas for example. Short-term exposure to fatty diet instantly alters intestinal microbiota towards a more pathogenic composition. However, whether host defense mechanisms also adapt instantly remains unknown.

In order to establish a first line of protection the epithelium secretes mucins to form the mucus layer and also anti-microbial peptides. Another defense mechanism is constituted by the immune system: secretion of IL-17A and IL-22 especially by CD4 T-cells, maintains intestinal barrier integrity.

Our data showed that 3-day western-type diet (WTD) feeding induced mucus which was toxic to CD45⁺ lymphocytes *in vitro* and *in vivo*. mRNA analysis revealed diminished expression of IL-17A and IL-22 within the small intestine and impaired expression of mucins and antimicrobial peptides. Newly developed fate mapping reporter mice (Foxp3^{RFP}IL-10^{eGFP}IL-17A^{Kata}IL-22^{BFP}IL-17A^{CRER26^{YFP}}) allowed us to analyze multiple T-cell subsets and the fate of T_H17 cells in response to the short dietary change. In mucosa-associated lymphoid tissues, we observed immediate changes in cytokine expression by T_H17 after three days of WTD. Specifically, release of IL-10 by T_H17 decreased. It further decreased after 3 days of chow recovery phase. Additionally, WTD-feeding seemed to convert T_H17 to pathogenic T_H1

because exT_H17 acquired elevated IFN γ production.

Considering our preliminary results, we conclude that proximity of toxic mucus to underlying immune cells after short exposure to WTD generates an immunodeficient barrier. We envision that such alterations predispose to bacterial invasion and/or chronic inflammatory bowel disease.

F.73. Building an Immune Single Cell Atlas of Ileum Crohn's Disease

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Triggering events of Crohn's disease (CD), one of the two main forms of inflammatory bowel disease, involve a complex interplay between environmental factors and genetic susceptibilities, culminating in uncontrolled immune responses against luminal antigens. Numerous efforts have attempted to dissect the key cellular and molecular modules of CD inflammation but most of the targets selected from these studies failed to demonstrate significant effect in clinical trials. Moreover, therapeutic response to approved drugs targeting inflammatory mediators is highly variable, and up to 40% of CD patients experience primary non-response to TNF blockade. This suggests that immune heterogeneity exists at the lesional site among CD patients despite clinical similarities. In this study, we built an immune cell atlas of ileum CD by performing an unbiased, multi-dimensional characterization of matched uninvolved and involved ileums and peripheral blood mononuclear cells (PBMCs) of 14 patients, using single cell RNA sequencing (scRNAseq) and cytometry by time-of-flight (CyTOF). We identified complex immune patterns in involved ileums that correlated with PBMCs profiles and stratified the patients into two groups. We validated the patterns dichotomy by transposing scRNAseq findings to independent larger cohorts of CD tissues sequenced in bulk. Finally, we provided evidence suggesting that the dichotomic enrichment of these patterns in biologics naïve patients could predict responsiveness to anti-TNF therapy. In conclusion, our study demonstrates that CD patients can be stratified according to their immune cellular and molecular profiles at the lesional site to predict disease behavior, and should be integrated in the design of future clinical trials.

F.111. Anti-TNF α Therapy Increases Suppressor Regulatory T Cells in Patients with Crohn's Disease

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CD39 and CD73 are two cell markers of Regulatory T cells (Tregs) and have been considered pivotal in generation of immunosuppressive microenvironments for catalyzing ATP into adenosine. Crohn's Disease (CD) is an inflammatory bowel condition, which has the pathogenesis incompletely understood but seems to involve multiple factors including hyperactivity of the immune system. Anti-TNF α demonstrated their efficacy for induction and maintenance of remission in a significant proportion of patient refractory to conventional therapy but the mechanisms by which this happens are not known. We hypothesized that anti-TNF α might be acting increasing Suppressor Tregs, leading to a greater control of inflammation. To elucidate this, we analyzed peripheral blood of health donors (HD) (n=28) and patients with CD that are under Anti-TNF α therapy (n=24) or Conventional Therapy (CT) (n=19). Suppressor Tregs were characterized by the surface expression of CD3⁺CD4⁺CD25^{hi}CD127⁺FOXP3⁺CD39⁺CD73⁺ and measured by multiparametric flow cytometry. We observed that Anti-TNF α was able to increase the percentage of Suppressor Tregs (p = 0, 0031) and decrease expression of CD38 in TCD4⁺ (p = 0, 0035) when compared to CT. Furthermore, when compared the expression of CD39 on Tregs, patients that are undergoing Anti-TNF α present a higher mean fluorescence intensity of this marker when compared to CT (p = 0,045). In summary, Anti-TNF α is able to recover Suppressor Tregs and up regulate CD39,

the limiting enzyme of this pathway. We suggest that this may be one of the mechanisms involved in the effectiveness of Anti-TNF α and a target for treatment follow up.

F.112. Metabolic Inhibition of Microbiota-Reactive CD4 T Cells for the Prevention and Treatment of Inflammatory Bowel Diseases

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Microbiota-reactive CD4 T memory (T_M) cells are generated during intestinal infections and inflammation, which can potentially serve as a reservoir for pathogenic CD4 T effector (T_E) cells thus drive the progression of inflammatory bowel diseases (IBD). Unlike T_E cells, T_M cells keep a low rate of metabolism unless they are activated by re-encountering cognate antigens. Inhibition of antigen-specific CD4 T cell metabolism during cell activation by targeting key metabolic regulators such as mTORC and AMPK will lead to cell death and anergy, and favor the generation of regulatory T (Treg) cells. Thus we hypothesize that metabolic inhibition of microbiota-reactive CD4 T cells could decrease the pathogenic T_M reservoir and serve as a promising immunotherapy of IBD. We show that application of metabolic inhibitors during activation of CD4 T cells led to dampened cell survival and anabolism, but enhanced induction of Treg cells *in vitro*. *In vivo*, simultaneous metabolic inhibition during microbiota antigen encounter significantly impaired T_M formation with an increased Treg/ T_E ratio. And metabolic inhibition during T_M re-activation ablated microbiota-specific CD4 T cells. This approach also successfully prevented the development of intestinal inflammation in a T cell transfer colitis model, limiting microbiota-specific T_M cells as well as decreasing their differentiation into pathogenic T_E cells in the gut. Our results suggest that targeting the metabolism of microbiota-reactive CD4 T cells serves as a method to eliminate pathogenic CD4 T_M and induce Treg cells, thus it has promise as an immunotherapy for the prevention and/or treatment of IBD.

T.25. Alpha Kinase 1 Controls Intestinal Inflammation via Suppression of the IL-12/IL-23 Axis

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Inflammatory bowel diseases (IBD) are heterogeneous inflammatory disorders of the intestine. In mice, genetic variants in a region of chromosome 3 called the *Hiccs* locus have been linked with susceptibility to IBD-like pathology, but the causative genetic element in this locus has not been identified. We now report that a poorly characterized kinase encoded in the *Hiccs* locus, Alpk1 (alpha kinase 1), is a potent negative regulator of intestinal inflammation. Following infection with the commensal pathobiont *Helicobacter hepaticus* (*Hh*), Alpk1-deficiency in both lymphocyte-replete and Rag2-deficient mice causes an exacerbated inflammatory response and intestinal pathology. This heightened response is characterized by robust production of cytokines characteristic of type 1 immunity including IL-12 and IFN- γ , but modest expression of type 2 and type 17 cytokines, resulting in a highly polarized type 1/Th1 immune response. Exacerbated colitis in Alpk1-deficient mice is dependent on both IL-12 and IL-23 signaling. Although Alpk1 has been implicated in pathogen sensing by epithelial cells, bone marrow chimera experiments demonstrated that it controls intestinal homeostasis via the hematopoietic system, in which it is highly expressed by macrophages and dendritic cells. In bone marrow-derived macrophages, Alpk1-deficiency selectively promotes expression of *Il12a* and *Il12b*, but not *Il23a*, downstream of toll-like receptor 2 (TLR2) activation in response to *Hh*. Overall, we identify Alpk1 as a novel guardian of intestinal homeostasis that controls the intensity of type 1 immunity following microbial challenge.

T.36. A Novel Whole Blood Assay Detects Flagellin-Specific CD4⁺ T Cells in Patients with Inflammatory Bowel Disease

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Precisely which antigens drive pathogenic T-cell responses in IBD remains unknown. We investigated whether bacterial flagellins are major antigens for CD4⁺ T-cells in humans. Our initial cohort comprised n=48 Crohn's Disease (CD); n=7 Ulcerative Colitis (UC) and n=23 healthy controls. Our validation cohort comprised n=20 CD; n=20 UC and n=20 healthy controls. We used a whole blood assay to detect flagellin-specific cells, defined by induced CD25/OX40 co-expression after 48h incubation with antigen. We tested *Lachnospiraceae*-derived A4-Fla2 or *E. coli* H18 FliC antigens. We observed strong responses to Fla2 and FliC antigens in both CD and UC patients, a distinct difference from previous studies of anti-flagellin antibodies. However, in our initial IBD cohort the frequencies of flagellin-specific T cells was not different from healthy controls. In concordance with a recent study we found both FliC and Fla2-specific CD4⁺ T cells predominantly exhibit a Th17 cell phenotype. Of note, many IBD patients had detectable responses to *C. difficile* toxin TcdB, indicating patients may frequently encounter this pathogen even without a recognized diagnosis of *C. difficile* infection. We also investigated whether anti-TNF α therapy affects the presence of flagellin-specific CD4⁺ T cells by collecting blood pre- and post-therapy. For n=13 patients we observed a significant loss of FliC-specific, but not Fla2-specific, cells by week 8 post-therapy that persisted until the end of observation at 6 months. The majority (80%) of these patients had improved/steady symptoms. Our data confirm the presence of circulating flagellin-specific Th17 cells in IBD patients, with no difference between CD and UC.

T.78. Mesenchymal Stem Cells have Attenuated Differentiation and Clonogenic Potential in Ulcerative Colitis

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Ulcerative Colitis (UC) is one of the major forms of the Inflammatory Bowel Disease (IBD) caused by an abnormal immune response to gut microbiota and resulting in the chronic inflammation the colonic mucosa. Mesenchymal Stem Cells (MSCs) therapy shown promise to treat IBD and attributed to the MSC tolerogenic and regenerative capacities. However, there is lack of knowledge on the fate of the intestinal mucosa resident MSC during UC development. We previously noted that in UC, CD90⁺ mesenchymal stromal cells, (myo-)fibroblasts and their progenitor MSCs, switch their activity from tolerogenic toward inflammatory. However, the stem-like properties in this population are poorly characterized. Our objective was to determine whether changes in the colonic tissue MSCs occur in UC mucosa. An increase in the mRNA expression of stem cell marker Oct3/4 and ALDH1A was observed in the UC inflamed colonic mucosa and shown a positive association with an increase in expression of mesenchymal lineage marker Grem1 (Pearson correlation analyses, pln situ analysis demonstrated an increase in MSC-like cells co-expressing Oct3/4 and ALDH1A in UC when compared to the normal colonic mucosa. We also observed an increase in expression of myofibroblast differentiation marker, α -SMA, by the MSC-like cells in UC. When compared to normal controls, a dramatic reduction in differentiation and clonogenic capacities of UC derived CD90⁺ mesenchymal stromal cells was observed. Our data provide a rationale for MSC therapy and suggest that while the number of MSC-like cells is increased in inflamed UC, their regenerative capacity is decreasing.

W.56. Analysis of Cecal Immune Response in a Murine Model of Colitis Using Intravital Imaging

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Background & Aim: Accumulating epidemiological studies have suggested that appendectomy may reduce the risk of ulcerative colitis. Thus, ileocecal immune response may be considered to affect the development of inflammatory bowel diseases (IBD), but the mechanism is still unclear. Therefore, we analyzed the ileocecal immune response in a murine model of IBD under various conditions and background, including 5D intravital imaging.

Methods & Results: Wild type C57BL6 (WT) mice were sensitized with oxazolone to induce colitis. Subsequently, this resulted in remarkable ulcerations in the early stage of colitis development especially at the follicular-associated mucosa on the expanding cecal lymphoid follicle (CLF), which became the focus of our study. Mice underwent appendectomy before oxazolone treatment revealed attenuated colitis with regards to both clinical disease activity and histopathology in association with decrease of pro-inflammatory cytokine production such as IL-4. Similar clinical and histopathological changes were also observed in μ MT mice, which lack mature B cells. Real-time analysis of B cell activity in the CLF was accomplished by using mice with B cell-specific Ca^{2+} biosensor, YC3.60. B cell activation with frequent Ca^{2+} influx was observed in the CLF during the early phase of colitis.

Conclusion: Abnormal Ig production was previously considered to be the major role of B/plasma cells in the pathogenesis of IBD. However, our current results imply that B cells in the CLF contribute to the induction of acquired immune responses associated with IBD during the early phase of colitis development.

W.78. Effect of Concurrent Systemic Glucocorticoid use on CSF T Lymphocytes in Patients with Moderate-to-Severe Crohn's Disease

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Background: Systemic glucocorticoid therapy decreases circulating lymphocyte levels; however, its effect on CSF lymphocytes in patients with no CNS disease is unknown. The phase 1 TOSCA study (NCT01387594), investigated the effect of immunosuppressive therapy (including glucocorticoids) on CSF lymphocytes in patients with Crohn's disease (CD) treated with the fully human anti-MAdCAM-1 monoclonal antibody SHP647.

Methods: Patients with moderate-to-severe CD and a history of immunosuppressant and anti-TNF use were treated with SHP647 every 4 weeks for 12 weeks (3x225mg doses). Treatment with immunosuppressants and glucocorticoids could continue. Lumbar punctures were performed before and 9-11 weeks after treatment initiation, and assayed using FACS for CD3^+ , $\text{CD3}^+\text{CD4}^+$ and $\text{CD3}^+\text{CD8}^+$ lymphocytes.

Results: Of 39 patients entering the study, 12 were taking oral glucocorticoids at baseline (median prednisone equivalent dose=15mg/day), 25 were not, and 2 had missing data. Baseline geometric mean (mL) CSF CD3^+ cell count was 409 (95% CI: 271-616) for patients not on glucocorticoids, and 284 (137-589) for those on glucocorticoids. Post-treatment, the counts were 646 (399-1047), and 237 (161-350), respectively. Similar patterns were seen for $\text{CD3}^+\text{CD4}^+$ cells but not $\text{CD3}^+\text{CD8}^+$ cells. Patients receiving glucocorticoid doses $\leq 15\text{mg}$ had higher baseline cell counts/mL than those receiving doses $>15\text{mg}$ (CD3^+ : 379 [99-1453] vs 213 [72-629] $n=6$ per group).

Conclusions: Higher glucocorticoid doses were associated with lower CSF lymphocyte counts in patients concomitantly receiving anti-MAdCAM-1 therapy. With the exception of $\text{CD3}^+\text{CD8}^+$ cells, all lymphocyte subsets were higher in patients not receiving glucocorticoids. Treatment with SHP647 was not associated with a meaningful change in any lymphocyte subpopulation.

W.84. Therapeutic Potential of Wuchereria Bancrofti Derived Macrophage Migration Inhibitory Factor-2 (rWb-MIF-2) in DSS Induced Colitis Mouse Model

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Several recent reports suggest the therapeutic potential of helminth derived molecules in autoimmune diseases. In this study, we evaluated the therapeutic potential of rWba-MIF-2 in dextran sulfate sodium (DSS)-induced colitis in BALB/c mice. Colitis was induced by oral administration of 5% DSS for 7 days (DSS-PBS group). These mice showed loss of body weight, presence of blood in feces, reduced colon length and histopathological changes in the colon. PBS alone or rWba-MIF-2 alone group served as controls. In rWba-MIF-2 (4 doses at 25 µg/dose/ip) treated mice, body weight loss was minimal and there was no blood in the feces compared to the DSS-PBS group. Similarly, the colon length was not reduced and there were minimal histopathological changes in the colon tissue of rWba-MIF-2 treated animals. Analysis of the cellular responses in the colon, mesenteric lymph nodes (MLN), spleen and peritoneal cavity showed that IL-10 producing CD3⁺CD4⁺CD25⁺FoxP3⁺ T regulatory cells (Tregs) were increased in the colon tissue and IL-10 producing B1 cells were increased in the peritoneal cavity following rWba-MIF-2 treatment. Pro-inflammatory cytokines (TNF-α, IL-1β, IL-2, IL-6, IL17a) and Th1/Th17 transcription factors (TBX21 and RORγ) were significantly decreased in the colon and peritoneal cells of rWba-MIF-2 treated animals compared to DSS-PBS group. In conclusion, rWba-MIF-2 treatment ameliorated the symptoms and pathology of DSS induced colitis and this immunoregulatory effect appears to be mediated by IL-10 produced by Tregs and B1 cells. These findings reveal the potential therapeutic effect of rWba-MIF-2 against colitis.

Key words: Macrophage migration inhibitory factor, Colitis, Tregs, Colon, IL-10.

Innate immunity

F.15. An Atlas of the Mosquito Immune System at Single-cell Resolution

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Malaria is a deadly, worldwide disease. Every year the *Plasmodium* parasite is responsible for an estimated 214 million cases of malaria and over four hundred thousand deaths. In order to infect humans, *Plasmodium* parasites must complete their life-cycle in mosquitoes. *Anopheles gambiae* is the main vector for *Plasmodium falciparum* in Africa, the most virulent of the parasite species causing malaria. The mosquito in turn relies on both humoral and cellular innate immune divisions to defeat invading pathogens, coordinated by the hemocytes. Hemocytes are considered the equivalent of vertebrate white blood cells, circulating in the hemolymph within the insects' body cavity. However, basic hemocyte cell biology and immunological effector mechanisms are largely unknown, mainly due to the low number of immune cells in each mosquito. Here we profile 5,292 individual *Anopheles* mosquito hemocytes in baseline, blood-fed and malaria-infected conditions with single-cell RNAseq. We identify previously unknown cell types and their gene signatures. We also report shifts in the activation level and composition of the immune cells repertoire of mosquitoes infected with malaria parasites. Analysis shows the existence of three polarized immunological states: suppressed, primed, and malaria-activated. Recently, progress in reducing malaria mortality and incidence has been challenged by the emergence of parasite and mosquito resistance to drugs and insecticides. Developing an in depth understanding of the mosquito immune system will clarify malaria transmission dynamics and prove useful in developing novel vector control strategies.

F.27. Unique Effects of Human Tr1 Cells on Suppressing Innate Immunity and Promoting Epithelial Health

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Regulatory CD4⁺ T-cells (Tregs) can be used as cell-based therapies to suppress various immune-mediated diseases, but the optimal therapeutic Treg population may differ depending on the disease. We previously showed that mouse Tr1 cells, but not FOXP3⁺ Tregs, secreted high levels of IL-10 and potently suppressed inflammasome activation in macrophages. To test if this was also the case in humans, we optimized a protocol to obtain *ex vivo* human Tr1 cells. Following 16h of anti-CD3/CD28 stimulation, Tr1 cells were sorted as IL-10-secreting cells using a cytokine capture assay. After 14d expansion these cells retained high IL-10 secretion and were IFN- γ ⁺IL-2⁻IL-4⁻, consistent with a Tr1 cell phenotype. Tr1 cells were equivalent to FOXP3⁺ Treg cells in their ability to suppress T-cell proliferation and IFN- γ secretion. We confirmed that human Tr1 cells were superior to FOXP3⁺ Tregs in suppressing inflammasome responses. RNA sequencing confirmed both a Tr1 cell phenotype and revealed that Tr1 cells, but not Tregs, secrete the cytokine IL-22. We thus investigated the ability of Tr1 and Treg cell conditioned supernatants to influence enterocyte function by co-culturing them with primary human intestinal organoids. Interestingly, Tr1 cell supernatants uniquely suppressed stem cell proliferation, instead promoting goblet cell differentiation and mucus production, protective functions previously attributed to IL-22. Finally, we show that our protocol is suitable for isolating Tr1 cells from IBD patients, that had a similar IL-10⁺IL-22⁺ phenotype. Our data suggest that a Tr1 cell-based therapy may have specific mechanistic advantages for the treatment of human inflammatory diseases, such as IBD.

F.31. Caspase-1 Laden Extracellular Vesicles from Cystic Fibrosis Lungs Activate Inflammasome Signalling in Neutrophils and Epithelial Cells

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Background: One of the hallmarks of neutrophil-driven airway inflammation in cystic fibrosis (CF) patients is robust inflammasome activation resulting in IL-1b, IL-1a, and IL-18 release. Recent reports have indicated that components of the inflammasome complex can be found extracellularly and be phagocytosed, thereby perpetuating inflammasome activation. Here, we hypothesized that CF airway neutrophils serve a source of extracellular caspase-1 via the release of extracellular vesicles (EVs), thus creating a pro-inflammatory loop activating epithelial cells and neutrophils freshly recruited to CF airways.

Methods: We assessed the relationship between caspase-1 activation and IL-1 signaling in CF airway neutrophils *in vivo*, and in an *in vitro* model of airway recruitment and pathological conditioning of neutrophils. In addition, we assessed the presence of active caspase-1, IL-1 β , and the core inflammasome component ASC, in EVs from CF sputum and plasma. Finally, we assessed the ability of EVs from CF sputum to induce inflammasome activation in naïve neutrophils and epithelial cells.

Results: We found that CF airway PMNs *in vivo* and *in vitro* increased intracellular caspase-1 activity and surface expression of IL-R1. Moreover, EVs from CF sputum were enriched in caspase-1, IL-1 β , and ASC compared to those from plasma, and these EVs induced caspase-1 activation when administered to naïve neutrophils and epithelial cells.

Conclusions: Collectively, our data suggest that in CF airways, caspase-1 laden EVs serve to perpetuate inflammasome activation, suggesting a possible new target to combat aberrant inflammation in this intractable disease.

F.51. Posttranscriptional Control of Microglia-derived Neurotrophic Factors

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Resident microglia of the “M2” phenotype are active in tissue repair and prevention of neuronal death in a wide spectrum of CNS inflammatory states, including following an ischemic stroke. These reparative and neuroprotective effects are attributed to the secretion of neurotrophic factors, predominantly Nerve Growth Factor (NGF) and Brain-derived Neurotrophic Factor (BDNF), which have been shown to reduce neuron death in a variety of ischemic stroke models. We established a phenotype resembling M2 microglia in the mouse BV2 microglial cell line via treatment with TGF- β , a well-established inducer of this pathway. We sought to examine whether BDNF/NGF were regulated at the posttranscriptional level. The BDNF 3'UTR, when cloned into the pEZX luciferase reporter plasmid and expressed in BV2 cells, significantly reduces luminescence. This suggests that microRNA-mediated posttranscriptional inhibition may play a clinically relevant role in these pathways. Using the oxygen-glucose deprivation (OGD) model of ischemic stroke, we observed increased BV2 BDNF and NGF mRNA levels in these ischemic-like conditions, and that TGF- β cooperatively enhances those neurotrophic transcript levels in OGD-exposed cells. Candidate BDNF and NGF 3'UTR target microRNAs are being examined as competitive (negative) modulators of this M2 microglia-mediated neuroprotection/repair pathway, with goals of miR-directed therapeutics for enhancement of these favorable post-CNS injury responses.

F.66. Opposing Roles of CD2 and 2B4 in iNKT Cell Cytotoxic Responses

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Invariant natural killer T cells (iNKTs) comprise a unique lineage of innate-type lipid-reactive T lymphocytes with important roles in host immunity, including defense against specific pathogens and cancers. iNKTs rapidly produce cytokines and mount potent cytotoxic responses following T cell receptor (TCR) engagement, but the mechanisms that control iNKT cell functions remain poorly understood. CD2 and 2B4 are receptors on iNKTs that both bind to CD48 on target cells and modulate immune activation by facilitating cell-cell interactions and transducing intracellular signals. We observe that CD2-deficient (*Cd2*^{-/-}) iNKTs fail to kill target cells while 2B4-deficient (*2b4*^{-/-}) iNKTs exhibit significantly enhanced cytotoxicity, both *in vitro* as well as *in vivo*. These novel findings suggest that CD2 is a positive and 2B4 a negative regulator of TCR-induced iNKT cell functions. We are currently investigating whether 2B4 exerts its inhibitory role by directly competing with CD2 for CD48 binding and/or by inducing a negative signal. As CD1d (the ligand for the invariant TCR) and CD48 (the ligand for CD2 and 2B4) are both widely expressed on hematopoietic tumors, further elucidation of the mechanisms by which CD2 and 2B4 regulate TCR-induced iNKT cell anti-tumor activity is of significant scientific and clinical importance. The results obtained from these studies will have an impact by establishing new paradigms for iNKT signaling and offering insights into how iNKTs can be best activated to enhance host immunity and treat hematopoietic cancers such as leukemia and lymphoma.

F.101. Diminished B Cell Response after Repeated Influenza Vaccination

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Antigenic drift of influenza viruses necessitates changing the components of seasonal influenza vaccines to better match the predicted circulating strains of the upcoming season. Annual influenza vaccination is, therefore, considered the most

effective approach for protection and to boost waning immunity. Past serological and meta-analysis reported conflicting results on the benefits of annual vaccination, whereas B cell response elicited by repeated influenza vaccination has not been analyzed in detail. Here, we monitored the B cell responses in subjects vaccinated yearly with inactivated influenza vaccines (IIV) from 2010/2011 to 2014. We focused on the plasmablast responses in peripheral blood samples collected at day 7 after vaccination, which represented B cells activated by the vaccination. In the subjects annually vaccinated from 2010/2011 through 2014, the vaccine-specific binding reactivity of plasmablast-derived polyclonal antibodies (PPAb) were significantly reduced after the second year of vaccination in comparison to the first year. This response did not increase in subsequent years. A similar trend was observed with the frequency of influenza-specific antibody secreting cells, and the fold increase of serum hemagglutination inhibition titers. Analysis of hemagglutinin-specific reactivity of PPAbs for the component viruses also showed the same trend suggesting that effective B cell responses that contribute to protection diminish with repeated vaccination. Additionally, significant differences in the avidity of the PPAbs against the H1 protein (unchanged in 2010-2014 IIV) were not detected in these years. The diminished B cell immune response to repeated influenza vaccination should be a factor to be considered in making influenza vaccination policy.

T.2. Immune-modulatory Role of miR-511-3p on Mannose Receptor Expression and Impact on Human DC Function

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MicroRNAs are small, single-stranded RNA molecules that have emerged as important regulators of gene expression in the immune system and play key roles in controlling immune modulatory events. MiR-511-3p is a multifunctional miRNA implicated in several human diseases and development of antigen presenting cells mainly DCs. Interestingly, miR-511-3p is embedded within the *MRC1* gene that encodes the mannose receptor (MR). We have previously shown that C-type lectins on DCs such as MR and DC-SIGN are involved in uptake of allergens leading to allergic sensitization. In particular, allergen binding to MR can modulate TLR4 signalling, affecting Th polarisation. Here we sought to examine the impact of miR-511-3p regulation on MR expression and downstream DC function. Using gene silencing technology and miRNA mimics, we could successfully suppress or induce expression of miR-511-3p in human DCs. Leveraging on this ability, we show for the first time that inhibiting or overexpressing miR-511-3p modulates the regulatory function of DCs at least partly due to changes in MR expression. We showed that DCs exhibited a tolerogenic phenotype characterised by upregulation of key phenotypic markers such as PDL-1 and production of IL-10, when miR-511-3p was inhibited compared to overexpressed conditions. Investigating the intracellular pathways mediating these events, we further highlight key molecules that impact downstream NF- κ B activation following modulation of miR-511-3p in these cells. Taken together, our data provides new insight into the complex nature of the immune regulatory network and could pave way for rational design of novel immune modulatory therapies.

T.9. Role of SLC7A5 in Metabolic Reprogramming of Human Monocyte/Macrophage Immune Responses

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Amino acids (AAs) are necessary nutrients which act not only as building blocks in protein synthesis, but also in crucial anabolic cellular signaling pathways. It has been demonstrated that SLC7A5 is a critical transporter that mediates uptake

of several essential amino acids (EAAs) in highly proliferative tumors and activated T cells. However, the dynamics and relevance of SLC7A5 activity in monocytes/macrophages is still poorly understood. We provide evidence that SLC7A5-mediated leucine influx contributes to proinflammatory cytokine production via mTORC1-induced glycolytic reprogramming in activated human monocytes/macrophages. Moreover, expression of SLC7A5 is significantly elevated in monocytes derived from patients with rheumatoid arthritis (RA), a chronic inflammatory disease, and was also markedly induced by LPS stimulation of both monocytes and macrophages from healthy individuals. Further, pharmacological blockade or silencing of SLC7A5 led to a significant reduction of IL-1 β downstream of leucine-mediated mTORC1 activation. Inhibition of SLC7A5-mediated leucine influx was linked to downregulation of glycolytic metabolism as evidenced by the decreased extracellular acidification rate (ECAR), suggesting a regulatory role for this molecule in glycolytic reprogramming. Furthermore, the expression of SLC7A5 on circulating monocytes from RA patients positively correlated with clinical parameters, suggesting that SLC7A5-mediated AA influx is related to inflammatory conditions.

T.27. Protective Roles of Myeloid SOCS3 to Neuroinflammation in Neurodegenerative Parkinson's Disease

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Activated central nervous system (CNS)-resident microglia and infiltrating immune cells contribute to neurodegeneration in Parkinson's Disease (PD), a chronic neurodegenerative disorder. We recently demonstrated that there is enhanced activation of the JAK/STAT pathway in an α -synuclein (α -syn) overexpression PD model, and inhibiting this pathway prevents neuroinflammation and neurodegeneration. Our previous studies identified that myeloid SOCS3, a negative regulator of the JAK/STAT pathway, plays a critical role in neuroinflammation. To better understand the function of myeloid SOCS3 in the pathogenesis of PD, mice with SOCS3 deletion in myeloid cells (SOCS3^{MyeKO}) were utilized in our α -syn-overexpressed PD model. Our results indicated that hyper-activation of JAK/STAT in SOCS3^{MyeKO} mice results in a significant enhancement of neuroinflammation and neurodegeneration in responses to overexpressed α -syn compared to SOCS3^{fl/fl} mice. To further investigate the therapeutic potential of inhibiting JAK/STAT activation, the JAK1/2 inhibitor AZD1480 was utilized in this project. Our results demonstrated that AZD1480 inhibits α -syn-induced neuroinflammation by preventing microglia/macrophage activation, reducing infiltration of CD4⁺ T-cells, and suppressing the peripheral IFN- γ ⁺CD4⁺ and IFN- γ ⁺CD8⁺ T cells in this PD model *in vivo*. Furthermore, our results indicated that inhibition of the JAK/STAT pathway with AZD1480 prevents the degeneration of dopaminergic neurons in both of SOCS3^{fl/fl} and SOCS3^{MyeKO} mice. Our studies demonstrated the critical function of the myeloid SOCS3 and related JAK/STAT pathway in the pathogenesis of neurodegenerative PD. Giving the importance of innate and adaptive immune responses in neuroinflammation and neurodegeneration, our results suggest that targeting the JAK/STAT pathway may become a potential therapeutic strategy in PD patients.

T.60. Mass Cytometry-Based Analysis of Human Lung Macrophages

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Macrophages play important roles in many lung pathologies including Chronic Obstructive Pulmonary Disease (COPD). These cells accumulate inhaled particles and are highly auto-fluorescent especially in smokers. Characterization of lung macrophages, using flow cytometry and fluorescent microscopy, has been challenging due to this autofluorescence. Objective: To develop methods for characterization of human lung macrophage subsets and their polarization & function. Methodology: Current and former smokers with & without COPD, scheduled to undergo lung resection for malignancy were recruited. Non-cancerous portions of resected lung tissue samples were collected within 15 minutes of resection and were enzymatically digested for immune cell isolation. A separate cohort of never-smokers, former smokers (stopped smoking for >12 months) and current smokers with and without COPD were recruited to undergo

bronchoalveolar lavage (BAL). Immune cells isolated from lung tissue and BAL were characterized using mass cytometry.

Results: We developed a 45-marker mass cytometry-based panel that allow us to characterize the phenotype and polarization status of lung macrophages isolated from lung tissue and BAL, regardless of their smoking status. The panel consists of 12 lineage markers, 16 activation or inhibitory markers, 9 cell adhesion markers, and 8 homing or co-stimulatory markers.

Conclusion: Diverse lung macrophage populations may be isolated and characterized for their origin, polarization, and function using the developed mass cytometry panel. This technique is helpful in studying lung macrophages with auto-fluorescence, thus superseding the limitations of traditional flowcytometry. Future studies will focus on characterizing the macrophage subsets and determining their function in lung tissue from COPD patients.

T.76. HMGB1 Redox Forms Differentially Mediate Innate-to-Adaptive Immune Switch after Ischemia-Reperfusion Injury in Human Orthotopic Liver Transplantation

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Ischemia-reperfusion injury (IRI) is a major risk factor of acute and chronic graft rejection in orthotopic liver transplantation (OLT); however, no clinical therapeutics or patient-specific diagnostics are currently available. Murine studies have shown the damage-associated molecular pattern (DAMP) high-mobility group box 1 (HMGB1) is released during OLT-IRI and binds to the pattern recognition receptor (PRR) TLR4 on parenchymal and/or infiltrating innate immune cells, inducing pro-inflammatory cytokine production. We measured HMGB1 in plasma obtained pre-, intra- and post-transplant from OLT patients (n=70), and found it was significantly elevated in initial liver flush (LF) obtained after reperfusion. Yet, although HMGB1⁺ LF from patients identified as IRI⁺ by histopathology (n=36) increased TNF α production in third-party PBMC-derived monocytes compared to IRI⁻ HMGB1⁺ LF (n=34), HMGB1 presence alone did not predict IRI. Further investigation indicated HMGB1⁺ LF from IRI⁺ patients increased activation of HEK-BlueTM hTLR4 and/or hTLR9 transfected cells, whereas IRI⁻ patient LF primarily increased hTLR7 activation. Western blot analysis revealed patient LF samples contained varying levels of different HMGB1 redox forms, which can initiate different downstream immune effects through different PRRs. Three-dimensional subcellular reconstruction of multiparameter immunofluorescence confocal images taken of 100um-thick tissue sections demonstrated IRI⁺ biopsies contain mostly chemotaxis-inducing all-thiol HMGB1 pre-transplant, and a large inflammatory infiltrate consisting of disulfide-HMGB1-secreting monocytes/macrophages post-transplant, whereas IRI⁻ biopsies show predominantly non-immunogenic sulfonyl-HMGB1 from ischemic parenchymal cells. Together these data highlight important diagnostic differences of HMGB1 redox states in donor organs that should be considered in recipient matching and/or therapeutic intervention during transport for improving OLT outcome.

T.85. The Induction Mechanism of Interferon Regulatory Factor 5 in HMGB1-induced Macrophage Polarization

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Stimulation of monocytes with High mobility group box 1 (HMGB1) triggers distinct pro-inflammatory M1-like macrophages while concomitant exposure to C1q opposes the HMGB1-mediated M1 polarization program. The transcription factor interferon regulatory factor 5 (IRF5) is a major regulator of pro-inflammatory M1 macrophage polarization. Leukotrienes are known to contribute to inflammation and to be secreted by M1 macrophages. How these factors influence each other remains unclear. We found that both IRF5 expression and leukotriene B4 (LTB4) production were high in HMGB1-exposed human monocytes, while HMGB1 plus C1q exposed cells secrete more lipoxin A4 and less LTB4. We also found that decreasing IRF5 led to a decrease in HMGB1-induced LTB4 suggesting that IRF5 is involved in HMGB1-induced LTB4 production as well as in the production of pro-inflammatory cytokines. Antagonists of the leukotriene receptor or 5-LO inhibitors suppressed the induction of IRF5 in HMGB1-exposed cells. Moreover, when mice were given HMGB1 ip, a peritoneal exudate developed characterized by M1-like macrophages while in mice given HMGB1 plus C1q ip, there were more M2-macrophages (identified as CD163⁺ and Mer^{high}) with IRF5 enhancing. Specifically, Mer^{high} cells secreted more resolvin D2 and more IL-10 transcript and less LTB4 and IRF5 transcript. Intra-peritoneal injection of an LTB4 receptor antagonist or a 5-LO inhibitor suppressed M1 polarization of macrophages in HMGB1-peritonitis *in vivo*. Our data suggest a cross-talk between the leukotriene pathway and IRF5 expression in HMGB1-mediated macrophage polarization with IRF5 enhancing leukotriene production and leukotrienes enhancing IRF5 expression. C1q present together with HMGB1 opposes the M1 polarization program.

T.103. IL-5 Signaling in Structural or Hematopoietic Cells Protects Mice Against Acute Lung Injury

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Acute respiratory distress syndrome (ARDS) affects 200,000 people in the US each year with a mortality rate of 20-40%. ARDS occurs as a result of acute lung injury (ALI)-induced damage to pulmonary epithelium and/or endothelium. In a bleomycin model of ALI, we previously have shown exogenous treatment of mice with IL-5 reduces lung edema and mortality. We now demonstrate that IL-5 signaling is critical in protection, as IL-5R α ^{-/-} mice had significantly increased lung edema and mortality. IL-5R α ^{-/-} mice had severely reduced numbers of lung eosinophils before and after challenge compared to WT mice, suggesting that eosinophils may provide protection from ALI. However, we found no difference in survival between eosinophil-deficient PHIL mice and WT mice. In WT mice, IL-5R α was constitutively expressed by lung neutrophils as well as eosinophils. Surprisingly, bleomycin challenge upregulated IL-5R α on myeloid cells and epithelial cells, indicating that IL-5 may regulate multiple cell populations during inflammation. To examine what cell types mediated protection, we used bone-marrow chimeric mice and found that expression of IL-5R α on structural cells alone or hematopoietic cells alone was sufficient to protect mice from mortality. We examined human primary lung epithelial cells and found high expression of IL-5R α protein, implicating a role for eosinophil-independent IL-5 responses in human disease. Our data suggests that epithelial cells, or possibly other cell types, have an unappreciated role in the IL-5 response. Thus, targeting the non-eosinophilic effects of IL-5 may be a novel therapeutic strategy to protect against lung injury following pulmonary infection, sepsis, or trauma.

T.108. Mycobacterium Leprae Proteins, GroES and MMP-II, Induce Extracellular Traps in Human Neutrophils

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Neutrophils are important cells in innate immune responses, one effector mechanism of these cells is the formation of NETs. These are structures formed by DNA and microbicidal proteins. A variety of microorganisms and antigens are capable of trigger NETs.

Two proteins of *Mycobacterium leprae* have shown the ability of stimulate both cellular and humoral responses. GroES protein is a chaperonin that helps in the folding of proteins, while MMP-II is identified as the bacterioferritin of *M. leprae*.

Aim

To determine if the proteins of *Mycobacterium leprae*, GroES and MMP-II, have the capability of induce the release of NETs.

Methodology

Neutrophils from peripheral blood were obtained by Ficoll Histopaque 1119/1077 density gradient. Induction of NETs *in vitro* was carried out with the proteins GroES and MMP-II at different concentrations 0.01, 0.1 and 1 µg/µL. LPS at 60 µg/µL was used as positive control and SSF as a negative control. 2.5×10^5 neutrophils were added to polystyrene plates and stimulated with the different concentrations of proteins, incubated for 3 hrs and then fixed with paraformaldehyde. Finally, DNA and elastase were stained. The samples were observed in a fluorescence microscope. DNA/NETs quantification was performed by fluorometry.

Results

We observed that GroES and MMP-II induced NETs in a dose-dependent manner.

Conclusions

Previously it has been reported that GroES and MMP-II proteins are capable of stimulate immune adaptative responses. In this work we demonstrated that both proteins trigger NETs release by neutrophils, thus capable to initiate the innate immune response.

W.13. Neutrophils Shed Tethers that form a New Class of Elongated Shear-derived Particles (SDP)

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When neutrophils roll along the endothelium at high shear stress, microvilli are pulled out behind the cell into several micron long, thin membrane tubes called tethers that stabilize rolling. Here, we report that these tethers are the source of a new class of blood microparticles. Detached neutrophil tethers remain elongated shear-derived particles (SDP) and can be imaged in mice via Ly6G antibody labeling. Intravital microscopy of mouse cremaster showed *in vivo* the formation of SDPs with median length of 6.5 µm (range 1.9-112 µm, n=234 collected from 8 mice). Most SDPs appeared in venules, stuck to the endothelium, or in 18% of cases “sneaking” along the vessel wall with the blood flow at a median velocity lower than neutrophil rolling velocity (1.13 µm/s vs. 3.47 µm/s). Free-floating SDPs were visible in the arterioles and in the harvested blood of these mice. Isolated human neutrophils showed SDP production (visualized by CD16 antibody) in flow chambers (>10 dyn/cm²). A standardized confocal scanning method showed that plasma of septic patients (n=24) compared to healthy donors (n=20) contains more and longer CD16⁺ SDP; count 705(0-1400)/µl vs. 115(0-420)/µl, median length 5.4(1.7-23)µm vs. 4(1.7-12.8)µm. Imaging cytometry (Amnis Image Stream) confirmed these findings and showed that CD16⁺ SDPs are ~1000-fold less common than CD16⁺ spherical particles. Some SDPs may fragment into spherical vesicles. Confocal microscopy and Amnis showed that CD16⁺ SDPs are negative for the

exosome markers CD9, CD63, CD81 and for the apoptotic body marker Annexin5. All significances: $p < 0.0001$ with Student's T-test.

W.22. STING Associated Vasculopathy with Onset in Infancy (SAVI) and Chronic Atypical Neutrophilic Dermatitis with Lipodystrophy and Elevated Temperature (CANDLE) are Autoinflammatory Diseases Driven by Active Type I Interferons

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SAVI is caused by gain-of-function mutations in *TMEM173*/STING and CANDLE loss-of-function mutations in proteasome subunits. In both diseases, pan-interferon (IFN)- α serum levels are 8 and 25-fold increased, respectively and patients (pts) had 12-fold increase in IFN response gene signatures (IRG) compared to healthy controls (HC). To determine the cellular origin of IFNs, we evaluated sorted PBMCs and lesional skin biopsies from pts and HC. SAVI pts constitutively expressed IFNs and IRG even post-treatment, QPCR results showed 450-fold and 280-fold *IFNB1* [IQR 0.5-61, 60.6], and 3.5-fold and 4.4-fold *IFNA7* [IQR 0-2.93, 2.93] monocytes (n=5) and dendritic cells (n=3) respectively, compared to CANDLE and HC. During disease flares, CANDLE monocytes increased *IFNB1* by 4.6 and 184-fold and *IFNA7* by 2.5 to 110-fold (n=2). Compared to SAVI, CANDLE monocytes had higher levels of cytoplasmic vacuoles 6.4 ± 2.1 SD compared to SAVI 3.5 ± 2.5 SD ($p > 0.05$) and HC 2.2 ± 1.2 SD ($p < 0.004$). RNA sequencing showed 2-4-fold increase in mitochondrial stress genes compared to SAVI and HC. We also detected 30-400-fold increase of *CXCL10* in CANDLE lesional skin expression, suggesting higher contribution to the IFN score from the skin compared to blood. Constitutive activation of IFNs in SAVI monocytes inhibited differentiation into macrophage and within 24-hours, monocytes experienced rapid cell death which was associated with increased cleaved caspase-1 compared to HC and CANDLE. In conclusion, the source of IFN production varies in CANDLE and SAVI, understanding cellular origin, interferon signaling pathways and biomarkers will aid in the diagnosis of yet genetically uncharacterized autoinflammatory diseases and the design of targeted treatments.

W.29. Implications of Silver Nanoparticles on Phenotypic Changes in Murine Bone-marrow Derived Dendritic Cells

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Introduction. DCs are key on activation of the immune response. To regulate them, have been proposed the use of some nanomaterials as silver nanoparticles (AgNPs). Objective. To analyze the immunophenotype and cytotoxicity on mouse BMDCs treated with AgNPs. Material y Methods. The bone marrow cells were extracted from mice C57BL/6 of 8 weeks old and differentiated to BMDCs for 9 days with RPMI-1640 medium, with 10% of FBS, and 30% transfected CHO culture supernatant with GM-CSF. The assays were done at 24 and 48 h. From MTT assays, the concentrations of AgNPs were selected. The immunophenotype (CD40, CD274, and MHCII at CD11c (BMDCs)) and cytotoxicity were analyzed by flow cytometry. The levels of IL-6, 10, 12, and TNF- α were determined by ELISA. Results. The AgNPs at 62.5, 125, and 250 ng/ml had the major effect. None of them showed a cytotoxicity. The CD11c⁺ cells were superior to 50%. MHCII and CD40 showed low expression, meanwhile in CD274 was high. The IL-6 and 12, and TNF- α were low at

24 h, and high at 48 h. On the other hand, IL-10 was higher at 24 h, and lower at 48 h. Conclusion. The AgNPs had effect at 62.5, 125, and 250 ng/ml concentrations in BMDCs which do not show cytotoxicity. The phenotype markers indicate BMDCs were immature. Although, the high levels of IL-6 and 12, and TNF- α , and the low levels of IL-10, at 48 h, show a tendency to develop of an immunogenic phenotype.

W.33. Adhesion and Degranulation Promoting Adaptor Protein Regulates Both Direct and Indirect Anti-Tumor Responses of Invariant Natural Killer T Cells

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Invariant natural killer T cells (iNKTs) comprise a sub-lineage of T lymphocytes that express an "invariant" T cell receptor (TCR) with specificity for glycolipid antigens (Ag) such as α -galactosyl ceramide (aGC), when presented by MHC class I molecule CD1d. Following TCR engagement, iNKTs rapidly secrete cytokines and trans-activate the anti-tumor functions of dendritic cells (DC), natural killer (NK), T and B cells as well as directly kill tumor cells. Although TCR-CD1d interactions are generally required for iNKT cell functions, the signaling mechanisms that co-operate with the TCR to promote maximal iNKT anti-tumor responses remain poorly understood. Our previous studies demonstrate that the adaptor protein SAP and its signaling partner, Fyn (a Src-tyrosine kinase) is critical for iNKT cell anti-tumor responses. We recently observed that the adhesion and degranulation promoting adaptor protein (ADAP)-deficient (*Adap*^{-/-}) iNKTs exhibit marked defect in aGC-induced cytokine production and bystander immune cell activation. Importantly, we observed that ADAP is essential for T-cell receptor (TCR)-induced iNKT cell cytotoxicity against leukemia targets *in vitro* and iNKT-cell-mediated control of T-cell leukemia growth *in vivo*. Given that ADAP is a known Fyn-binding protein, our data suggest that ADAP is a critical downstream regulator of Ag-induced iNKT cell cytotoxic responses. Studies are currently underway to elucidate the mechanism(s) by which ADAP regulates iNKT cell functions. These studies will have an impact by establishing new paradigms for iNKT cell signaling and offering insights into how iNKTs can be best activated to enhance host immunity and treat cancer.

W.43. Inflammatory Phenotypes of Monocytes/Macrophages in Acute CPP Arthritis: a Comparison with Acute Gouty Arthritis

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Objectives: Deposition of calcium pyrophosphate (CPP) crystals can be presented as acute inflammatory arthritis referred to as acute CPP crystal arthritis (also called 'pseudogout'); resembling acute gout. However, difference in the monocytes/macrophages phenotypes between acute CPP crystal arthritis and acute gouty arthritis has not yet been investigated. Therefore, we examined the immunological characteristics of synovial monocytes/macrophages in patients with acute CPP arthritis and acute gouty arthritis.

Methods: The expression of markers for infiltrated monocyte and tissue-resident macrophages were measured in CD14⁺ cells from synovial mononuclear cells of patients during acute attack. Expression of pro- and anti-inflammatory cytokines and markers was examined by flow cytometry following stimulation with or without LPS.

Results: Synovial monocytes/macrophages of acute CPP arthritis showed the phenotypes of infiltrated monocytes as shown by expression of CD88, CCR2, MRP8, MRP14 but not MERTK. CD14⁺ cells from patients with acute CPP arthritis had similar high levels of IL-1 β and TNF- α production, but significantly lower IL-10 and M2 marker (CD163) expression than those from gout attack. In addition, monocytes/macrophages had capacity to induce IL-8 production in response to CPP crystals.

Conclusion: Pro-inflammatory features were more dominant in monocytes/macrophages during acute CPP arthritis

compared with those during acute gout. These CD14⁺ cells can produce IL-8, possibly contributing to the neutrophilic inflammation in acute CPP arthritis.

W.63. High Throughput Screening of IRAK4 Small Molecule Inhibitors in TLR Ligand Stimulated Whole Blood

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The IRAK4 kinase is a key regulator of TLR and IL1R signaling. Small molecule inhibitors of IRAK4 represent an important advance in targeting these pathways. We developed a high throughput whole blood assay to identify potent IRAK4 inhibitors through the inhibition of TLR ligand (R848, LPS, and Gardiquimod) induced inflammatory cytokine production (IL6, TNF, IFN α). Extensive optimization was carried out to ensure rigorous measurement of small molecule potencies, percent maximum inhibition, and cytokine endpoint correlations to clearly distinguish compounds from one another in the context of human whole blood variance. Whole blood treated with IRAK4 inhibitors resulted in partial inhibition of TLR induced cytokines. Whole blood is a complex mixture of cell types and serum proteins. Our analysis shows various dependency of TLR signaling on IRAK4 kinase activity varies among various immune cells allowing for residual inflammatory outputs. *Ex vivo* whole blood stimulation assays have proven to be an important tool in drug discovery to identify potent IRAK4 small molecule clinical candidates and further define the role of IRAK4 signaling in immune responses.

W.98. Innate Lymphoid Cells are Activated in Acute Dengue Infection and Persist Through Clinical Resolution

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Introduction: Dengue virus (DENV) infection is a significant global health problem. Disease manifestation vary from asymptomatic, a flu-like illness called Dengue Fever (DF) and more severe form, Dengue Hemorrhagic Fever (DHF). Despite extensive studies in disease pathogenesis, the underlying innate immune mechanisms have not been fully elucidated. Innate Lymphoid Cells (ILCs) are lymphocytes capable of early cytokine production and are divided into 3 groups resembling their Th counterparts. They were shown to playrole in both homeostasis and inflammation. However, the role of ILCs in human DENV infection is not known.

Objective: To study the impact of human DENV infection on ILCs number and activation status.

Method: PBMC are collected from 3 DF and 3 DHF patients at acute and convalescent phase and 12 healthy controls after informed consent. Flow cytometry is used for phenotyping ILCs (CD45^{hi}Lin-CD127⁺) and assessment of their ILC activation status by CD69 expression.

Results: Our preliminary results show that all ILC subsets were activated in acute phase, when compared to healthy control, while the number and subset distribution remain unchanged. Moreover, ILCs from DHF patients show trend of higher activation than DF patients, however, more samples is needed to confirm statistical significant difference.

Intriguingly, ILCs in convalescent phase remained activated, consistent with previous reports in HIV infection.

Conclusion and perspective: ILCs are activated in human DENV infection. Additional samples as well as functional assessment of the ILCs are needed to further our understanding of their protective or detrimental role in DENV infection.

W.116. Glutathione Peroxidase 8 Negatively Regulates Caspase-4/11 to Protect Against Colitis by Restraining NLRP3-inflammasome Activation

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Caspase-4/11 serves as an innate immune receptor for intracellular lipopolysaccharide, but how caspase-4/11 is regulated remains unknown. By phenotypic characterization of glutathione peroxidase (GPx) 8 knockout mice, we demonstrated that GPx8^{-/-} mice exhibited exacerbated colitis and were more susceptible to endotoxic shock. Furthermore, GPx8-deficient cells displayed enhanced caspase-11 activity resulting in increased pyroptosis and IL-1 β production. GPx8 compromised the activation of caspase-4/11 directly through disulfide bonding mediated by cysteine 79 of GPx8 and cysteine 118 of caspase-4. Purified oxidized GPx8, but not C79S mutant, inhibited the oligomerization of caspase-4 *in vitro*. Consistently, a caspase-4 inhibitor, VX-765, suppressed caspase4/11-dependent inflammasome activation and reduced colitis in Gpx8-deficient mice. Significantly, a positive correlation was found between lower Gpx8 and higher caspase-4 expression in the colon tissue of ulcerative colitis patients. Taken together, these results indicate that GPx8 negatively regulates caspase-4/11 activity in mice and humans and highlights the importance of GPx8 in the noncanonical inflammasome pathway.

Organ transplantation

F.59. Profile of Human Alloreactive T Cells Stimulated by CD40L-stimulated B Cells and Monocyte-Derived Dendritic Cells

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Activated B cells and mature dendritic cells (DCs) are potent antigen-presenting cells (APC) that contribute to alloimmune responses. We compared the phenotype of CD40L-stimulated B cells (sBcs) and cytokine-matured monocyte-derived DCs (moDCs), and the characteristics of sBc- and moDC-stimulated alloreactive T cells. moDCs promoted more robust proliferation of alloreactive conventional CD4⁺ T cells (Tconv), CD8⁺ T cells, and Tregs compared to sBcs, correlating with moDCs expressing higher CD80 and CD86 MFI. Both moDCs and sBcs produced large amounts of the chemokines CCL22 and CCL5. Neither APC type produced much IL-1, IL-6, IL-12p70, IL-23, nor TNF α that can polarize Tconv and destabilize Tregs. Additionally, moDCs, but not sBcs, produced IL-1R antagonist. sBc- and moDC-stimulated alloreactive Tregs (arTregs) expressed similar high percentages of Treg-defining markers (FOXP3, HELIOS, CD25, CD27, and CD62L). Unsupervised clustering analysis of Nanostring gene expression showed sBc- and moDCs-arTregs were most similar, but distinct from primary Tregs, and further separated from Tconvs. Interestingly, TCR sequencing analyses showed that repertoires of moDC-stimulated and sBc-stimulated alloreactive Tconv, CD8⁺ T cells, and Tregs were mostly distinct. T cells in a kidney biopsy with acute T-cell mediated rejection overlapped more with moDC-stimulated alloreactive CD8⁺ T cell repertoire than other T cell repertoires. Ongoing experiments will investigate how PBMC-stimulated T cell repertoire compare to moDC- and sBc- T cell repertoires, 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.72. Phenotypic and Clonal Analysis of Recipient B Cells and Plasma Cells Repopulating Graft Mucosa Reveals an Association with Rejection After Human Intestinal Transplantation

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Alloantibodies produced by recipient B cells are associated with rejection after intestinal transplantation (ITx). Although traditionally attributed to plasma cells (PCs) in lymphoid tissues and bone marrow, some studies suggest that antibody produced by recipient B cells within heart and kidney allografts may contribute to rejection. We serially analyzed B cell chimerism, phenotype, and clonotypes in the allograft and peripheral blood of ITx recipients. Surveillance ileal biopsies demonstrated eventual (months to >1 year) replacement of donor lamina propria (LP) B cells by recipient B cells. In patients with donor T cell blood macrochimerism (>4%), who have lower DSA and rejection rates, replacement of donor graft B cells by recipient occurred over several months. In contrast, recipient B cells rapidly replaced donor graft cells in patients without T cell macrochimerism, 6/7 of whom had early moderate to severe rejection. Increased recipient PC frequencies were detected in grafts during rejection episodes (4/4 patients) and recipient-derived surface IgG⁺ and IgA⁺ B cells appeared in long-term grafts of two DSA⁺ patients, but not a DSA⁻ patient's graft. IgH V region sequencing of recipient B cells revealed clonal evolution among ileal allograft biopsies over time. In summary, graft B cell repopulation rates correlated inversely with blood T cell chimerism, while recipient PC and class-switched B cells in the graft associated with rejection and DSA development. Graft-repopulating recipient B cell clones may expand and acquire effector B cell phenotypes, thereby promoting organ-specific alloimmunity.

T.14. B Cell Immune Repertoire Sequencing Identifies Pre-transplant Rejection Risk

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The diversity of the B cell immune repertoire (IR) may drive humoral injury and rejection in organ transplantation (tx). We studied functionally correlated variations in the VDJ-CDR3 regions by B cell sequencing, examining 124 DNA and RNA samples from the same tx patient before tx, and at 6 and 24 mo post-tx. Three patient groups sorted into non-progressors (NP), progressors/ no rejection (PNR) and progressors/ with rejection (PR) were scored by progressive changes in the chronic allograft damage index (CADI). Pre-tx IR diversity was significantly greater in patients that went on to reject their tx (PR vs NP, $p = 0.008$). Post-tx, there was a strong interaction between clinical outcome (CADI score, AR) and time, with a significant reduction in clonal diversity ($p = 0.01$) in PR, and an increase in NP. We defined a network for each sample considering the clone definition (same V and J segments, same CDR3 length and 90% nucleotide identity between CDR3s). We used Gini index for quantification and found relevant changes at 24 mo post tx (PR vs. NP, $p=0.003$ and $p=0.001$). The diversity of recipient IR plays a major role in driving AR and progressive chronic tissue injury, and this effect can be observed even prior to engraftment of the tx organ. Selected dominant clones expand in patients who reject allografts, and the preservation of these dominant clones results in a reduction of their diversity. Pre-tx prediction of recipient risk of rejection, provides a powerful approach for precision medicine in tx.

T.17. Identification of Transplant Injury Specific Metabolites in the Kidney Transplant Urine By Metabolomics

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Introduction: Urine metabolomics could identify metabolites as biomarkers for graft rejection and dysfunction and provide clues to graft failure after kidney transplantation.

Method: A total of 340 archived urine from kidney transplant patients were analyzed that included 106 acute rejection (AR), 111 stable graft function (STA), 81 chronic allograft injury (CAI), 22 BKV nephropathy, and 20 non-transplant controls. Metabolomics on urine samples was performed using GC-MS approach. The data was normalized with urine creatinine before analyzing the data for transplant injury specific panel for kidney transplant injury. Data analysis tools such as SAM, PAM, Metaboanalyst, and Ingenuity Pathway Analysis were used for statistical and bioinformatics analysis. A t-test p-value

Results: 152 metabolites were differentially present in AR compared to STA. A panel of 59 metabolites was able to distinguish AR urine (n=70) from STA (n=70) in a training set with 87% sensitivity and 93% specificity. The same panel was able to identify a test set of 36 AR and 41 STA with 92% sensitivity and 93% specificity.

A panel of 8 metabolites was able to distinguish BKVN urine from STA urine in a training set with 14 BKVN and 16 STA urine with 82% sensitivity and 94% specificity. The same panel was tested on a test set of 8 BKVN and 114 others was able to classify BKVN urine with 100% sensitivity and 93% specificity.

Pathway analysis revealed that galactose and aspartate metabolism being the most impacted in AR injury.
Conclusion: Urine metabolomics identified metabolites that can differentiate AR and BKVN in kidney transplantation.

T.66. The Preferential Expression of Donor-HLA Molecules by 'Cross-Dressed' Cells Following Clinical Liver Transplantation is Mediated by Extracellular Vesicles

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Mounting data from animal models suggests that T-cell alloreactivity early after transplantation is triggered by recipients APCs that present intact donor-MHC on their surfaces. These 'cross-dressed'; APCs acquire donor-MHC either by direct cell contact, or via allograft-derived extracellular vesicles (EVs). Our aim is to ascertain the presence and origin of cross-dressed cells in the context of clinical liver transplantation. PBMCs and platelet-poor plasma were collected from liver transplant recipients pre-transplant, and at post-transplant days 1, 4, 10, and 90. Advanced imaging flow cytometry with Amnis'; ImageStream was used to perform multiparametric phenotyping of PBMCs and EVs. The percentage of PBMCs displaying donor-HLA peaked at day 1 post-transplantation, and declined until undetectable at 90 days. In all cases, the majority of cells expressing donor-HLA also expressed recipient-HLA (cross-dressed). CD14⁺, CD16⁺ and CD11c⁺ cells accounted for the majority of cross-dressed cells, while proportions of these subsets expressing only donor-HLA (passenger leukocytes) were significantly lower (P<0.01). EVs expressing donor-HLA peaked at day 1 following transplantation (0.24-9.05% of total small-EVs). Moreover, the percentages of circulating donor EVs was directly associated with increased proportions of cross-dressing (P<0.001). PD-L1 expression among the cross-dressed cells was significantly higher than in recipient cells (only recipient-HLA)(P<0.001). Furthermore, PD-L1 molecules colocalized with donor-HLA on the surfaces of cross-dressed cells indicating EVs origin. Therefore, our data suggest that the kinetics of EV release in the early post-operative period are associated with the appearance of cross-dressed cells, and their enrichment on PD-L1 could modulate the allograft-targeted immune responses in clinical liver transplantation.

T.72. Endothelial Expression of MHC Class I is Necessary for Transfusion-Related Acute Lung Injury

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Transfusion-related acute lung injury (TRALI) is the most common cause of death from blood transfusion therapy. In a mouse model of TRALI based on MHC Class I (MHC I) mAb (H-2K^d) challenge, the critical site of cognate antigen expression has not been elucidated. We generated conditional MHC I knockout mice by crossing floxed beta2 microglobulin mice (*B2mFlox*) to a congenic H-2K^d expressing C57Bl/6 mouse (*C-H2^d*) and to cell-specific Cre strains to delete MHC I in myeloid cells (*LysM-Cre*), endothelial cells (*VECadherin-CreERT2*) and platelets (*PF4-Cre*). We found that *C-H2^d* mice are fully susceptible to TRALI with no difference in lung injury compared to BALB/c wild-type controls. In *C-H2^d x B2mFlox x PF4-Cre* mice, MHC I is efficiently deleted from platelets and these mice are fully susceptible to TRALI compared to *C-H2^d x B2mFlox* controls. *C-H2^d x B2mFlox x LysMCre* mice efficiently delete MHC I in neutrophils but not monocytes. *C-H2^d x B2mFlox x LysMCre* mice have no differences in lung injury compared to controls. MHC I is resistant to deletion in *C-H2^d x B2mFlox x VECadherinCreERT2* adult mice dosed with tamoxifen, however neonatal tamoxifen administration reduces lung endothelial MHC I expression to <25% of control, which protects mice from lung injury. In conclusion, TRALI results from cognate antibody recognition of endothelial MHC I. The vast endothelial surface area of the lung and the first-pass of transfused blood products through the lung may explain the pulmonary-restricted organ injury after transfused cognate antibody.

W.34. Tr1 Cell Immunotherapy Promotes Transplant Tolerance via *De Novo* Tr1 Cell Induction and is Safe and Effective During Acute Viral Infection

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Tr1 cell therapy is considered an emerging approach to improve transplant tolerance and enhance allogeneic graft survival. However, it remains unclear how Tr1 cells promote transplant tolerance and whether they will be safe and stable in the face of an acute viral infection. By employing a mouse model of pancreatic islet transplantation, we report that Tr1 cells promoted transplant tolerance via *de novo* induction of Tr1 cells in the recipients. Acute viral infection with lymphocytic choriomeningitis virus (LCMV) had no impact on Tr1 cell number and function, neither on the Tr1 cells infused nor on those induced, and that was reflected in the robust maintenance of the graft. Moreover, Tr1 cell immunotherapy had no detrimental effect on CD8 and CD4 anti-LCMV effector T cell responses and viral control. Together, these data suggest that Tr1 cells did not convert to effector cells during acute infection with LCMV, maintained transplant tolerance and did not inhibit antiviral immunity.

W.44. Identification of Leukocyte Subpopulations as Potential Biomarkers of Long-Term Survival with Normal Allograft Function After Lung Transplantation

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Background: Long-term survival after lung transplantation (LT) is limited by the development of chronic lung allograft dysfunction (CLAD). Despite this fact, a small number of lung transplant recipients are long-term survivors with a good allograft function (LTS). The study of this particular population could be a first step to search for transplant tolerance biomarkers that can lead to the reduction of immunosuppressive drugs in treatment plans and improve personalized medicine. The objective of this study was to identify leukocyte subpopulations as potential tolerance biomarkers in LTS after lung transplantation.

Methods: Sixty-two double lung transplant recipients were included in this multicenter cross-sectional study: 31 patients with CLAD and 31 patients with stable lung allograft function after 10 years from LT (LTS). Six leukocyte profiling panels computing 4 to 10 markers were used for whole blood leukocyte subset profiling by flow cytometry. The percentages of the different leukocyte subpopulations were compared between both groups.

Results: LTS patients showed significant increases in CD14^{high}CD16⁻ monocytes, CD56⁺CD16⁻ NK cells, CD4-CD8- $\alpha\beta$ T cell subset and CD62L⁺ granulocytes (p-values of 0.046, 0.02, 0.002 and 0.007 respectively). Regarding CLAD group, CD14^{high}CD16⁺ monocytes, CD56⁺CD16⁺ NK cells and V δ 1⁺ $\gamma\delta$ T cell subpopulation were significantly elevated (p-values of 0.009, 0.004 and 0.027 respectively). There were no significant differences in the frequencies of regulatory T cells and B cell subpopulations between the LTS and CLAD groups.

Conclusion: These results suggest that CD14^{high}CD16^{+/-} monocytes, CD56⁺CD16^{+/-} NK cells, CD4-CD8- $\alpha\beta$ and V δ 1⁺ $\gamma\delta$ T cell subpopulations could be potential biomarkers of long term allograft survival in lung transplant patients.

Other

T.88. Fatty Acid Binding Protein 5 is Required for Treg Metabolism and Function

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Regulatory T cells are critical for maintaining immune homeostasis and are intimately involved in immunological disorders and cancer. In contrast to CD4 T helper subsets, which primarily rely on aerobic glycolysis for proliferation and function, regulatory T cells rely on oxidative metabolism, fuelled by lipids. Central to Treg activation are changes in lipid metabolic pathways, permitting the acquisition of free lipids to support both biosynthetic reactions and the ligation of intracellular receptors. Owing to their hydrophobic nature, lipid chaperones are required to facilitate the intracellular trafficking of lipids. Fatty acid binding proteins (FABPs) are a class of lipid chaperones, with FABP5 highly expressed in T cells. Acute pharmacological inhibition of FABP5 results in impaired lipid saturation and subsequently decreased basal metabolism in Tregs, with a subsequent loss of spare respiratory capacity as a result of disruption of mitochondrial cristae structure and loss of ETC integrity. Interestingly, these cells had increased suppressive capacity. Mitochondrial damage resulted in the induction of type 1 interferon signaling and increased expression of the IL2 receptor subunit α (IL2R α /CD25). *In vivo* blockade of FABP5 reversed disease progression in EAE, through modulation of Treg metabolism. The link between type 1 interferon induction and increased Treg suppression may provide mechanistic insight into interferon therapy for MS patients.

Reproductive immunology

F.40. IgA Antiphospholipid Antibodies: Exploring Their Role in the Diagnosis of Antiphospholipid Syndrome in Recurrent Reproductive Failure Patients.

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Background

IgA antiphospholipid antibodies (aPL) are not currently included in the criteria for the Antiphospholipid Syndrome (APS) classification. Several studies have pointed out the potential role of IgA-aPL in the pathophysiology of APS (thrombocytopenia, thrombosis), especially on seronegative and SLE patients. This role has also been reported in pregnancy morbidity.

Objective:

To assess the role of IgA anti-cardiolipin and IgA anti- β 2-glycoprotein-I aPL in recurrent reproductive failure (RRF) patients and to correlate it with the presence of IgG and IgM-aPL.

Methods

Sixty-two patients, mean age 36.08 ± 4.58 (recurrent miscarriage $n=47$, 75.81%; foetal death, $n=9$, 14.52%; recurrent implantation failure $n=14$, 22.58%) and 30 healthy controls, mean age 32.2 ± 4.49 (with proven fertility) were tested for IgA, IgG and IgM anti-cardiolipin and anti- β 2glycoprotein-I (Bioplex® 2200, Bio-Rad).

Results:

Global incidence of IgA-aPL in our cohort was low ($n=4$, 6.78%). 13 out of 59 patients have IgG or IgM-aPL positive (22.03%). Only two of them were positive for IgA-aPL (3.39%), while two had only IgA positive aPL without IgG or IgM (3.39%). We observed a strong correlation between β 2GPI-IgA with β 2GPI-IgG (0,91), ACA-IgG (0,91) and with ACA-IgA (0,95). The correlation was low between β 2GPI-IgA with ACA-IgM (0,19) and β 2GPI-IgM (0,23).

Conclusion:

In our cohort of 62 RRF patients, IgA-aPL helped to discriminate 6.78% of seronegative APS patients, which has relevant clinical implications. A strong correlation between IgA and IgG-aPL antibodies was observed. We suggest further investigation using a bigger RRF cohort according to classical aPL to warrant these observations.

T.12. Helios Drives Preferential Regulatory T Cell Differentiation in Fetal Naïve T Cells

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Human fetal CD4⁺ naïve T cells are primed to differentiate into CD25^{hi}FOXP3^{hi} regulatory T (Treg) cells upon T cell receptor (TCR) stimulation without TGF β supplementation, thus contributing to the generation of immunotolerance in the developing human fetus. In addition to expression of the lineage-determining factor *FOXP3*, commitment to the Treg cell fate is preceded by the acquisition of permissive epigenetic modifications at Treg-specific enhancers associated with the transcriptional control of Treg signature genes. Through ATACseq (Assay for Transposase-Accessible Chromatin Sequencing) and H3K27ac ChIPseq (Chromatin Immunoprecipitation Sequencing), we reveal that this intrinsic predisposition for Treg differentiation in fetal naïve T cells is correlated with increased chromatin accessibility and H3K27ac enrichment at 906 and 109 Treg-specific enhancers respectively. We show by RNA sequencing that fetal naïve T cells subsequently have increased transcription of the underlying Treg-associated genes such as Helios (*IKZF2*). Using flow cytometry, we confirmed that Helios expression is higher in fetal naïve T cells relative to adult naïve T cells. CRISPR (clustered regular interspaced short palindromic repeats)-Cas9 (CRISPR-associated protein 9) mediated

knockdown of Helios expression in primary human fetal naïve T cells reduced induction of CD25^{hi}FOXP3^{hi} Treg cells in response to TCR stimulation alone, indicating that high baseline Helios expression contributes to the priming of Treg differentiation in fetal naïve T cells. Overall, a novel Treg-associated epigenome and transcriptome within fetal naïve T cells establishes preferential Treg differentiation as a mechanism to maintain immunotolerance *in utero*.

T.24. Meta-Analysis of Maternal and Fetal Transcriptomic Data Elucidates the Role of Adaptive and Innate Immunity in Preterm Birth

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Preterm birth (PTB) is the leading cause of newborn deaths around the world. Spontaneous preterm birth (sPTB) accounts for two-thirds of all preterm births; however, there remains an unmet need of detecting and preventing sPTB. Although the dysregulation of the immune system has been implicated in various studies, small sizes and irreproducibility of results have limited identification of its role. Here, we present a cross-study meta-analysis to evaluate genome-wide differential gene expression signals in sPTB. A comprehensive search of the NIH genomic database for studies related to sPTB with maternal whole blood samples resulted in data from three separate studies consisting of 339 samples. After aggregating and normalizing these transcriptomic datasets and performing a meta-analysis, we identified 210 genes that were differentially expressed in sPTB relative to term birth. These genes were enriched in immune related pathways, showing upregulation of innate immunity and downregulation of adaptive immunity in women who delivered preterm. An additional analysis found several of these differentially expressed at mid-gestation, suggesting their potential to be clinically relevant biomarkers. Furthermore, a complementary analysis identified 473 genes differentially expressed in preterm cord blood samples. However, these genes demonstrated downregulation of the innate immune system, a stark contrast to findings using maternal blood samples. These immune related findings were further confirmed by cell deconvolution as well as upstream transcription and cytokine regulation analyses. Overall, this study identified a strong immune signature related to sPTB as well as several potential biomarkers that could be translated to clinical use.

T.42. Prevalence of Antiphospholipid Antibodies Among Groups of Reproductive Failure. Association with Natural Killer Cells and Another Immunological Alterations.

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Classification criteria of obstetrical antiphospholipid syndrome (OAPS) were settled in 2006, before significant changes in laboratory diagnosis were done.

Objectives: To describe the incidence of low-titer antiphospholipid antibodies (aPL) in different groups of reproductive failure; and the correlation with expanded natural killer (NK)/NKT cells.

Methods: A retrospective observational study in 651 consecutively studied women (273 recurrent miscarriages, RM, 87 fetal death, FD, 264 recurrent implantation failure, RIF, 27 sterility, St) was carried out. Anticardiolipin (aCL) and anti-beta-2-glycoprotein I (ab2GP) antibodies were measured by Multiplex (Bioplex, BioRad Lab, USA) and lupus anticoagulant; blood NK cells (cytotoxic CD3⁺CD56⁺CD16⁺, cytNK) and NKT-like (CD3⁺CD56⁺) proportions by flow-

cytometry (BD, San José CA). Cut-offs levels of aPLs were defined as negative (0-5), low (5-20) and moderate/high positive (20->80).

Results: Median age of RRF women was 38±4 years-old without differences among groups. Low aPL were detected in 122 (46.2%) of RM; 111 (43.7%) RIF; 41 (48.2%) FD; 12 (44.4%) St women, without differences among groups. Moderate-high aPL were detected in 29 (11.0%) RM; 29 (11.0%) RIF; 1 (3.7%) FD; and 4 (4.7%) Ste, without differences. 56 (38.6%) of women showed expanded cytNK and 19 (35.8%) expanded NKT with low aPL; while 14 (9.7%) and 7 (13.2%) with moderate/high aPL, respectively

Conclusions: aPL were the most common immunological finding in all reproductive failure groups, which urges the revision of current classification criteria. Expanded cytNK or NKT cells were associated with aPL antibodies. Low aPL increase 45.4% the proportion of patients that could benefit of prophylactic antithrombotic strategy.

W.7. Peripheral Blood Cell MicroRNA Predicts Preeclampsia and Preterm Birth when Collected Early in the First Trimester of Pregnancy

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Major disorders of pregnancy that include preeclampsia and preterm pose a significant risk to both maternal and infant health and cost billions of dollars annually. The absence of an early and precise screening test is a major obstacle to adequate treatment and prevention. Previous studies directed to quantification of plasma biomarkers fail to predict pregnancy outcome with high precision in the first trimester. MicroRNA is a class of regulatory RNAs that are involved in a wide range of cellular activities. We quantified a panel of peripheral blood cell microRNAs drawn from pregnant women at five weeks gestation through to the end of the first trimester of pregnancy in a series of clinical studies. Area under the ROC curves for pregnancy outcome prediction were calculated and ranged from 0.91 for preeclampsia to 0.95 for preterm birth. Our test is the first to use microRNA in peripheral blood cells to successfully predict placental bed disorders with high sensitivity and specificity. Peripheral blood microRNA may offer hope for early pregnancy screening and disease prevention.

W.26. AIRE Supports Maternal-fetal Tolerance During Early Pregnancy

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Numerous mechanisms prevent maternal-fetal immune conflict during healthy pregnancy. Autoimmune regulator gene (AIRE) is a crucial component of tolerance to self-antigens, but its relevance for maternal-fetal tolerance has not yet been explored. AIRE contributes to both central and peripheral tolerance via the presentation of tissue specific antigens (TSAs), leading to the clonal deletion of self-reactive T-cells. These TSAs include placental antigens, which we call “distant self” antigens, since they are encoded in the maternal genome, but have not been expressed since the mother was herself an embryo with a placenta. We hypothesize that maternal tolerance of “distant-self” TSAs are critical for successful pregnancy. Using RNAscope, we found AIRE transcripts in the uterine draining lymph nodes (udLNs) and significantly more AIRE expression in udLNs of pregnant vs. nonpregnant littermates ($p=0.01$). To test AIRE function during pregnancy, we used AIRE-diphtheria toxin receptor (DTR) transgenic mice to ablate AIRE-expressing cells during the first nine days of pregnancy. We found smaller litter sizes ($p<0.0001$) and smaller embryos ($p=0.005$) in a subset of AIRE-DTR pregnancies (30% of $N=10$), compared to wildtype (WT) pregnancies ($N=11$). Flow cytometry showed AIRE-DTR pregnancies had more CD4⁺CD25⁺ T-effectors and fewer FoxP3⁺ T-regs in the uterus, lymph nodes, and peripheral blood ($p<0.05$). AIRE-DTR mice were less likely to be pregnant at E9.5 after plug (67% AIRE-DTR vs 92% WT),

suggesting decreased fertility or early pregnancy loss. Thus, AIRE-expressing cells may provide an additional mechanism of maternal-fetal tolerance by deleting reactive maternal T-cells and promoting maternal T-reg differentiation at the maternal-fetal interface.

W.51. Integrative Analysis of miRNA and mRNA Sequencing in Spontaneous Preterm Birth

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Spontaneous preterm birth (sPTB) is a major complication of pregnancy and a leading cause of morbidity and mortality in neonates. Although previous studies have examined transcriptomic differences that characterize preterm and term labor neonates, most studies have focused on mRNA expression. Here, we integrate cell-free miRNA sequencing from cord blood plasma from 7 sPTB and 10 term infants with a publically available mRNA sequencing dataset from cord blood of 12 sPTB and 12 term infants (GSE73685). From the publicly available mRNA data, we identified 473 genes that were differentially expressed ($|\log_2(\text{FC})| > 1.3$ and $\text{FDR} < 0.05$) in sPTB relative to term birth with downregulation of genes related to neutrophil degranulation, TLR signaling, NK-kB signaling, and Fcγ mediated phagocytosis, suggesting a dysregulation of the innate immune system in the fetus. Upon examining miRNA expression, we found 14 miRNAs that were differentially expressed in sPTB relative to term birth ($\text{FDR} < 0.05$). Of these 14 miRNAs, three miRNAs (miR-26b, miR-148b, miR-149) had experimentally validated target genes which were enriched in the set of 473 differentially expressed genes ($\text{FDR} < 0.05$). Gene ontology analysis revealed that enriched target genes of these three miRNAs were related to neutrophil degranulation ($\text{FDR} < 0.01$). This analysis suggests that dysregulated neutrophil degranulation may play a role in sPTB with changes in both mRNA expression as well as the upstream miRNA regulators. By integrating different types of molecular measurements, we identify a strong fetal innate immune signature related to sPTB.

W.59. Activation and Expansion of CD8⁺ T cells in Cord Blood and in the Maternal-fetal Interphase During Preterm Labor

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Preterm labor (PTL) is associated with disturbance of maternal-fetal tolerance of the semi-allogeneic fetus. We have recently reported that fetal T cell responses against maternal antigens contribute to the pathophysiology of PTL. We now examine T cell profiles in cord blood and in the decidua (the maternal tissue adjacent to the placenta) in patients with PTL.

We performed RNA-seq of sorted naïve (CD45RA⁺CCR7⁺) CD8⁺ cord blood T cells from term (N=5) and preterm (N=5) infants and found 198 upregulated and 38 downregulated genes between these groups ($\text{FDR} < 0.1$). The most highly upregulated genes in preterm T cells ($\log_2$ fold change > 4) were related to pathways involved in CD8 chemoattraction (CCL3, CCL4) and activation (XCL2), while also implicating pathways involving NK cell activity (Granzyme H, killer cell immunoglobulin-like receptor, killer cell lectin-like receptor). We investigated the immune repertoire composition by applying MIXCR to the RNA-seq data and found a significantly higher ratio of gamma/delta to alpha/beta TCR expression in preterm infants compared to term controls ($p < 0.1$). Consistent with the finding of fetal T cell activation, we found an increased frequency of maternal decidual CD8⁺ T cells (64.2 ± 11.8 , N=2) in PTL when compared to term controls (25.1 ± 3.1 , N=4), suggesting parallel changes on the maternal side during PTL.

These results suggest that the numerous mechanisms that usually prevent T cell activation (such as decidual chemokine

gene silencing) may be perturbed during preterm labor, ultimately resulting in a breakdown of maternal-fetal tolerance by both maternal and fetal T cells.

Therapeutics/pharmacology

F.10. Identification of Potent RORc Inhibitors for the Treatment of IL-17-mediated Inflammatory Diseases: Is it Worth the Risk?

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The retinoic acid-related orphan receptor gamma (RORc) is a nuclear hormone receptor that is essential for the expression of IL-17. Elevated IL-17 has been associated with inflammatory diseases, including rheumatoid arthritis and psoriasis. RORc has thus generated considerable interest as a drug target. We have developed potent and highly selective RORc inverse agonists, including the clinical candidate GDC-0022 and a chemically diverse back-up compound with attractive *in vitro* pharmacology properties.

These molecules inhibited IL-17A production from various human and murine cellular sources, such as Th17, gdT and MAIT cells, as well as the expression of other RORc-dependent genes. Compound treatment also inhibited Th17 differentiation in mouse and human. In a mouse model of collagen-induced arthritis, dosing with RORc inverse agonists resulted in significantly reduced clinical scores and IL-17A production.

However, RORc-deficient mice are known to exhibit massive thymocyte apoptosis due to the downregulation of the survival factor Bcl-2. Excessive thymocyte proliferation is observed as well, and the mice eventually succumb to thymic lymphoma. The precise mechanistic link between thymocyte apoptosis, proliferation and lymphoma development in RORc-deficient mice and its potential translation into other species is largely unclear. We have inducibly deleted RORc in adult mice and observed the development of thymic lymphomas within 4 months following tamoxifen-treatment. More importantly, we describe both apoptosis as well as, for the first time, excessive thymocyte proliferation in monkeys treated with GDC-0022. These observations and related safety concerns strongly caution against the development of RORc inverse agonist for treatment of inflammatory disorders.

F.87. *In Vitro* and *In Vivo* Pharmacodynamic Profile of DV281, a TLR9 Agonist, in Development as an Inhalational Therapeutic for Lung Cancer

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To support the development of the CpG-motif-containing oligodeoxynucleotide toll-like receptor (TLR) 9-agonist DV281 as an inhalational immunotherapeutic in combination regimens for lung malignancies, we performed pharmacological studies *in vitro* and *in vivo* in mice and non-human primates. DV281 induced dose-dependent secretion of IFN- α from human and cynomolgus PBMCs, IL-6 from human B cells, and TLR9-dependent IL-6 from mouse splenocytes. Intranasal administration every 2 weeks (4 doses) of DV281 to naïve mice induced bronchoalveolar lavage (BALF) cytokines and cell infiltration into the lungs, as quantified 24 hours post 4th dose. These responses were reversible as demonstrated by absence of BALF cytokines and attenuated lung histopathological scoring two weeks after the last dose. In addition, lung responses to DV281 were strictly TLR9-dependent as BALF cytokines, histological changes, and dosing-related effects on body weight were absent in TLR9^{-/-} mice. To bridge these findings to humans, we measured responses to DV281 delivered by nebulization to non-human primates. Cynomolgus macaques responded

to single escalating doses of aerosolized DV281 (10-100 mcg/kg) with dose-dependent IFN-inducible genes and IFN- α protein in the BALF, indicating that DV281 was pharmacologically active in monkey lungs. To evaluate whether combining DV281 and anti-PD-1 would exaggerate immune responses, the combination was evaluated in human PBMC *in vitro*. Blocking the PD-1/PD-L1 pathway did not modulate DV281-induced cytokines or B cell proliferation. In summary, these studies indicate that DV281 potently and specifically stimulates pharmacodynamic responses in rodents and non-human primates, supporting its clinical development as an inhaled therapeutic for lung cancers in combination with anti-PD-1.

F.90. Development of New Hollow Mesoporous Silica Nanoparticles Coated with mAb Trastuzumab to Treat Breast Carcinoma

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Breast carcinoma is the most frequent cancer in women with more than one million new cases reported in the USA each year. Although current therapeutic interventions have increased the 5-year survival rate for women with the disease, there is still an urgent need to develop new strategies to fight breast cancer. Hollow mesoporous silica nanoparticles (HMSNs) are one of the most promising carriers for effective drug delivery due to their large surface area, high volume for drug loading and excellent biocompatibility. For this reason, we previously developed and described hollow mesoporous silica nanoparticles with antibody Trastuzumab (HMSNs-TZ) in its surface. These NPs were then modified with Eudragit (HMSNs-TZ-RL) in order to regulate drug release.

The aim of this work was to investigate the effect of HMSNs-TZ-RL carrying doxorubicin on BT474 spheroids, as an *in vitro* model of human breast carcinoma. First, the intracellular delivery of HMSNs-TZ was evaluated. Spheroids were treated with the NPs (20 μ g/mL) for 6 h. Subsequently, the cells were washed extensively with PBS, fixed in 4% paraformaldehyde, and visualized immediately. We observe the entry of a large number of nanoparticles inside the tumor.

HMSNs-TZ modified on their surface with Eudragit allowed the release of doxorubicin with the change of tumoral pH. Therefore, we evaluated the effect of these NPs on BT474 spheroids. Our results showed a significant decrease in tumoral proliferation when cells were incubated in the presence of HMSNs-TZ-RL. There was also a significant increase in HSP-70 levels together with an increase of apoptotic cells.

F.93. A Synthetic Biology Approach for the Treatment of Cancer and Inflammation

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At Synlogic we apply synthetic biology to non-pathogenic bacteria (*E. coli* Nissle) to develop "Synthetic Biotic Medicines" capable of manipulating multiple pathways relevant for the treatment of cancer and autoimmunity. Our synthetic biology platform allows us to design bacterial strains capable of executing metabolic conversions (production or consumption of metabolites), secretion of proteins (chemokines, cytokines, enzymes) and secretion or display of single-chain Fv (scFv) molecules (to interfere with ligand-receptor interactions).

We have applied this synthetic biology platform to modulate immune responses in the context of cancer and inflammation. In cancer we effectively trigger innate and adaptive immune responses by intratumoral expression of a variety of effectors, such as STING agonists, TNF α and IFN γ , and reverse immunosuppression by consumption of suppressive metabolites. These strains show robust anti-tumor activity in B16F10 and CT26 syngeneic mouse models,

as single agents or in combination with checkpoint inhibitors. In inflammation, we have successfully built strains that produce an array of immunomodulatory metabolites (such as short chain fatty acids and tryptophan derivatives) as well as immunomodulatory cytokines. Given orally, these strains demonstrate robust modulation of immune cellular subsets and inflammation, both locally in the gut as well as systemically.

Taken together, these results establish our synthetic biology-based platform as a robust system for the localized and sustained delivery of immunological payloads to the tumor microenvironment as well as the gut, and support the development of Synthetic Biotic Medicines as a novel approach to treat immune-mediated diseases, both in cancer and inflammation/autoimmunity.

F.98. Fostamatinib, a Spleen Tyrosine Kinase (Syk) Inhibitor, for Treatment of Warm Antibody Autoimmune Hemolytic Anemia (wAIHA): Preliminary Results of the SOAR Phase 2, Multicenter, Open-label Study

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Background: Syk signaling is implicated in macrophage destruction of red blood cells in patients with wAIHA. In animal studies, the Syk inhibitor fostamatinib protected against antibody-induced anemia.

Methods: Eligibility criteria included primary/secondary wAIHA, ≥ 1 failed prior therapy, hemoglobin (Hgb) < 10 g/dL, IgG⁺ direct antiglobulin test, haptoglobin < 10 mg/dL, lactate dehydrogenase $> \text{ULN}$. Fostamatinib (150 mg BID orally) was administered for 12 weeks. Primary endpoint: Hgb > 10 g/dL and 2 g/dL increase from baseline by Week 12 without rescue medication. Using Simon two-stage design, achievement of primary endpoint in ≥ 4 patients during Stage 1 ($n=17$) enabled opening Stage 2 ($n=20$). Stage 1 results are presented.

Results: 19 patients enrolled (63% female; 79% white; median age 60y [range, 27-88], median 2y duration of wAIHA). Median Hgb at baseline: 9.1 g/dL (range, 6.8-9.9). Six of 17 (35%) evaluable patients met the primary endpoint, allowing opening of Stage 2. Four of the 6 subjects met the response criteria within 2 weeks; by week 4, responders¹; median Hgb increase: > 3.0 g/dL; median duration of response: > 9 weeks (range 1 to > 9 weeks). Three additional patients who continued fostamatinib in an extension study responded after Week 12, for a total response rate of 53%. 18/19 (95%) patients experienced AEs (most common: diarrhea, hypertension). SAEs occurred in 3 (16%) patients: 2 fatal, none related to fostamatinib.

Conclusions: Fostamatinib treatment led to marked increases in Hgb in 53% of the patients with wAIHA in this study. Most responses were rapid and sustained. There were no unexpected safety findings.

F.116. Gene Therapy Trial for Rare, Severe Neuromuscular Disease: Preliminary Efficacy, Safety and Immunological Findings

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X-linked myotubular myopathy (XLMTM) is a rare disease caused by mutations in the *MTM1* gene, characterized by profound muscle weakness, respiratory failure, and early death. ASPIRO is a Phase 1/2, open-label, randomized study to evaluate safety and preliminary efficacy of AT132 (rAAV8-Des-hMTM1), an AAV-mediated, investigational gene therapy product for XLMTM patients. ASPIRO participants are randomized into ascending dose cohorts to receive a single intravenous (IV) administration of AT132 or act as delayed treatment control (DTC; $n=1$ per dose cohort). Participants must be younger than 5years, without AAV8 NAb greater than 1:20.

Treated patients demonstrated considerable improvements in neuromuscular (CHOP-INTEND) and respiratory (maximal inspiratory pressure) co-primary endpoints; airway clearance, secretion management, limb/trunk strength, velocity/accuracy of movement, and ability to vocalize. At time of preliminary data cut, patient data (n=3 treated, n=1 DTC) included a total of six AEs. Three AEs were deemed probably or possibly related to drug, and two were deemed serious AEs (SAEs), both of which occurred in Patient 3, who responded to immunosuppression and supportive care.

Previous gene therapy studies have documented T-cell-mediated hepatic inflammation and adverse events (AEs) that are immunological in nature. Therefore, the ASPIRO study design includes prophylactic prednisolone immunosuppression (beginning 1-day prior to AT132 dosing, continuing for 15-weeks), and regular safety monitoring for potential T-cell-mediated responses. To further characterize potential immunogenicity of AT132 and of AAV gene therapies in general, comprehensive safety labs include collection ALT, AST, CRP, cK, troponin, and antibody levels (MTM1-protein and AAV-capsid). Updated data will be shared at FOCIS 2018.

T.1. Monocyte NOTCH2 Expression Predicts Interferon-beta Immunogenicity in Multiple Sclerosis Patients

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Multiple sclerosis (MS) is a multifocal demyelinating disease of the CNS affecting about 2.5 million people worldwide. In the last 25 years many disease-modifying drugs (DMD) have become available for patients with MS, which aim to prevent rather than repair tissue injury. The biopharmaceutical IFN- β is a well established treatment for the relapsing remitting form of MS (RRMS), however, between 5 and 30% of IFN- β -treated patients develop neutralizing ADA (nADA) leading to reduced drug efficacy. Mechanisms driving anti-drug immunogenicity remain uncertain and reliable biomarkers to predict immunogenicity development are lacking. Here, high-throughput flow cytometry was used to identify a unique phenotypic signature associated with the development of nADA to IFN- β in RRMS patients. NOTCH2 expression on CD14⁺ monocytes and increased frequency of pro-inflammatory monocyte subsets were identified as baseline predictors of nADA development in IFN- β treated RRMS patients. The association of this monocyte profile with nADA development was validated in two independent cross-sectional RRMS patient cohorts and a prospective cohort followed before and after IFN- β administration. Reduced monocyte NOTCH2 expression in nADA⁺ RRMS patients was associated with

NOTCH2 activation measured by increased expression of Notch-responsive genes, polarization of monocytes towards a non-classical phenotype and increased pro-inflammatory IL-6 production. Thus this work has identified a novel mechanism influencing the development of immunogenicity to biopharmaceuticals and a potential biomarker to predict anti-drug responses. Our findings have clear implications for treatment of patients with multiple sclerosis and potentially other patient groups treated with biopharmaceuticals such patients with rheumatoid arthritis or systemic lupus erythematosus.

T.8. Repairing Foxp3 Mutations in Scurfy T Cells Restores Regulatory T Cell Function

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Adoptive cell transfer is a powerful approach to treat various diseases including infectious diseases and certain blood cancers. Emerging genome engineering tools enable direct genetic manipulation of primary immune cells. This opens new therapeutic opportunities for monogenic T cell diseases. We recently reported an efficient protocol for CRISPR-mediated T cell editing. Here, we report the successful correction of two different pathogenic *Foxp3* mutations in primary murine T cells. Both repairing the cause of the scurfy syndrome, a 2bp insertion in *Foxp3*, and repairing the clinically relevant *Foxp3*^{K276X} mutation restored *Foxp3* expression in primary T cells *in vitro*. *Ex vivo* gene-repaired T cells adoptively transferred to lymphodeficient mice survive and expand *in vivo*. We show that the gene-corrected cells are viable, express high levels of *Foxp3*, CD25 and other regulatory T cell (Treg) markers and are responsive to IL-2 treatment upon adoptive transfer *in vivo*. Importantly, repaired Treg cells prevent the development of dermatitis illustrating regained suppressive capacity *in vivo*. These results suggest that gene therapy of T cells might constitute an alternative to gene therapy in hematopoietic stem cells.

T.11. Direct Control of Inflammation in Rheumatoid Disorders with Tolerogenic Nanoparticles

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Treatment with biodegradable tolerogenic nanoparticles carrying rapamycin (synthetic vaccine particles, SVP) and antigen has the unique capacity to induce durable B cell tolerance resistant to multiple immunogenic challenges. Only administration of encapsulated and not free rapamycin can lead to tolerance induction. We have exploited this attribute to induce tolerance to biologic drugs and avoid anti-drug antibody (ADA) responses to coagulation factor VIII, adalimumab (or Humira®), recombinant human acid- α -glucosidase (rhGAA or Myozyme®), adeno-associated viruses (AAV) and the immunotoxin-antibody conjugate LMB-100. Injections of SEL-212, a combination drug for the treatment of gout encompassing SVP (or SEL-110) and a recombinant pegylated uricase (pegsitricase, pegadricase or SEL-037) leads to the establishment of durable immunological tolerance to the biologic. In absence of ADAs SEL-037 conserves its native activity and half-life, and can be repetitively dosed in a preclinical model of hyperuricemia. The anti-inflammatory effects of rapamycin delivered by the SEL-110 nanoparticles were explored. Here we show that SEL-110 administration alone can prevent LPS- and monosodium urate crystals-induced IL-1 β secretion in mice, joint damage in arthritis-prone huTNF α transgenic animals, revert hypergammaglobulinemia, lymphoproliferative disorder and improve overall survival of animals with established lupus disease. The double benefit of lowering uric acid and inhibiting inflammation with SEL-212 is currently being evaluated in a Phase 2 clinical trial in patients with gout.

T.18. A Novel Inhibitor (N5-1) of Interferon Regulatory Factor 5 (IRF5) Ameliorates Disease Severity in NZB/W F1 Mice

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Systemic lupus erythematosus (SLE) is associated with high levels of inflammatory cytokines and autoantibodies. Accumulating evidence in multiple murine models of lupus shows that the interferon regulatory factor 5 (*Irf5*) deficiency ameliorates disease severity. In the current study, we assessed the therapeutic outcome of an inhibitor (N5-1) targeting IRF5 activation in the NZB/W F1 murine model that has not previously been examined for IRF5 function.

N5-1 binds to full-length recombinant IRF5 protein with high affinity (Biacore T200). Western blot confirmed that N5-1 decreases IRF5 nuclear translocation in a dose-dependent manner after LPS stimulation in RAW 264.7 cells. N5-1 blocks R848 induced IL-6 production in Balb/C mice. NZB/W F1 mice received 5 equal doses of 100 µg N5-1 from 8-10 weeks of age. The treatment resulted in significantly reduced proteinuria as lupus disease progressed. At week 20, anti-dsDNA antibody titers were significantly suppressed in N5-1-treated mice. At week 27, while 55% of mice in the control group showed positive antinuclear antibody (ANA) staining on HEp-2 cell, all N5-1-treated mice were negative. Overall survival monitored until 36-weeks age was significantly improved by N5-1 ($p=0.017$). Last, there was no significant change in body weight between groups, suggesting good tolerance of N5-1.

These data support the *in vivo* utility of N5-1 in protecting NZB/W F1 mice from spontaneous lupus disease onset and mortality. To date, this is the first report of a targeted IRF5 inhibitor showing therapeutic efficacy in an *in vivo* model of autoimmune disease, which holds significant promise for patients with SLE.

T.51. Building CRISPR/Cas9-based Platforms for Therapeutic Target Identification and Validation

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The immune system helps to protect the body against invaders that cause infection and disease. On the other hand, disorders of the immune system can lead to autoimmunity, inflammatory disease and cancer. The clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated (Cas) protein 9 system (known as CRISPR/Cas9) is a powerful new technology with a wide range of applications in biomedical research, including the potential to treat human genetic disease. To better understand the immune system and discover novel therapeutics for autoimmune diseases, we have built CRISPR/Cas9-based platforms for new target identification, target exploration and validation, and evaluation of the efficacy and toxicity for early targets. In this work, we will describe the methodologies we have established for delivering the CRISPR/Cas9 system to the cell types of interest, and the *in vitro* and *in vivo* model systems we have built using CRISPR/Cas9 to study gene function. With CRISPR/Cas9-based platforms, we aim to build a mechanism to continuously screen new targets from various data sources and to explore novel biology involved in the immunological diseases.

T.96. Fostamatinib, an Oral Spleen Tyrosine Kinase (Syk) Inhibitor, in Adult Persistent/Chronic Immune Thrombocytopenia (ITP): Safety and Efficacy Results from Patients who Converted from Placebo onto the 049 Open-label Extension Study

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Background: The Syk signaling pathway is important for autoantibody-mediated platelet destruction by macrophages in ITP. In two phase 3, randomized studies in patients with persistent/chronic ITP, the Syk inhibitor fostamatinib produced stable responses (platelet count $\geq 50,000/\mu\text{L}$ at ≥ 4 of 6 visits without rescue therapy weeks 14-24) in 18% of patients vs. 2% on placebo ($P=0.0003$) and produced a 43% platelet response rate with count $\geq 50,000/\mu\text{L}$.

Methods: Placebo patients from the phase 3 studies initiated fostamatinib at 100mg BID PO (increasing to 150mg BID if platelets <50,000/ μ L after 1 month) in an open label extension (OLE) study. Response was prespecified as platelet count $\geq$ 50,000/ μ L within 3 months without rescue therapy in the preceding 28 days.

Results: The OLE study included 44 patients with chronic (93%) or persistent (7%) ITP, a median disease duration of 8.4y, and median age 52y (range, 20-78). Of 44 patients, many heavily pretreated, 19 (43%) achieved responses to fostamatinib, with a median time to response of 29 days. At time of analysis, 11/19 (58%) maintained a platelet response (median count 70,000/ μ L) with estimated median duration not reached at >21 months. The most common AEs were diarrhea (22%), hepatic disorders (17%), and hypertension (16%). Treatment-related AEs occurred in 52% of patients, all mild/moderate and resolving over time. Five (11%) patients discontinued due to AEs. Serious bleeding events occurred in 28% of nonresponders vs none who responded to fostamatinib. Updated results will be presented.

Conclusion: Syk inhibition with fostamatinib represents a promising treatment option for persistent/chronic adult ITP.

T.101. Depletion of Membrane LT α Expressing Cells in Combination with TNF α Neutralization Provides Superior Efficacy in Rheumatoid Arthritis Preclinical Model

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Lymphotoxin alpha (LT α) belongs to tumor necrosis factor (TNF) super family. It can form soluble homotrimer (LT α 3), which binds to TNFR1 and TNFR2; and membrane bound heterotrimer LT α 1 β 2, which binds to LT β R. LT α is expressed by CD4⁺ T helper type 1 (Th1) cells, CD8⁺ cells, natural killer (NK) cells, B cells, and macrophages and its expression is upregulated upon cell activation. TNF α blockers have shown the breakthrough efficacy since their first approval in RA in early 2000. But there are still significant patients who are not responding well to the anti-TNF α treatment.

In the current study, we generated surrogate anti-mouse LT α antibodies with various Fc effector functions which were confirmed by *in vitro* ADCC assay. These variants of anti-LT α mAbs fully maintained their antigen binding and antagonistic properties. In the anti-CD3 induced T cell activation model, both Fc active anti-LT α antibodies decreased LT α expressing CD69⁺ activated T cells, but not Fc dead version, even with the comparable circulating exposure. In the mCIA study, the treatment of Fc enhanced anti-LT α post disease onset significantly decreased disease severity in a dose dependent manner. The efficacy is also dependent on the Fc function, as Fc null version of anti-LT α failed to show any efficacy. More importantly, the combination of low dose Fc enhanced anti-LT α with anti-TNF α demonstrates better efficacy than anti-LT α or anti-TNF α monotherapy.

In conclusion, our study demonstrated that the combination of anti-LT α and anti-TNF α might be a promising approach to achieve the better efficacy in RA patients.

T.112. Antibody-Mediated Protection from an Adenovirus-Vectored RSV Vaccine

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Background: RSV vaccine development is a priority. We previously described GC46.F0, a novel non-human adenovirus engineered to express RSV F0. These studies demonstrated induction of neutralizing antibody and protection against RSV challenge in both upper and lower respiratory tracts of immunized mice and cotton rats. Adenovirus-induced immunity is traditionally thought to be predominantly T cell-mediated. By targeted interference with immune function, we evaluated individual contributions of GC46.F0-induced antibody and T cell-mediated immunity in protection against RSV challenge.

Methods: RSV clearance in GC46.F0-immunized mice was determined at 8 hours to 5 days after challenge. Vaccine-induced T cells were evaluated in mice immunized with GC46.F0 by *in vivo* T cell depletion at challenge. Similarly, antibody-deficient Jh transgenic mice were immunized, then challenged with RSV. Neutralizing antibody, RSV titers, and T cell function were measured in all experiments.

Results: In control mice, GC46.F0 immunization fully protected lungs with partial protection in noses. T cell depletion did not affect GC46.F0-induced protection. However, early after challenge, RSV titers were significantly elevated in lungs and noses of GC46.F0-immunized Jh mice relative to controls. By day 5 post-challenge RSV clearance was delayed in lungs, but not noses, of immunized Jh mice. RSV F-specific CD4 responses, but not CD8, were reduced in GC46.F0-immunized Jh mice.

Conclusions: Vaccine-induced antibodies appeared to be the major protective factor. However, T cells contributed to viral clearance in the absence of antibody. Protection induced by intramuscular immunization with GC46.F0 protected the lung and nose, although protection was more robust in the lung.

T.113. Retrovirus-derived Virus-like Particles as Tolerogenic Vaccines for Food Allergy Treatment

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The rise of immune disorders in the last decades paved the way for the development of new therapeutic approaches such as tolerogenic vaccination. The rationale is to administer allergens in a specific formulation thus reinforcing allergen-specific Th1 and/or regulatory T cell responses. Among the different vector systems used to formulate allergens, we selected and designed a retrovirus-derived Virus-Like Particles (VLP) to carry allergens into the core and display an inhibitory molecule on their surface. A chimeric form of CTLA-4 was used to assure enhanced membrane anchoring onto the VLPs.

Recombinant tolerogenic CTLA-4⁺ VLPs (tVLPs) with ovalbumin (OVA) as model antigen were produced and we investigated their regulatory activity on both human and murine dendritic cells (DCs). We showed that tVLPs downregulate the surface expression of costimulatory molecules (i.e. CD80, CD86) on DCs and induce a pro-tolerogenic cytokine secretion profile. The therapeutic efficacy against allergy was assessed in a murine model of OVA-induced food allergy. BALB/c mice sensitized to OVA were vaccinated with tVLPs and then challenged by OVA *p.o* administrations. tVLP vaccination significantly reduced the clinical signs of allergy and protected against anaphylactic reactions. Notably, we demonstrated that the induced protection is maintained over a 5-months period in vaccinated mice and was dependent on regulatory T cells.

Altogether, our results support the proof of concept for the efficacy of a VLP-based tolerogenic vaccine against food allergy and the characterization of related mechanisms are under investigation.

W.36. Exploration of Autophagic Modulators of A β Production for Inactivating the Targeted Inflammasomes

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Exploration of autophagic modulators of A β production for inactivating the targeted inflammasomes

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Innate immunity and inflammatory response plays an important role in the pathogenesis of Alzheimer's disease (AD). AD is a neurodegenerative condition in which the neuropathological hallmarks are the deposition of amyloid- β (A β) and hyperphosphorylated tau protein coated neurofibrillary tangles. Recently, a number of investigations linking the microglia-mediated activation of NLRP3 inflammasome to AD pathogenesis has emerged and demonstrated that excessive and toxic A β can raise an ignition in NLRP3 inflammasome and eventually induce neuronal damage and AD pathology. Here, we explore a type of autophagic modulators of A β production that can inactivate the targeted inflammasome and would largely protect from memory loss and decrease A β deposition. Thus, targeting A β production can downregulate NLRP3 inflammasome and to be a potential strategy for AD therapy.

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W.68. Antisense Spherical Nucleic Acids Targeting IL-1 β for Inflammatory Diseases

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Spherical nucleic acids (SNAs) are a novel class of therapeutic molecules consisting of densely packed oligonucleotides arranged radially around a spherical nanoparticle core. As a consequence of their 3-dimensional structure, SNAs display increased cellular uptake and avidity for targets compared with the same oligonucleotide sequence as a conventional linear oligo. IL-1 β is a pro-inflammatory cytokine that is a well validated therapeutic target for a number of systemic and local inflammatory diseases. Here, we designed, synthesized, and assessed antisense SNAs targeting IL-1 β mRNA to regulate inflammatory pathways. SNAs exhibited dose-responsive IL-1 β mRNA knockdown up to 85-90%, which was enhanced relative to linear oligos of the same sequences in human foreskin keratinocyte (HFK) cultures. SNAs did not show cytotoxicity and off-target effects in HFK or immunotoxicity in human PBMC cultures. SNA-induced IL-1 β mRNA knockdown was observed as early as 4 hours to longer than 96 hours post-treatment in HFKs. Secondary to IL-1 β mRNA knockdown by SNA, expression levels of IL-6, IL-8, TNF and other inflammatory mRNAs were also reduced, confirming functional knockdown of IL-1 β signaling in HFKs. Epidermolysis bullosa (EB) is a dermatological condition in which elevated IL-1 β levels are implicated in disease maintenance. In dystrophic EB patient-derived fibroblasts, SNA treatment lead to more than 90% reduction of IL-1 β mRNA levels. These data confirm the utility of SNAs for targeting IL-1 β disease pathways and suggest that SNAs have great therapeutic potential for regulating clinically relevant inflammatory diseases.

W.86. Addition of Anti-CD2 to Anti-CD3/CD28-conjugated Nanoparticles Increases Expansion of Human CD4⁺ T Cells and IL-10-transduced CD4⁺ Type 1 Regulatory T Cells

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Type 1 regulatory cells (Tr1) are a promising therapy for the prevention of GvHD in hematopoietic stem cell transplantation due to their ability to promote immunological tolerance in an antigen-specific manner. Tr1 cells also have the unique ability to exert a direct graft versus leukemia effect by lysing myeloid cells, demonstrating an additional therapeutic synergistic effect of Tr1 with other common immunotherapies. We have shown that Tr1 cells can be produced *in vitro* through lentiviral transduction of CD4⁺ T cells with human IL-10 gene (CD4^{IL-10}). These cells are currently expanded using irradiated peripheral blood mononuclear cell (PBMC) from healthy donors. To minimize

variability in CD4^{IL-10} expansion and translate our therapy for clinical applications, we are aiming to convert our expansion protocol towards GMP-compliant anti-CD3 and anti-CD28-conjugated synthetic particles. We are investigating whether microscale (3.5 – 4 μ m) or nanoscale (200 – 500 nm) particles can be used to achieve comparable expansion of CD4^{IL-10} cells while maintaining their suppressive functional characteristics. Given the upregulation of surface CD2 on CD4^{IL-10}, we are also testing whether conjugation of anti-CD2 to anti-CD3 or anti-CD3/28 particles increases expansion or provides distinct costimulatory signaling for Tr1 cells. We demonstrate that addition of anti-CD2 increases efficacy of anti-CD3/28 conjugated nanoparticles, as shown by increased induction of IL-2, IL-4, IL-10 and IFN- γ secretion and CD4⁺ T cell proliferation. Addition of anti-CD2 also induces optimal proliferation of CD4^{IL-10}, warranting further investigation into the optimization of cell expansion parameters for clinical trials with Tr1 cell therapy.

Transplantation

F.9. HCV-induced Heterologous Immunity Regulates Alloreactive T-cell Exhaustion in Liver Transplantation

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Selected liver transplant recipients can discontinue immunosuppression and maintain allograft tolerance despite persistent hepatitis C virus (HCV) infection. This phenomenon is associated with increased HCV-induced CD8⁺ T-cell exhaustion, suggesting that HCV infection might exert beneficial effects by restraining anti-donor responses instead of increasing alloreactivity by heterologous immunity. The current availability of directly-acting anti-viral (DAA) agents offers a unique setting to investigate whether HCV-specific immunity influences donor-specific responses. T-cell lines from HCV-infected liver transplant recipients before and 6 months after DAA treatment were derived from FACS sorted HCV-dextramer positive and negative CD8 T-cells. PBMCs, HCV⁺ and HCV⁻ CD8 T-cell clones were stimulated with K562 cell lines expressing specific HLA molecules to assess the extent of cross-reactivity. Our data revealed for the first time that HCV-specific CD8 T-cells cross-react with allogeneic HLA molecules. In liver transplanted recipients, we detected an enrichment of donor HLA-reactive cells among the HCV⁺ clones compared to 3rd party HLAs. In addition, the increased expression of the exhaustion markers CTLA4 and PD1 in CD8 T-cells significantly correlated with a reduction in donor-specific alloreactivity. HCV clearance resulted in a reduction in the CD8 T-cell exhaustion phenotype. Moreover, the extent of anti-donor HLA alloreactivity increased 6 months after successful DAA treatment. The use of PD1/CTLA4 co-blockade augmented donor-specific responses, but exclusively before viral clearance, indicating that T-cell exhaustion induced by HCV infection regulates T-cell alloreactivity. These findings indicate that the capacity of persistent HCV infection to regulate alloreactive T-cells responses is reversed following viral eradication.

F.24. Durable Mixed Hematopoietic Chimerism Through Megadose Bone Marrow and Regulatory T Cells for the Induction of Immune Tolerance in Non-human Primates Across MHC-barriers

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Transient mixed chimerism (MC) leads to renal allograft tolerance in 60% of Cynomolgus macaques (CM) when the donor kidney (unlike other solid organs) is co-transplanted with MHC-mismatched bone marrow (BM). Infusion of Tregs has prolonged MC and skin graft tolerance in mice. We hypothesize that high BM dose and Treg administration will enhance the duration of donor MC in CM.

CM received total body irradiation (1.25Gyx2), thymic irradiation (7Gy), ATGAM, anti-CD40L and two months of rapamycin. BM ($14.56 \pm 3.7 \times 10^6$ CD34⁺ and $68.8 \pm 9.1 \times 10^6$ CD3⁺ cells/kg) and the first dose of polyclonal recipient Tregs were infused on day 0 or 2. On average, $46.68 \pm 4.2 \times 10^6$ Tregs/kg were delivered between five infusions. Tregs were expanded with artificial APCs and donor PBMCs or CD40L-stimulated-B cells from multiple allogeneic donors.

Five of the Treg-treated animals developed high and long-lasting MC. Among those, the recipients of CD40L-B cell-Tregs achieved full-donor chimerism, which in one was associated with GVHD. The sixth Treg recipient lost chimerism earlier in association with substantial CMV viremia and immune activation. Two of 5 controls lost MC early post-BMT. In an additional control, the MC started to decrease at the time of sacrifice (due to infection) and the other two controls developed full-donor chimerism and GVHD. *In vitro*, Treg-treated animals were donor-hyporesponsive while chimeric.

In conclusion, megadose BMT prolongs MC across MHC barriers, which is further improved by administering Tregs. Two controls and one Treg-treated animal developed GVHD. Further studies should emphasize infusion of high BM doses with lower T cell levels along with increased Treg numbers.

F.25. BPX-501 Cell Infusion After Alpha/Beta T-cell and B-cell Depleted HLA-haploidentical HSCT (haplo-HSCT) in Children with Primary Immunodeficiencies (PIDs): High Rate of Cure with Low Risk of Acute and Chronic GvHD.

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Allogeneic HSCT remains the standard of care for children with PIDs. In this setting, haplo-HSCT was inferior to HLA-matched transplant. Here, we report the outcome of 59 PIDs (42 EU, 17 US) enrolled in a multicenter EU/US prospective trial on alpha/beta T-cell/B-cell depleted haplo-HSCT followed by addback of donor lymphocytes genetically transduced with the iC9 suicide gene (BPX-501 cells). SCID, WAS and CGD (19, 9, 7 patients respectively) represented 60% of diagnoses. The conditioning regimen was mainly Treosulfan-based for EU patients, while US children received a Busulfan-based preparative regimen. As graft rejection prophylaxis, all patients were given rabbit anti-thymocyte globulin (Grafalon/Neovii® EU, Thymoglobuline/Sanofi® US). The median time for PMN and PLT engraftment was 16 and 11 days, respectively. Four children experienced graft failure (3 HLH, 1 CID). Ten patients developed grade II-IV aGvHD (17%). The dimerizing agent AP1903 was administered in 6 patients (2 CRs, 2 PRs, 1 NE, 1 no response). Two of the 57 patients at risk developed cGvHD. 3/59 patients died after transplantation, leading to a 9.6% CI of TRM. At 487 days median f/u, probabilities of OS and DFS for the entire cohort of patients are 89% and 88%. BPX-501 cells infused at a median time of 15 days after HSCT boosted the immune recovery, being the mean number of CD3⁺/BPX-501 cells at 1, 6 and 12 months 123/0.5, 1007/21.7, 1840/21.5/uL, respectively. Our data suggest that selectively T-cell depleted haplo-HSCT followed by BPX-501 infusion represents a highly effective transplantation strategy in PIDs.

F.32. Complement Induces Inflammasome Assembly in IFN-γ-primed Human Endothelium: Mechanisms and Consequences for Allograft Rejection

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Allograft vasculopathy (AV), a major cause of late allograft loss, results from IFN- γ -producing host T cells within the graft vessel wall responding to non-self HLA on graft endothelial cells (ECs). AV progression is exacerbated by binding of complement-activating donor specific antibodies to graft ECs. We modeled this process by binding high titer panel-reactive antibody to cultured human ECs, primed with IFN- γ to reinduce HLA expression, resulting in complement activation. This treatment induced new EC gene expression dependent upon internalization of membrane attack complexes (MAC), stabilization of NF- κ B-inducing kinase (NIK) on MAC⁺ endosomes and activation of non-canonical NF- κ B signaling. MAC activation of IFN- γ -primed ECs increased the CD4⁺ T cell response by activating additional alloreactive T cell clones.

Here we report IFN- γ treatment significantly induces IL-1 β mRNA (≥ 200 fold) and pro-protein and NLRP3 mRNA (~3-5 fold) and protein, assessed by qRT-PCR and immunoblotting, respectively. MAC internalization induces assembly of an NLRP3-ASC-caspase-1 inflammasome, assessed by ASC speck formation using immunofluorescence microscopy or immunoblotting for activated caspase-1. Inflammasome assembly depends on endosome-associated NIK and is not replicated by NIK stabilization in response to LIGHT. Blocking caspase-1 activity or IL-1 receptor binding inhibits subsequent autocrine canonical NF- κ B activation, EC inflammatory gene expression and MAC augmentation of the allogeneic T cell response (≥ 2 fold decrease), but not NIK stabilization or non-canonical NF- κ B activation. These data suggest that IFN- γ -mediated gene induction and MAC-mediated inflammasome activation in ECs combine to intensify the host T cell response to graft ECs by increasing local production of IL-1, thereby potentiating AV.

F.42. Precision Phenotyping of Histologically Stable Kidney Transplants

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Current methods of detection of acute rejection (AR) of allografts are subjective and suffer from a lack of quantitative assays therefore there is substantial variation in histological diagnosis among pathologists. We analyzed histologically stable (hSTA) kidney transplants and identified those that confirm the phenotype molecularly.

We leveraged publicly available gene expression data of kidney allograft biopsies on a total of 3,004 samples across 2929 NCBI GEO datasets, which focused on AR (585 samples), hSTA (1,386 samples), and normal donor (620 samples) allografts. Gene expression analysis (FDR < 0.01, FC > 1.3) revealed 1,732 differentially expressed genes in AR. Hierarchical clustering based on this gene expression signature identified a sizable fraction (30%) of hSTA as molecularly false positives, with highly similar profiles to AR (hSTA/mAR). Next, we utilized xCell, a new tool for cell type enrichment, to identify a unique signature of 27 cell types (B-H adj. p-value < 0.01) that specifically distinguished allografts with AR. Two major cell types were confirmed to drive the major changes in gene expression in AR: CD8⁺ Tem and CD8⁺ Tcm cells (log FC = 37). We then scored each STA sample based on a combination of AR specific expression and cell type enrichment features, which allowed us to more precisely phenotype hSTA allografts into hSTA/mSTA and hSTA/mAR. These results were independently validated on longitudinal cohorts of kidney tx bx.

Our study of precision phenotyping of stable transplants shows the possible causes of discrepancies in allograft phenotyping and may revolutionize the way kidney biopsies are evaluated.

F.110. Potential for Immunity and Tolerance After Bilateral Orthotopic Lung Transplant (BOLT) in Tandem with a CD3⁺/CD19⁺ Depleted Vertebral Bone Marrow Transplant (BOLT+BMT) from 1 of 8 HLA-Matched Cadaveric Donors

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Primary immunodeficiency patients may develop pulmonary complications & most are ineligible for either lung transplant or BMT. We report on our first 2 subjects receiving tandem BOLT& BMT deceased UNOS donors (*Clinicaltrials.gov* NCT01852370) **offering a chance for survival with potential for tolerance.**

Case 1: A 14y/o girl with IL-7R SCID & recurrent pneumonia underwent BOLT. Marrow suspension prepared from T11-L4 VB was CD3⁺/CD19⁺depleted and frozen with ~20x fewer cells from the ileum. At 3m post-BOLT BMT conditioning commenced. She engrafted with 100%donor cells. Autologous CD3⁺ and myeloid recovery prompted DLI ~10w later (5E4 T cells/kg) resulting in >95% donor T cell chimerism and stable mixed myeloid (10%). Lymphs and DC subsets in BAL exhibited different kinetics. T/B cells, TCR/BCR repertoires, & sjTREC exceeded pre-BMT values by 3-6m. Mild skin GVHD post-DLI cleared in 2 weeks. She is off ISD x 11 months without rejection or GVHD. At 18m post-BMT (2m post-ISD withdrawal), circulating T cells (donor) were unresponsive to host DC. In vitro tolerance was Treg & Tr1 independent.

Case 2: A 38y/o woman with CVID on ECMO underwent BOLT. 14m later after 3 episodes of lung rejection became eligible for BMT. She engrafted by D⁺12 however, high grade adenoviremia necessitated repeated DLI leading to grade 3-4 GVHD.

Case 1 is the first in human to demonstrate 1) durable engraftment, 2) immune competence, and 3) acquisition of tolerance from deceased donor VB marrow 4) matched only at a single MHC allele.

T.23. Reshaping Undesired Immune Reactivity by Regulatory T Cells - Experience from Kidney Transplantation (One Study) and Chronic GvHD (PegTreg)

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Recently, we could demonstrate that only regulatory T cells (nTreg) were able to induce long-term allograft acceptance in an advanced rat kidney transplant model simulating the clinical challenges of donor-specific memory, if combined with T-cell depletion and short-term CNI treatment. Based on these promising data, we developed an effective and robust manufacturing process (worldwide the only protocol starting with 1,000-fold expansion). Humanized models of skin transplantation and GvHD confirmed its high efficacy. We received manufacturing authorization and approval for a First-In-Human phase I/IIa dose-escalating study in kidney LD recipients. The study was part of the European One Study that compared distinct cell therapy protocols and reference group patients, all on the same consensus basal triple-drug immunosuppression and validated monitoring. The 1-yr follow-up of all 12 study patients was finalized. The therapy seems to be safe; most importantly, about 80% of patients could be kept on CNI monotherapy as early as after month 6 suggesting regulatory efficacy despite the increase of circulating Treg was only mild and short. In addition, four children with therapy-resistant chronic GvHD after allogeneic HSCT were treated on individual case basis by stem cell donor-derived nTreg product with impressive data on clinical signs of GvHD and support of engraftment, showing for first time also potency of nTreg to reverse lasting immune pathogenesis. Despite promising data, limited survival of Treg pushed us to generate a 2nd-generation of nTreg resistant to immunosuppressive drugs to prolong survival and function in immunosuppressed patients.

T.32. Ex-vivo Expansion of Regulatory T Cells from Long-term Belatacept-Treated Kidney Transplant Patients Restores Both Their Phenotype and *In Vitro* Suppressive Function

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Regulatory T cells (Tregs) are important in establishing peripheral tolerance and have been shown to prevent or delay graft rejection in animal models. We recently reported that Tregs cells from long-term Belatacept-treated kidney transplant patients displayed an altered phenotype compared to Tregs from controls but remains unknown whether their Tregs can be expanded for use in immunotherapy. In the present work, Tregs cells from Belatacept-treated were expanded *in vitro* with anti-CD3/anti-CD28-coated beads in the presence of rapamycin and IL-2. After four weeks of expansion, no significant differences in fold expansion was found between Tregs cells from patients compared to healthy controls. In addition, Tregs from Belatacept patients recovered the normal expression of typical Treg markers including FOXP3, CD25, CTLA4, Helios and CCR7 were similarly to expanded Tregs from control individuals. Moreover, expanded Tregs from patients displayed strong *in vitro* suppressive activity comparable to Tregs expanded from normal controls. Most importantly, Tregs from patients maintained their phenotype and function in the presence of the pro-inflammatory cytokines IFN- γ , IL-6, TNF α and IL-4. In conclusion, Tregs cells from long-term Belatacept-treated kidney transplant patients can be expanded *ex vivo* without loss of their phenotype and *in vitro* function. These data demonstrate that despite the reported alterations of Tregs from transplanted patients maintained long-term with immunosuppressive therapy, it is possible *ex vivo* expand their Tregs cells with therapeutic potential for induction of allograft tolerance.

T.50. CRISPR/Cas9-Mediated Ablation of Class I and II Major Histocompatibility Complex Antigen Expression on Human Endothelial Cells

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Human cord blood derived endothelial cells (ECs) retain the capacity to self-organize into vessels and have replicative potential to undergo clonal expansion for use in tissue engineering. Like other human ECs, these cells express both class I and II MHC and costimulatory molecules that initiate rejection by direct presentation to host alloreactive T effector memory cells. We performed CRISPR/Cas9-mediated biallelic ablation of both β 2-microglobulin and CIITA in these ECs to eliminate class I and II MHC expression. β 2-microglobulin^{null}/CIITA^{null} cells retain junctional expression of CD31 and CD144, normal TNF- α -dependent upregulation of E-selectin and ICAM-1, and form perfused vessels when embedded in a collagen gel and implanted into an immunodeficient mouse. RNA sequencing of β 2-microglobulin^{null}/CIITA^{null} cells did not reveal significant changes in expression of genes unrelated to MHC molecule expression. β 2-microglobulin^{null}/CIITA^{null} EC clones show diminished capacity to induce proliferation of allogeneic CD8⁺ (3.4% WT vs. 1.3% β 2M^{null}/CIITA^{null} CFSE^{low} cells, β 2M^{null}/CIITA^{null} CFSE^{low} cells, effector memory T cells *in vitro* without enabling activation of natural killer cells (TNF- α (pg/mL): 0.0 WT vs. 0.0 β 2M^{null}/CIITA^{null} vs. 20.4 K562 cells). β 2-microglobulin^{null}/CIITA^{null} ECs showed marked reductions in antibody binding and complement activation when incubated with high titer panel reactive antibody from allosensitized patients (IgG cMFI: 26235 $\pm$ 1644 WT vs. 2008 $\pm$ 131 β 2M^{null}/CIITA^{null}, C4d cMFI: 817 $\pm$ 106 WT vs. 72 $\pm$ 12 β 2M^{null}/CIITA^{null}, p<0.01). These data suggest that tissue

engineered constructs lacking professional APCs and perfused through vessels lined by allogeneic ECs lacking MHC molecules will be significantly less prone to rejection.

W.10. Multiplexed Immunofluorescence to Investigate the Immune Response to BK Virus in Kidney Transplant Recipients

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Background: BK virus-associated nephropathy (BKVN) is a significant complication in renal transplantation that leads to allograft dysfunction or failure. We have used a novel multiplexed immunofluorescence technique to define spatial relationships and colocalization among immune cell phenotypes within the kidney microenvironment. We hypothesized that there will be a distinct signature of immune cell infiltrates in BKVN in transplant recipients.

Methods: Co-Detection by inDEXing (CODEX) employs DNA barcoded antibodies to enable high parameter profiling of tissues using a single stain, multiple image approach. We performed CODEX on BKVN positive and BKVN negative kidney tissue using an immune panel consisting of 40 markers including SV40 large T antigen to identify BKV, immune cell subsets (T cells, B cells, NK cells, macrophages, plasma cells, neutrophils) and structural markers (blood vessels, renal tubules).

Results: CODEX is able to identify structural features of the kidney, such as renal epithelial cells, tubules, blood vessels, and glomeruli. SV40 Large T antigen expressing epithelial cells are detectable in BKVN positive samples. Dense lymphocytic infiltrate consisting of CD4 and CD8 T cells, some of which co-express CD45RA. Interestingly, few NK cells or dendritic cells were present.

Conclusions: Our preliminary studies show promising data that CODEX can identify important spatial relationships and phenotypic characterization of immune cells in BKVN. Further studies using CODEX are needed to characterize the immune signature of the various stages in BKVN. These findings can identify potential immune cells of interest and cellular markers that can translate to the design of future diagnostic and therapeutic approaches in BKVN.

W.50. Generation of Human/Pig Hybrid Thymus to Achieve Immune Tolerance to Pig Antigens with Optimal Immune Function

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Thymus transplantation is a promising approach to induce T-cell tolerance for xenotransplantation. Humanized mice generated with human hematopoietic stem cells (HSCs) and swine (SW) thymus grafts (SW/HU mice) are tolerant to human hematopoietic antigens. However, our data suggest that they are not tolerant to human tissue-restricted antigens (TRAs), presumably due to the lack of expression of human TRAs by SW thymic epithelial cells (TECs). This problem and suboptimal positive selection of T-cells recognizing antigens presented by HLA might be overcome by creating a human/pig hybrid thymus. Thymic stromal cells of human fetal (gestational age 20 weeks) and pediatric (4-month old) thymi were injected into freeze/thawed fetal SW thymic tissue and transplanted to NSG mice with human fetal liver CD34⁺ cells. HuTEC-injected SW thymi were functional and supported human thymopoiesis. Human mTECs and cTECs were admixed with pig TECs in hybrid thymi generated by both human fetal and pediatric stromal donors. Peripheral T-cells of mice generated with fetal TEC-injected SW thymi were hyporesponsive to TEC donor-derived DCs. In similar studies using expanded thymus-derived huTECs and thymic mesenchyme cells from a 17-year-old donor, human TECs were detected in long-term SW/HU thymi. In conclusion, injection of human thymic stromal cells into pig thymus can generate a human/pig hybrid thymus. Human TECs from older thymi can be expanded *in vitro* with our protocol and

persist long-term in SW thymic grafts.

W.80. Virus Specific T Lymphocytes Expansion for Adoptive Immunotherapy

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Adoptive immunotherapy with virus-specific T lymphocytes (VST) can prevent immunodeficient patients from virus reactivation or *de novo* infection.

However, this treatment has several limitations, such as HLA-compatibility restriction, high costs and time required in the production of personalised medicines. These limitations can be overcome by the generation of a third-party VST bank through large-scale expansion compliant with Good Manufacturing Practice (GMP) standards.

Cytomegalovirus (CMV) peptides were used to stimulate lymphocytes specifically against these antigens. Readouts after expansion included cell counting, purity, in terms of CD8⁺ and CD4⁺, and specificity (interferon gamma (IFN γ) expression). Moreover, selection of specific T cells initially and further expansion was also tested. These strategies were compared to methods found in the literature that also used peptides for specific stimulation.

Physiological VST stimulus was tested, consisting of CMV pp65 peptide-pulsed autologous dendritic cells (DCs) derived from monocytes. Furthermore, anti-CD3+anti-CD28 antibodies were added in order to enhance VST proliferation. The combination of cytokines IL-2+IL-7+IL-15 in a G-Rex cell culture system resulted in optimum conditions for VST expansion.

The use of DCs for VST activation and the addition of anti-CD3+anti-CD28 in the presence of IL-2 resulted in greater than 40-fold expansion at day 14 and 90% specificity was achieved after a purification step based on Cytokine Capture System (Miltenyi Biotec).

A GMP scalable process and with high values of VST was successfully defined. Quality controls will be established as ELISPOT, cytotoxicity and alloreactivity assays and extensive immunophenotype. A further improvement to the approach would consider specificity for other viruses.

W.95. Clonality and Function of Cytomegalovirus Specific T Cells In Transplant Recipients

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Cytomegalovirus (CMV) reactivation is associated with increased morbidity and mortality in transplant recipients. We previously found that the frequency of CMV-responsive CD8 T cells significantly increased over the first year post-transplant in CMV seropositive (CMV⁺) recipients in the absence of detected viremia. Polyfunctionality has been shown to protect against CMV. The goal of these studies is to determine changes in T cell receptor (TCR) repertoire and functional changes within individual CMV responsive clones. Blood was obtained from healthy volunteers and from heart and kidney transplant recipients pre- and up to one-year post-transplant. Patients received standard of care anti-viral prophylaxis and immunosuppression. Some recipients also received T cell depleting induction therapy. Cryopreserved PBMC were stimulated with CMV IE1 peptides and analyzed for TCR repertoire and cytokine expression using an approach coupling paired single cell TCR $\alpha\beta$ sequencing to gene expression. In two patients, up to 80% of CMV-responsive CD8 T cells are clonally expanded in CMV⁺ transplant recipients. Clones present at day 90 persist until day 360 post-transplantation. IE1-responsive cells are more terminally-differentiated at day 360. Clonally expanded IE1-responsive cells are polyfunctional at day 90, co-expressing cytokines and cytolytic molecules (TNF α , TGF β , granzymeB

and perforin) and the number of functions per cell increases to day 360. In conclusion, CMV-responsive T cells maintain a clonal hierarchy until at least one-year post-transplantation. Despite the absence of CMV viremia, these cells continue to differentiate and increase polyfunctionality during this period. Analysis of these and additional patient samples will be presented.

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