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Allergy/Asthma

OR.50. Exosome Mediated T Helper Cell Polarization in Asthma Patho-progression

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Asthma is a chronic inflammatory disease of the airway affecting over 25 million people in the United States. Recent evidence suggests a role for exosomes, membrane vesicles that shuttle bio-active cargo, to fuel airway hyper-responsiveness by promoting mediators of inflammation. Herein, we describe exosomes-mediated $CD4^+$ T cell polarization and explore the potential for direct activation of these cells. Exosomes were isolated from bronchoalveolar lavage fluid (BALF), validated, quantitated and characterized by transmission electron microscopy, nano-particle tracking, and flow cytometry. Functional analysis was performed by co-culturing exosomes with autologous $CD4^+$ T lymphocytes, including time-lapse confocal imaging of the co-cultures. Exosomes isolated from BALF of mild asthmatic patients displayed a heterogeneous population of particles varying in size, granularity, and surface expression of markers (HLA-DR, CD63, CD54, CD36, and CD303a). Interestingly, CD36 was expressed markedly higher in asthmatic extracellular vesicles than in healthy subjects, and were exclusive to $HLA\text{-}DR^-$ subset of particles. Asthmatic BALF exosomes promoted $CD4^+$ T lymphocyte proliferation and enhanced Th2 and Th17 polarization. Exosomes isolated from $HLA\text{-}DR^+$ subsets of airway myeloid-derived regulatory cells enhanced polarization of Th17 and Th2/Th17 hybrid cells in an antigen specific manner. These data suggest a direct role for exosomes in antigen presentation and promotion of $CD4^+$ T lymphocyte proliferation, and implicate exosomes in asthma exacerbations.

W.01. An Antigen Specific Exosome Cascade Leads to Final APC-derived Peptide-MHC-specific Exosomes That Suppress Effector T Cells by Delivery of Inhibitory miRNA-150

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Introduction: Antigen (Ag)-specific-suppressive-exosomes from T cells inhibit hapten-induced-contact-sensitivity (CS) and ovalbumin (OVA) protein-induced-delayed-type hypersensitivity (DTH). OVA-DTH allowed analysis of the APC^- target of the Ag-specific-suppressive-exosomes that are coated with antibody (Ab) light chains that bind peptides in MHC on the APC surface. Here we examined the mechanism of subsequent APC suppression of the final targeted effector T cells.

Methods: APC pulsed with Ag-specific $CD8^+$T suppressor exosomes from non-Treg were cultured for release of a factor involved in suppression and monoclonal Ab were used to block interactions with the DTH-effector T cells.

Results: A 100,000g ultracentrifuged pellet from the supernatant of the T suppressor exosome-pulsed APC, itself suppressed the DTG-effector T cells. This was dependent on binding then transfer of APC-derived secondary suppressive exosomes acting directly on the DTH-effector T cells. This secondary suppression was blocked with monoclonal Ab to MHC Class II and Ab to Ag peptides. Similarly obtained APC-derived secondary suppressive exosomes from a system of tolerized miRNA150-/- mice were not suppressive, unless the APC were pre-treated instead with wild-type mouse T cell-derived suppressor exosomes delivering $miRNA^-150$.

Conclusions: We concluded that primary Ab light chain coated Ag-specific suppressor exosomes bound peptides in MHC on APC to transfer inhibitory miR-150 that induces the APC to release secondary suppressive exosomes with peptide-MHC-specificity. These directly suppress the DTH-effector T cells likely via interaction with their TCR and subsequent delivery of an unknown regulatory miRNA. We theorize that such multiple cell derived exosome cascades may underlie other immune and possibly non-immune functional cell interactions *in vivo*.

W.02. Thymic Stromal Lymphopoietin and Interleukin-33 Increase Mast Cell Production of Prostaglandin D_2 Contributing to Inflammation in Chronic Rhinosinusitis

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Chronic rhinosinusitis (CRS) is a prevalent condition often marked by eosinophilic mucosal inflammation. Prostaglandin D_2 (PGD_2) is the dominant cyclooxygenase (COX) product of mast cells and is known to induce eosinophil chemotaxis and tissue vasodilation. We assessed the role of the innate cytokines thymic stromal lymphopoietin (TSLP) and interleukin (IL)-33 acting on mast cells to generate PGD_2 and facilitate tissue eosinophilia and mucosal inflammation in CRS.

Mouse bone marrow mast cells (mBMMC) and human peripheral blood-derived mast cells were stimulated with IL-33 and TSLP *in vitro* to assess PGD_2 synthase (hPGDS) and COX mRNA expression and PGD_2 generation. Nasal polyp specimens from human CRS subjects were analyzed via qPCR. A urinary PGD_2metabolite (uPGD-M) was measured in patients with severe CRS with nasal polyps.

Recombinant IL-33 induced expression of COX-2 mRNA in mBMMC and human mast cells. Recombinant IL-33 and TSLP synergistically induced PGD_2 generation by cultured human mast cells. Mast cells sorted from sinus tissue expressed hPGDS mRNA at higher levels than did eosinophils sorted from the same tissue. Both sorted sinus tissue mast cells and eosinophils expressed more COX-2 than COX-1 mRNA. Sorted sinus tissue stromal cells expressed TSLP and IL-33 mRNA. Whole sinus tissue TSLP mRNA expression correlated with systemic uPGD-M (r=0.74) in a subset of patients with severe CRS with nasal polyps.

This study demonstrates that the innate cytokines IL-33 and TSLP induce mast cell generation of PGD_2 through a COX-2-dependent pathway. Sinus tissue studies suggest dysregulation of this system contributes to the pathophysiology of CRS.

W.03. A Rare Case of Hyper-IgE-Syndrome and Severe Food Allergy Treated with the Proteasome Inhibitor Bortezomib

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Introduction: Food allergy usually is caused by IgE-antibodies to comestibles. This may provoke symptoms like diarrhea, rashes, itching, shortening of breath, and even anaphylaxia.

Case: A 40-year-old patient presented to our Immunology Outpatient Clinic with rashes, itching of the whole integument and severe diarrhea. Initially he reacted only on a few foods. Gradually, allergic reactions exacerbated; he was on elementary diet already at first examination.

Since childhood, he suffered from atopic dermatitis. Familiy history was unremarkable standard rheumatological testing was unspecific. Total serum IgE-concentration however was >5000U/ml (upper limit of detection). Symptoms releaved somewhat undergoing 20mg of prednisolone. Addition of ciclosporine A did not have marked effect. Undergoing biannual rituximab, total serum IgE-levels fell to 4200U/ml, but with slight clinical effect. The patient was so severely impaired that he developed suicidal tendencies demanding a more aggressive treatment. Therefore, the proteasome inhibitor bortezomib, which can eliminate plasma cells, was started. After the first course of three injections all allergic symptoms markedly regressed. IgE-concentrations decreased to 2000U/ml. The patient could restart a low-allergy diet of solid food. After two more courses of bortezomib clinical symptoms stabilized; IgE-levels were constantly <2000U/ml and thereby low enough to start the patient on the anti-IgE-antibody omalizumab.

Thereby symptoms further improved. The patient gained body weight and got back to work, although clinical symptoms never fully resolved.

Conclusion: The proteasome inhibitor bortezomib, approved for the treatment of multiple myeloma, may represent a therapeutic option in very severe cases of otherwise intractable causes of IgE-mediated allergic diseases.

W.04. Worsening Asthma in a Patient with a History of Sarcoidosis

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Introduction: The purpose of this study is to describe asthma exacerbation and atopy in a patient with a sarcoid history and if both diseases can coexist.

Case Presentation: A 43-year-old African-American female was evaluated in January 2016 complaining of chronic cough and wheezing for uncontrolled asthma and atopy. She has asthma, COPD, hypertension and a sarcoid history, previously diagnosed based on age, sex, ethnicity and presentation. No confirmatory biopsy was done.

She has strong family history of sarcoidosis but denies any familial atopic diseases. She was a former smoker with a 15-pack-year. On physical examination, vital signs were stable. She had boggy nasal turbinate and wheezing in bilateral lung fields. Serum IgE was elevated 2251 kU/L; sIgE was positive for common ragweed, *Dermatophagoides*, maple, oak, peanut, banana, giant ragweed, mugwort, *Penicillium notatum*, *Cladosporium herbarum, Hevea brasiliensis*, grass, and dog danger. Chest X-ray showed streaky nodular reticular markings on bilateral lungs with chronic interstitial lung disease. PFT showed mixed obstructive and restrictive pattern with reduced diffusion lung capacity. She was prescribed oral steroids, ipratropium, budesonide/formoterol, and albuterol inhalers.

Discussion: Poorly controlled asthma and exacerbations can occur in a patient with a history of sarcoidosis. Possible sarcoid etiologies are infection and environmental agents. She has a strong family history of sarcoidosis. Exposure to molds has been associated with an increased disease risk. The presence of sarcoidosis could lead to deterioration of asthma control.

Conclusion: A sarcoid history can potentially lead to worsening asthma.

W.05. IgE-Mediated Signaling Induces TSLPR Expression on Basophils, but Not Plasmacytoid Dendritics Cells, Upon Food Allergen Challenge

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Food allergens propagate allergic inflammation by activating mast cells and basophils to release inflammatory mediators and cytokines. IgE-mediated signaling predominantly activates basophils in response to food allergens, yet pro-type 2 cytokine Thymic Stromal Lymphopoietin (TSLP) and interleukin-3 (IL-3) activate basophils independent of IgE signaling. TSLP activates a number of immune cells besides basophils, specifically dendritic cells (DCs). Furthermore, recent data reveals that cross-linking IgE on basophils induces TSLPR expression. To date, it is unclear 1) whether food allergens induce TSLPR induction, 2) how food allergens directly modulate response to TSLP via upregulation of TSLPR on immune cells subsets and 3) whether TSLP amplifies activation of basophils and dendritic cells subsets in concert with or independent of IgE-signaling. To address these questions, we removed IgE, using a disruptive anti-IgE inhibitor, from basophils and DC subsets and assessed TSLPR induction. Using a cohort of twelve (12) food allergic patients, we found that both basophils and plasmacytoid dendritic cells (pDCs) express high levels of FceRI and IgE as opposed to conventional DCs (cDCs). Subsequent treatment with the disruptive inhibitor effectively removed IgE from both pDCs and basophils. Overnight treatment with the food allergens induced

TSLPR expression on basophils. Preliminary data reveals a trend in TSLPR expression that is dependent on intact IgE signaling in basophils, but not pDCs, suggesting a distinct mechanism of TSLPR induction between these two immune subsets. Our initial results indicate that basophils upregulate TSLPR to enhance sensitivity to tissue-derived TSLP in amplifying type 2 allergic responses.

W.06. *In Vitro* Induction of Peanut-Specific Tr1 Cells

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Rationale: IL-10 producing type 1 regulatory T cells (Tr1) express the surface markers LAG3 and CD49b, can be induced *in vitro* and used as cell therapy to control undesired immune responses. Peanut allergy is a life-threatening condition with no curative treatment. Our aim is to induce peanut-specific Tr1s *in vitro.*

Methods: Healthy controls (HC) and allergic patients under peanut oral immunotherapy (>3years) were included in this study. Mature (mDCs) or tolerogenic (DC10) dendritic cells were differentiated in the presence of the main peanut allergens Arah1/2. Autologous $CD4^+$T cells were co-incubated for 14 days with DC10 and IL-10 ('T10') or with mDC ('Tm'). We assessed by flow cytometry the expression of the Tr1 markers LAG3/CD49b and of the gut-homing receptor GPR15; in addition, we tested anergy of T10 compared to Tm cells, and IL-4 IL-10, IFN-γ and GM-CSF production upon restimulation with Arah1/2.

Results: The percentages of $LAG3^+CD49b^+$T cells were higher in T10 compared to Tm cultures, and comparable between patients (9.6%) and HC (8.8%). T10 from HC and patients were anergic compared to Tm upon Arah1/2 restimulation. The percentage of $GPR15^+$ cells was higher in $LAG3^+CD49b^+$T cells compared to memory T cells (p=0.007), and higher in T10 from patients compared to HC (p=0.009). Preliminary results show a higher IL-10/IL-4 ratio in T10 than in Tm cultures.

Conclusions: We successfully induced $LAG3^+CD49b^+$ Tr1 cells that presented antigen-specific anergy from peanut-allergic patients and HC. $GPR15^+$ cells were enriched in this population, suggesting their gut-homing capacity. Further studies are ongoing to assess the suppressive properties of Tr1 cells.

W.07. Oncostatin M is Produced by Neutrophils, and Promotes Epithelial Barrier Dysfunction in Mucosal Airways Disease

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We have previously shown that Oncostatin M (OSM) was elevated in nasal polyp (NP) tissue from chronic rhinosinusitis (CRS) patients, and in broncho-alveolar lavage (BAL) fluid following segmental allergen challenge (SAC) in allergic-asthmatics. Levels of OSM detected *in vivo* were sufficient to impair epithelial barrier function in *ex vivo* cultured airway epithelium. To determine which cell type(s) was producing OSM, nasal polyp sections were stained for OSM and hematopoietic cell specific markers. OSM showed co-localization with neutrophil elastase (n=10), however OSM did not co-localize with markers for eosinophils, macrophages, T cells or B cells (n=3-5). OSM mRNA correlated with mRNA for neutrophil markers CD16 (r=.61,p<.01) and cathepsin G (r=.56,p<.01) in mRNA from CRS patients and controls. OSM also correlated with the percent neutrophils in the BAL of allergic asthmatics following SAC (r=.50,p<.05), but did not correlate with total cell counts or eosinophil counts. Flow cytometric analysis of live cells isolated from NP (n=10) showed that 14±6% of $CD45^+$ cells were OSM^+, and 85±6% of OSM^+ cells were $CD16^+$/Siglec 8-, indicating neutrophil lineage. GM-CSF and FSTL-1 have been shown to induce OSM, and both were found to be elevated in nasal polyps. Additionally, GM-CSF, alone or in combination with FSTL-1, was sufficient

to induce OSM protein in cell culture supernatants of *ex vivo* cultured blood neutrophils. These data suggest that OSM is produced by neutrophils in NP, and may mediate epithelial barrier dysfunction in mucosal airways disease.

W.08. Clinical Tolerance to a Low Dose of Peanut Protein in Sensitized Individuals is Characterized by a Distinct Gene Expression Profile in Peanut-specific CD4$^+$ T cells

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Individuals who produce peanut-specific IgE comprise a clinical spectrum from asymptomatic tolerance to exquisite clinical sensitivity. We hypothesized that part of the variability may be determined by immune responses that affect features independent of the humoral response. Conventional CD4$^+$ T cells orchestrate the immune response to exogenous antigens. We sought to phenotype CD4$^+$ T cells in two groups of 10 peanut-sensitized patients who were either clinically reactive or tolerant to a controlled exposure of 443 mg peanut flour. PBMCs were isolated from whole blood and cultured with peanut protein extract for 20h. Activated and resting memory CD4$^+$ T cells were sorted based on expression of CD40L. Total RNA was isolated and used for RNA sequencing. Gene expression was compared between activated and resting T cells from the same cultures, and between reactive and tolerant subjects. Peanut-specific memory CD4$^+$ T cells from tolerant individuals in comparison to the reactive, were less numerous and had a distinct transcriptional phenotype, characterized in part by lower expression of a cluster of genes including IL5, IL9, IL17F, IL22, and IL31, but higher expression of a cluster including IFNG. The expression of other Th cytokines including IL2, IL4 and IL13 was strongly induced by activation, but did not differ between subject groups. Principal component analysis based on Th2/Th9 vs Th1/Tfh genes yielded separation between the T cell responses from reactive and tolerant subjects. The expression of IL5 and IL9, which are known to promote effector cell reactivity and consequent intestinal permeability, may play a role in determining clinical sensitivity to peanut allergen.

W.09. Peanut Protein Induces Expression of RALDH2 in Human Dendritic Cells in a TLR2-dependent Manner

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Dendritic cells instruct naïve T cells to differentiate into various effector cells determining immune responses such as allergy or tolerance. Our objective was to determine peanut protein (PP)-induced changes in gene expression in human myeloid dendritic cells (mDC), identify possible signaling receptors, and assess the role of differentially expressed genes in the induction of T cell differentiation.

mDC and naïve Th cells were isolated from blood bank donors. mDC were incubated for 12-24h with medium alone, PP, or control stimulants. mRNA was isolated for use in microarray and RT-qPCR. To assess T cell differentiation, mDC were cocultured for 6d with autologous naïve Th cells and the RALDH2 substrate retinal.

PP induced a unique expression profile in mDC, including the gene that encodes RALDH2, a rate-limiting retinoic acid (RA)-producing enzyme. RT-qPCR confirmed the ~20-fold induction of this gene. PP-treated mDC also demonstrated a 7-fold increase in enzymatic activity of RALDH2. Naïve Th cells cocultured with PP-treated mDC showed a 4-fold increase in production of IL-5 and in expression of the RA-sensitive surface marker α4β7-integrin. Blocking antibodies against TLR2/TLR1, as well as siRNA targeting TLR2/TLR1, reduced expression of RALDH2 in PP-stimulated mDC by 70%.

PP induces RALDH2 in mDC by signaling through the TLR2/TLR1 heterodimer. This leads to production of RA, which acts on Th cells to induce IL-5 and gut-homing integrin. RA has been implicated in differentiation of gut-homing regulatory and effector T cells. RALDH2 induction by PP could therefore be an important factor determining allergic or tolerant responses to peanut.

W.10. Basophil Suppression in Sustained Unresponsiveness After Peanut Oral Immunotherapy Due to Induction of Effective Antibody Responses

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Rationale: Peanut oral immunotherapy (PNOIT) can increase peanut tolerance in individuals with IgE-mediated peanut allergy. Although some have tolerance or sustained unresponsiveness (SU) to peanut, at least 50% regain sensitivity, experiencing only transient desensitization (TD). We hypothesize that suppression of basophil reactivity during PNOIT in SU occurs due to effective blocking antibodies.

Methods: Direct basophil reactivity was measured by flow cytometry after peanut allergen Arah2-stimulation of peripheral blood. Reactivity is reported as log area-under-the-curve of percent $CD63^{++}$ basophils. To evaluate PNOIT-induced antibodies, indirect basophil reactivity was evaluated by flow cytometry, using normal donor blood cells sensitized with PNOIT plasma from SU and TD subjects and stimulated by Arah2 with or without pre/post-treatment plasma from PNOIT subjects. Indirect basophil activation, normalized against the matching controls stimulated without plasma, is reported as percent inhibition.

Results: Direct basophil reactivity to Arah2 is similar at baseline in both SU and TD subjects but is significantly suppressed one month into PNOIT, more in SU than TD (298.1 vs. 28.8; $p<0.001$), which persists through desensitization (18.0 vs. 37.8; $p<0.001$). One month after avoidance, basophil reactivity rebounds, significantly more in TD (235.7 vs. 79.1; $p<0.001$). Indirect basophil activation reveals comparable passive IgE-mediated reactivity in TD and SU subjects. Suppression by post treatment serum is significant (86.3% vs. 7.0%; $p=0.006$). SU post-treatment plasma more effectively suppresses basophils than TD (16.0% vs. 73.4%; $p=0.002$).

Conclusions: Arah2-induced basophil activation is effectively suppressed in SU subjects after PNOIT, possibly due to the induction of a more effective antibody repertoire.

W.11. Characterization of TCR Repertoire Diversity and Tissue Distribution of Drug-specific T Cells in DRESS

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Vancomycin is now recognized as the most frequent antibiotic cause of drug reaction with eosinophilia and systemic symptoms (DRESS). DRESS is a T cell mediated adverse drug reaction (ADR) with up to 10% mortality that is characterized by fever, rash, hematologic abnormalities, and multi-organ dysfunction. We aim to describe the phenotype, repertoire diversity and tissue distribution of drug-specific pathogenic T cells and establish how long these vancomycin-reactive T cells are maintained during the recovery phase of DRESS. Following *ex vivo* (18 hour) stimulation with vancomycin, $CD4^+$ T cells expressing markers of T cell activation (CD137, CD69) were isolated from the peripheral blood of a vancomycin DRESS patient at three time points during the recovery phase. These activated drug-specific $CD4^+$ T cells were shown to be of effector memory phenotype and to produce IFN-gamma and IL-2. Single $CD4^+$ T cells were sorted for surface expression of CD137 and sequenced for paired T cell receptor (TCR) alpha and beta genes. TCR sequences from candidate pathogenic clonotypes were introduced into a Jurkat-based TCR expression system to verify drug reactivity. To define the clonotypes present in the skin during vancomycin DRESS, we performed TCR Vb repertoire (bulk) sequencing on DNA isolated from an archived formalin-fixed paraffin embedded skin biopsy obtained during the acute cutaneous reaction. We compared the TCR repertoire from affected skin to that found in the recovery phase of peripheral blood following *ex vivo* drug stimulation to determine if common clonotypes are present in these two compartments and if these are maintained over time.

T.81. Drug Hypersensitivity Leukocytoclastic Vasculitis After Two Different Antibiotics

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Background: Hypersensitivity or leukocytoclastic vasculitis (LCV) is a small vessel vasculitis with different etiologies, most common being medications, infection, malignancy, autoimmune and collagen vascular disease. Common known medications include: diuretics, antibiotics, anticonvulsants and NSAIDS.

Case report: 31 year old female presented to the ER with an acute generalized raised purpuric rash suggestive of LCV on upper and lower extremities. She had an episode of UTI 3 days before and had taken Bactrim. In ER, she was treated for a possible allergic reaction and was admitted to the medicine floor. A rheumatology work up was negative for SLE or any underlying systemic autoimmune disease or paraproteinemia. She was hospitalized again after 4 weeks with a LCV rash after using ciprofloxacin for another UTI.

Conclusion: Adverse reactions to medications are extremely common and display a characteristic morphology such as fixed drug eruption, Stevens-Johnson syndrome, urticaria, morbilliform exanthem, hypersensitivity syndrome, pigmentary changes, lichenoid, dermatitis, acute generalized exanthematous pustulosis, photosensitivity, vasculitis etc. Cross reactivity between fluoroquinolones and sulfa drugs has not been reported which can be the etiology in our case and a possible risk factor could be the concurrent infection.

LCV can be acute or chronic, involving arterioles and small vessels with circulating immune complexes as an underlying factor. The disease has both systemic and skin symptoms with a range of 7 to 10 days after exposure. The diagnosis is made by clinical findings and presence of offending agents in the history. Treatment consists of discontinuing offending medications or treating the underlying disease.

Autoimmune Neurologic Disease

OR.02. Regulatory B Cell Induction by a Human Gut Commensal Antigen Protects Against CNS Inflammation and Demyelination

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B lymphocytes have the ability to provide both positive and negative regulation of host immunity. While the classic antibody-mediated functions of B cells drive inflammation, regulatory B cells (B_{regs}) are involved in the maintenance of immune homeostasis during inflammation. While phenotypically heterogeneous, B_{regs} can be commonly identified by their production of the immunosuppressive cytokine interleukin-10 (IL-10). While artificial and exogenous microbial stimuli of B_{regs} have been described, environmental and endogenous cues for the induction of B_{regs} have yet to be described. Previous reports from our lab have identified that modulation of the microbiome during experimental autoimmune encephalomyelitis (EAE) leads to the induction of $CD5^+$ B_{reg} cells that can significantly reduce EAE disease severity. Here we show that treatment with polysaccharide A (PSA) derived from the intestinal symbiont *Bacteroides fragilis* also promotes an increase in systemic $CD5^+$ B_{regs} following the onset of EAE. PSA-induced $CD5^+$ B_{regs} produced IL-10 and were able to protect naïve recipients against EAE upon adoptive transfer. Specific deletion of IL-10 within B cells using IL-$10^{fl/fl}$CD19-Cre transgenics abrogated the protective effect of PSA. Importantly, we found that IL-10 production by B cells was dependent on TLR2 expression *in vitro*. *In vivo*, PSA was unable to modulate EAE disease severity when the B cell compartment was deficient in MyD88 signaling suggesting a B cell-intrinsic requirement for the ability to detect and respond to the intestinal microbiome. We have shown that the

regulatory functions of B cells triggered by a specific gut commensal can restrain autoimmune inflammation affecting the CNS.

OR.17. Identification of Eomes-positive T Helper Cells as a Pathogenic Factor in Chronic Neuroinflammation

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Multiple sclerosis (MS), the autoimmune disease of the central nervous system (CNS), frequently manifests a relapsing/remitting course (RRMS), later shifting to a secondary progressive form (SPMS). Pathogenesis of SPMS is poorly understood and lacks effective therapies. We have previously reported that NR4A2 is upregulated by circulating T cells in RRMS patients and is NR4A2 is expressed by CNS-infiltrating Th17 cells cells during experimental autoimmune encephalomyelitis (EAE).

Mice with T cell-specific NR4A2 deficiency (NR4A2 cKO) did not develop early/acute clinical EAE symptoms, but instead developed an unexpected late/chronic EAE. Disease reduction was associated with reduced NR4A2-dependent Th17 cells, whereas in late disease, non-Th17 CNS-infiltrating T cells from NR4A2 cKO were pathogenic on transfer.

The absence of NR4A2-dependent T cell responses revealed a novel population of $CD4^+$ T cells accumulating in CNS during late/chronic EAE that expressed the transcription factor eomes. Eomes RNAi treatment *in vivo* and CD4-specific eomes knockout suppressed the late/chronic symptoms in NR4A2 cKO. Such $eomes^+$ Th cells were implicated in granzyme B-mediated cytotoxicity potentially acting directly on PAR-1 receptors expressed by neurons; treatment with PAR-1 activating peptides restored late/chronic EAE in the absence of eomes. Strikingly, $eomes^+$Th cells were also remarkably increased in peripheral blood and further enriched in cerebrospinal fluid of SPMS patients.

Our findings raised the possibility that these previously unappreciated $eomes^+CD4^+$ T cells may be a novel therapeutic target for SPMS. NR4A2 cKO may also provide a useful animal model for investigating pathogenesis of late/chronic CNS autoimmunity.

OR.25. Wnt-activated APCs Regulate T Cell Response in Multiple Sclerosis

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Blood-derived myeloid cells account for a significant proportion of cells found within perivascular infiltrates in CNS lesions of multiple sclerosis (MS) and its animal model, experimental allergic encephalomyelitis (EAE). These antigen-presenting cells (APCs) promote the reactivation of myelin reactive T lymphocytes and contribute to the immune-mediated injury observed in MS and EAE. In addition to their capacity to induce and sustain inflammation, APCs are also critically involved in homeostasis and in promoting and maintaining peripheral tolerance. Recently, it was shown that Wnt signaling in APCs could play a central role in regulating the balance between inflammatory versus regulatory responses in the gut. Our goal was to verify the role played by the Wnt pathway in controlling neuroinflammatory disorders such as EAE. We found that Wnt agonist increased PD-L1 and PD-L2 expression on dendritic cells (DCs) and macrophages. Moreover, IL-1β, IL-12 and IL-23 were reduced on these populations while phagocytic capacities and pSTAT3 were increased following Wnt activation. These data were confirmed using human monocytes. In addition, purified naïve MOG_{35-55}-pecific T cells from 2D2 mice expressed less IL-17, GM-CSF and IFN-γ when cultured with MOG_{35-55}-pulsed APCs treated with the Wnt agonist. Finally, treatment with Wnt agonist decreases the clinical severity of EAE in MOG_{35-55}-induced disease and inhibited relapses in $PLP_{139-151}$-induced disease, reducing immune cell infiltration into the CNS, and most notably infiltration of myeloid cells and $CD4^+$ T lymphocytes. Altogether our data demonstrate that Wnt activation on APCs reduces the ability of T cells to mount an inflammatory response.

OR.47. CCAAT-Enhancer-binding Protein B Controls the Pathogenesis of EAE through Regulation of IL-23R Expression

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IL-23 driven inflammation mediated by IL-17-producing cells is essential in the development of experimental autoimmune encephalomyelitis (EAE). However, the mechanisms that control expression of IL-23R and subsequent IL-17-mediated inflammation have yet to be elucidated. CCAAT/Enhancer Binding Protein β (C/EBPβ) is a key transcription factor that orchestrates immune responses by regulating gene expression. C/EBPβ is activated by various cytokines, including IL-17. Despite its role in inflammation, very little is known on the significance of C/EBPβ in an autoimmune setting. Here, we demonstrate that C/EBPβ is absolutely required for induction of EAE. C/EBPβ-deficient mice exhibited dramatically reduced inflammation demonstrated by decreased numbers of lymphocytes, antigen presenting cells and reduced levels of proinflammatory cytokines. Most notably, C/EBPβ-deficient mice failed to upregulate IL-17 in response to IL-23, which was associated with reduced IL-23R in the lymph nodes of immunized mice. C/EBPβ bound to the IL-23R promoter in both Th17 differentiated cells and IL-17-activated BMDM, demonstrating a previously unrecognized function for C/EBPβ in regulating the expression of IL-23R. These findings establish C/EBPβ as a key factor mediating autoimmune inflammation in EAE and suggest a novel role for C/EBPβ in regulating IL-23R expression.

OR.56. Dietary Fatty Acids in Multiple Sclerosis: Therapeutic Potential of Propionic Acid

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We have recently shown that dietary fatty acids, depending on their chain length, exert opposing effects on T helper (Th) cells: while long chain fatty acids (LCFA) lead to enhanced differentiation and proliferation of Th1/Th17 cells and impair their intestinal sequestration, short chain FA (SCFA) enhance regulatory T cell (Treg) differentiation and expand gut Treg. Using experimental autoimmune encephalomyelitis (EAE) as a model of T cell mediated autoimmune diseases like multiple sclerosis (MS), LCFA consistently decrease SCFA in the gut and exacerbate disease. In contrast, treatment with propionate (PA), a SCFA, significantly ameliorates EAE by promoting the differentiation of Treg.

In a proof of concept study we transferred our observations on PA to healthy individuals and MS patients by orally administrating PA – an approved food additive with no safety concerns - in capsules for 14-60 days (1g daily). A detailed flow cytometry analysis of T cell subsets before and at several time points after PA intake revealed first beneficial *in vivo* effects of PA in humans: Treg frequencies increased up to 25-30% in all treated individuals, what was accompanied by a significant decrease of Th17 cells. Increased Treg frequencies were maintained for 2-3 weeks after withdrawal of PA and similar to baseline frequencies after 2 months of washout.

Our results thus underline the influence of nutritive FA on systemic immune response und may be included in an add-on regimen of PA in addition to established first line MS drugs.

OR.58. IL-10 Producing B Cells as Preferential Targets of Atacicept (TACI-Fc): a Potential Explanation for Disease Exacerbation in MS

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B cell depletion therapy using CD20 monoclonal antibody substantially decreases new MS disease activity. In contrast, Atacicept, a fusion protein of TACI and Ig that depletes B cells by blocking B cell survival factors

(BAFF/APRIL), surprisingly increased MS relapses. Previous studies from our group have shown that the balance between anti-inflammatory (regulatory) B cells and pathogenic pro-inflammatory (effector) B cells is important for MS disease activity. We hypothesized that Atacicept may preferentially deplete regulatory B cells thus contributing to a pro-inflammatory shift in disease-relevant B cell responses. To test this hypothesis, the effect of atacicept on human B cells was assessed under various conditions known to promote IL-10 production from B cells. We demonstrate that Atacicept significantly decreases the CpG induced IL-10 expressing B cells of both healthy controls and MS patients, while not impacting pro-inflammatory cytokine expression by the B cells. To further prove that TACI-Fc directly impacts IL-10 expressing B cells, we isolated live-purified cytokine-defined B cell subsets (IL-10$^+$, TNFa$^+$ and GM-CSF$^+$ and triple negative B cells) using a newly-optimized cytokine-secretion approach. Interestingly, we found that compared to the other B cell subsets, IL-10$^+$ B cells are more prone to apoptotic death. Adding exogenous BAFF rescued IL-10$^+$ B cells from cell death, and this rescue was blocked by pre-incubation of BAFF with TACI-Fc but not with control Fc. Together, these data indicate that Atacicept may preferentially deplete regulatory B cells while relatively sparing pro-inflammatory effector B cells, thus revealing a potential mechanism underlying Atacicept's disease-exacerbating effect in MS.

OR.59. Gut Intraepithelial Autoreactive CD4$^+$T Cells Influenced by Environment Suppress Central Nervous System Autoimmunity Via LAG-3

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Objective: Multiple sclerosis (MS) is an autoimmune disease that targets the myelin of the central nervous system (CNS). Increasing incidence of MS in Japan let us question which components of the immune system are altered by environmental factors. Gut mucosa is by far the largest immune organ that interacts with the external environment. Since T cells are pivotal in controlling CNS inflammation, we investigated the existence of myelin reactive T cells that might be induced in the gut and their influence on CNS autoimmunity.

Methods: Gut-T cells from MOG(35-55) specific T cell receptor transgenic (2D2) mice were analyzed by FACS. Sorted gut-T cells were adoptively transferred to experimental autoimmune encephalomyelitis (EAE) induced by immunization with MOG(35-55). Blocking antibodies were used for functional analysis. Gut microbiota was modulated by oral antibiotics treatment. Diets including poor or rich aryl hydrocarbon receptor ligands (AHRL) were fed to mice.

Results: Here we demonstrate that the gut epithelium of 2D2 mice is inhabited by intraepithelial T-lymphocytes (IEL) that can inhibit EAE upon transfer. The regulatory IEL had the phenotype of CD4$^+$'induced'IEL (CD2$^+$CD5$^+$), exhibited T_H17-like profile and had ability to infiltrate the inflamed CNS tissue. The IEL constitutively expressed Ctla4 and Tgfb1, and markedly upregulated Lag3 in the CNS, thereby inhibiting the inflammation. We also demonstrated that the IEL are induced by gut microbiota and AHRL.

Conclusions: Gut environment favors the generation of autoreactive T cells that have unique regulatory function, potentially important for preventing MS. Now we started to investigate the cerebrospinal fluid of MS.

W.59. Genetic Polymorphisms Associated with Loss of Immunologic Self-tolerance in Myasthenia Gravis

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Myasthenia gravis (MG) is a B cell driven, T cell dependent autoimmune neuromuscular disorder characterized by a relapsing-remitting disease pattern. The presence of polyclonal IgG anti-acetylcholine receptor antibodies (anti-AChR) or anti-muscle specific kinase antibodies suggests that loss of immune self-tolerance is key to the pathogenesis of MG. Autoreactive AChR specific CD4$^+$ T cells interact with B cells to produce anti-AChR antibodies. It is postulated that regulatory T cells lose their suppressive capabilities due to reduced cellular expression of

transcription factor forkhead protein 3 (FOXP3) hence mRNA and protein expression of FOXP3 is reduced, leading to production of pathogenic T helper 17 cells. Increased levels of interleukins 10 and 17 lead to chronic inflammation, contributing to T cell impairment. MG associated thymomas lack functional tolerogenic autoimmune regulators, expression of human leukocyte antigen class II molecules! and tolerogenic AChR positive thymic myeloid cells. For this reason, AChR positive myasthenia gravis improves after thymectomy.

Myasthenia gravis may be associated with genetic polymorphisms, microRNAs such as miRNA-146a whose expression may be significantly increased. The TT homozygous genotype of DNMT3B-579 T allele is associated with risk of thymomas, but not to other myasthenic features. FOXP3 IVS9^{+}459 G is protective against myasthenia. Dysregulated FOXP3 may be the cause of failure of self-tolerance. This review sums the key genetic polymorphisms recently described that affect the immunopathogenesis of myasthenia gravis. Further knowledge of such mechanisms can aid in patient classification for prognosis and therapeutic management.

W.60. TLR2 Tolerance in EAE

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TLR2 expression is enhanced in MS and EAE, but its role in these diseases is controversial. In adoptive transfer EAE, exogenous TLR ligands are not administered yet TLR2-deficient mice have been reported to develop attenuated disease. Despite this evidence for a disease-promoting function of TLR2, others have shown that administration of TLR2 ligands can inhibit EAE in WT mice. To understand this paradox we tested the postulate that although TLR2 signaling may be EAE-promoting, repeated TLR2 ligand administration can lead to TLR2-tolerance and attenuated disease.

We first showed that administration of Pam2Cys to mice for 5 days induces TLR2- tolerance that persists for 3-5 days as reflected in decreased serum TNFα in response to a second TLR2 ligand ($p<0.0001$ day 1; $p<0.01$ day 3; $p=0.069$ day 5). Next, this approach was used to induce TLR2-tolerance in the context of EAE. Mice received PLP-reactive LNCs (“day 0”) and tolerance was induced at various time points. Surprisingly, initiating the 5-day TLR2-tolerance protocol at day -2 or day +8 showed no effect on disease. In contrast, initiating the protocol between day +4 and +6 lead to significant inhibition of disease onset and severity that lasted a minimum of 5 days ($p=0.025$) and was associated with TLR2-tolerance ($p<0.0001$).

In sum, we have used TLR-tolerance as a novel probe to identify a narrow kinetic window (day 4-6) in which TLR2 is required for EAE. Additionally, we have demonstrated that TLR-tolerance may be relevant as a new therapeutic approach for EAE and potentially MS as well.

W.61. ROLE of CD46 GLYCOSYLATION in Tr1 DIFFERENTIATION

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Autoimmune diseases result not only from autoreactive T cells against the target antigen but also from a lack of regulation of these autoreactive T cells. Defects in both conventional CD4^{+}CD25^{+}Foxp3^{+} and Tr1 regulatory cells, which are characterized by their secretion of large amount of interleukin 10 (IL-10) and their low secretion of IFNg, have been demonstrated. T cell activation by the complement regulator CD46 promotes a switch of IFNg-secreting Th1 cells towards IL-10-secreting Tr1 in healthy T cells, and requires the shedding of CD46 upon its activation. The Th1-Tr1 switch is defective in chronic inflammatory diseases such as multiple sclerosis (MS). Herein, we report that CD3 activation induces changes in CD46 glycosylation. By using an O-glycosylation inhibitor or expressing a GFP-tagged CD46 glycosylated mutant deprived of O-glycosylation sites in primary human T cells, we demonstrate the key role of O-glycosylation in controlling CD46 shedding and the IFNg-IL-10 switch. Preliminary data suggest that

CD46 glycosylation controls its recruitment to the immune synapse and downstream signaling. These glycosylation changes are defective in CD3-activated MS T cells, leading to altered levels of CD46 expression. We propose that a defect in the regulation of CD46 glycosylation contributes to the defective CD46 pathway in MS.

W.62. Functional Heterogeneity of Inflammatory Antigen-reactive T cells in Human Autoimmune Diseases

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Autoreactive T cells have been identified in both healthy subjects and human autoimmune diseases, such as multiple sclerosis (MS) and myasthenia gravis (MG). However, the contribution of these autoreactive T cells to disease pathology remains unknown. Amplified T cell libraries generated from MS or MG patients were interrogated for their auto-antigen reactivity. Libraries derived from $CCR6^+$ myelin-reactive T cells from MS patients exhibited significantly enhanced production of pro-inflammatory cytokines (IFN-γ/IL-17/GM-CSF), but less IL-10 compared to healthy controls. Similar results were also presented in acetylcholine receptor-reactive T cells from MG patients. Moreover, MHC/peptide tetramers-based single-cell clones also secreted more multiple pro-inflammatory cytokines but less IL-10 relative to controls. Tetramer based single-cell analyses confirmed the heterogeneity of myelin-specific T cells by differential expression of chemokine receptors. In addition, RNA sequencing of myelin-specific T cells from MS patients demonstrated a transcriptome homology with encephalitogenic T cells isolated from mice with experimental autoimmune encephalitis and revealed a transcriptional profile that distinguishes MS-derived myelin-reactive T cells. Myelin-specific T cells from healthy subjects were highlighted by the up-regulation of CTLA-4 inhibitory pathway. Correlatively, inflammatory central nervous system demyelination and enhanced myelin-reactive T cell responses were observed in melanoma patients after treatment with Ipilimumab. Taken together, we were able to identify the functional and phenotypic properties that distinguish pathogenic T cells in MS or MG patients. These findings enhance our understanding of autoimmune pathogenesis as well as guide the development of focused therapies that optimize the risk: benefit profile of future interventions.

W.63. The Effect of Early-life Microbiota Disruption on Experimental Autoimmune Encephalomyelitis

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Early-life intestinal microbiota participates in educating the developing immune system, and disruptions from antibiotic exposure can have lasting effects on immunity and systemic diseases. The microbiota is hypothesized to play a role in multiple sclerosis (MS), and antibiotic treatment in humans has been associated with either protection or worsening of symptoms, depending on the study. The microbiota has been clearly demonstrated to play a role in experimental autoimmune encephalomyelitis (EAE), the animal model for MS, as germ-free mice or mice treated with high-dose broad-spectrum antibiotics have less severe EAE scores. Prior animal studies with high-dose antibiotics result in a massive depletion of the microbiota, nearly mimicking the germ-free state, providing an important proof-of-principal of microbiome involvement in EAE. However, this substantial elimination prevents the identification of key microbes that could play a role in EAE. To specifically examine these interactions, we used a milder perturbation by administering low-dose penicillin (LDP) at a level that does not reduce total microbial populations. We gave C57BL6 mice LDP from 4 to 8 weeks of age, and then induced EAE by administering myelin oligodendrocyte glycoprotein (MOG). We recorded EAE scores, collected longitudinal microbiota samples, and collected host tissues for immune expression profiling. Mice pretreated with LDP before MOG injection showed delayed and less severe EAE symptoms. Further studies examining the microbiota and the immune system are ongoing. We can conclude that LDP, an antibiotic regimen administered at a substantially lower dose than reported studies, can ameliorate EAE, and likely functions via modulating the microbiota.

W.64. The Role of the Receptor Mas in Autoimmune Neuroinflammation

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Blocking of classical renin-angiotensin system (RAS) pathways may target immune cells and improve symptoms in experimental autoimmune encephalomyelitis (EAE), a murine model for multiple sclerosis (MS). Recently, a novel RAS pathway has been described, which involves binding of angiotensin-(Ang) 1-7 to its receptor mas. This Mas axis may counterbalance AngII mediated pro-inflammatory effects. Here we thus investigate the role of the receptor Mas during autoimmune neuroinflammation.

Methods: (1) *In vitro* polarization, transwell and coculture experiments with murine bone marrow derived macrophages, peritoneal macrophages and naïve T cells from MasKO versus wildtype mice. (2) EAE induction in 10 week old C57BL/6 and MasKO mice by myelin-oligodendrocyte glycoprotein $(MOG)^{35-55}$/CFA and pertussis toxin. (3) *Ex vivo* investigation of various pro-inflammatory macrophage markers during EAE.

We found expression of the receptor Mas in pM, M0 and M1 macrophages on the mRNA and protein level. Mas deficiency led to increased M1 but decreased M2 polarization *in vitro*. Notably, it also enhanced macrophage migration ($p<0.05$, n=3) and macrophage induced T cell proliferation *in vitro* ($p<0.05$, n=3). In contrast, Mas did not impact on cytokine production and phagocytic activity of macrophages. *In vivo*, Mas deficiency aggravated MOG induced EAE (score 2.4+/-0.4 vs. 4.4+/-0.5, $p<0.01$, n=7 per group). Mas deficient mice displayed an increased expression of pro-inflammatory macrophage genes like *inos* and *il6* in the spinal cord and spleen ($p<0.01$, n=7).

In summary, the Mas axis was identified as an important factor in macrophage function during neuroinflammation and therefore may constitute an interesting new therapeutic target in MS.

W.65. Cystatin C Mediates Gender Dimorphism in Experimental Autoimmune Encephalomyelitis

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Multiple sclerosis (MS) is an autoimmune disease characterized by infiltration of myelin reactive immune cells into the central nervous system (CNS). The disease is associated with the enhanced and down-regulated expression of scores of genes and proteins but the function of most of these molecules is poorly understood. Cystatin C (CysC) is a gene whose level is enhanced in the brains of MS subjects. We have also found increased CysC protein expression in the CNS of mice with experimental allergic encephalomyelitis (EAE), an animal model of MS. CysC is an inhibitor of cysteine proteases including cathepsins B, H, K, L, and S and is mostly localized in astrocytes, neurons and macrophages. Because of discordance in the literature regarding the function of CysC (neurotoxic vs neuroprotective, pro-inflammatory vs immunosuppressive) and its expression in MS patients, we aimed to clarify the role of CysC in EAE. Using CysC knockout and over-expressing mice, we unexpectedly found that CysC has a detrimental effect in EAE but only in female animals. This female preference was not due to estrogens but was instead attributed to defects in the activation status of antigen presenting cells such as macrophages that subsequently disrupt T cell function.

W.66. Evaluation of Post-translational Modifications in the Mice Spinal Cord in an Experimental Autoimmune Encephalomyelitis (EAE) Model of Multiple Sclerosis (MS)

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Rational: Multiple sclerosis (MS) is a chronic inflammatory de-myelinating and incurable disease, characterized by the targeted immune-mediated destruction of central nervous system (CNS) myelin. Immune system-induced post-translational modifications in myelin proteins including methylation and acetylation are thought to be implicated in the molecular pathogenesis of MS-induced myelin damage. Myelin basic protein (MBP) and proteolipid protein (PLP) are two key proteins both of which have essential role in structural integrity and function of myelin. Therefore, our specific research using the experimental autoimmune encephalomyelitis (EAE) animal model of MS is primarily focused on post-translational modifications of these two proteins.

Hypothesis: We hypothesize that EAE-induced immune system induction results in post- translational modifications in MBP and PLP that correspond to the degree of neurological disability and myelin damage.

Aims and methods: we use myelin oligodendrocyte glycoprotein (MOG) induced model of EAE to identify immune system-induced post-translational modifications of myelin proteins in spinal cord (SC). Using liquid chromatography tandem mass spectrometry (LC-MS/MS), we quantified the specific types of post-translational modifications in MBP and PLP. In addition, we also aim to correlate these post- translational modifications to neurological disability scoring (NDS) of EAE animals.

Preliminary results and conclusion: Our initial results confirmed that EAE-induction was responsible for modifying myelin proteins. Specifically, we found a significant increase in relative amounts of acetyl-lysine, mono-di and tri methyl-lysine in SC of EAE mice compare to NC group during the peak inflammatory phase of the disease. These findings correlate with peak NDS.

W.67. Suppression of Regulatory T Cells: a Pathogenic Role of Exosomes in Multiple Sclerosis

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Background: Exosomes are extracellular vesicles which are involved in intercellular communications by delivering a variety of molecules such as miRNAs.

Objective: To determine differentially expressed miRNAs in exosomes and a role of exosomes in multiple sclerosis (MS).

Methods: Exosomes were collected from plasma of MS patients and healthy controls (HC). Disease-associated miRNAs in exosomes were investigated by microarray analyses and RT-qPCR. The function of exosomes was examined by co-culture with T cells followed by intracellular staining against cytokines and transcription factors.

Results: Microarray analysis and RT-qPCR clarified four miRNAs(miR-A,B,C,D) which were significantly increased in exosomes of MS patients (n=19) relative to those of HC (n=6) (26.3±14.0 vs 11.2±4.6, 122±56.2 vs 55.1±32.8, 38.1±17.4 vs 15.7±7.3, 334±174 vs 138±81.9 [AU], respectively, $p<0.05$). There was a significantly decreased frequency of $Foxp3^+$ regulatory T cells (Treg cells) among T cells which were co-cultured with exosomes of MS patients compared to HC (15.1±1.2% vs 16.8±1.1%, $p<0.05$). Furthermore, the frequency of Treg cells was inversely correlated with the amount of miR-A in the exosomes ($p<0.05$). There was also a significant reduction of Treg cells after transfection of miR-A compared to negative control (7.1±1.0% vs 10.4±1.9%, $p<0.05$).

Conclusion: This study indicated that miRNAs in exosomes might be new diagnostic markers. Furthermore, it was suggested that exosomes, through miR-A included in exosomes, might function as a novel player in the pathogenesis of MS by suppression of Treg cells.

W.68. Molecular-based Diagnosis of Multiple Sclerosis and its Progressive Stage

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Molecular taxonomy of cancers revolutionized oncology: biomarker-guided combination therapies inhibit dysregulated pathways and target-based patient preselection leads to economical clinical trials. In contrast, polygenic central nervous system (CNS) diseases, including multiple sclerosis (MS) lack molecular diagnosis. Contrary to the multifaceted pathophysiology of MS, patients are treated suboptimally with a single drug without understanding what molecular mechanism(s) drive their clinical disability. Therefore, the purpose of this study was to identify molecular signature of MS and its progressive stage(s).

Using commercially-available SOMAscan technology, we measured 1,128 proteins in the CSF of 85 untreated subjects from six diagnostic categories (10 healthy donors, and 15 subjects each from relapsing-remitting MS, primary- and secondary-progressive MS, non-inflammatory and other inflammatory neurological diseases) in the discovery cohort. These results led to a power analysis for an independent validation cohort consisting of 225 prospectively acquired untreated subjects from six identical diagnostic categories (40 patients per category + 25 healthy donors).

We identified and validated a cerebrospinal fluid-based diagnostic test that identifies MS with the area under the receiver operating characteristic curve (AUC) of 0.96, with immunological biomarkers dominating this MS diagnostic test. In contrast, CNS-derived biomarkers differentiate relapsing-remitting from progressive MS with validated AUC=0.91.

The identified molecular taxonomy opens opportunities for biomarker-supported drug development for novel neuroprotective and anti-inflammatory therapies in MS.

W.69. Tiam1/Rac1 Complex Controls *Il17a* Transcription and Autoimmunity

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Retinoic acid-related orphan receptor (ROR)γt, tightly regulates interleukin (IL)-17 expression and is one of the most promising drug targets for autoimmune diseases such as multiple sclerosis (MS). Tiam1 is a guanine nucleotide exchange factor for the Rho-family Guanine triphosphatase (GTPase) Rac1. Tiam1 and Rac1 act as molecular switches that have been implicated in regulating cellular motility, adhesion, proliferation, and survival. We identified a novel role of Tiam1 and its cognate, Rac1, in the regulation of Th17 cell development and autoimmunity. We found that Tiam1 expression is induced in Th17 cells in a STAT3-dependent manner, demonstrated using ChIP and reporter assays and confirmed using STAT3-/- mice. While *Tiam1* genetic deficiency only partially weakens IL-17A production and hinders disease development in experimental autoimmune encephalomyelitis (EAE), conditional deletion of *Rac1* in $CD4^+$ T cells exhibited more robust effects on Th17 cells and EAE. At the molecular level, Tiam1 and Rac1 form a protein complex with RORγt in the nuclear compartment of Th17 cells, and together bind the *Il17* promoter. This process is RORγt-dependent and enhances *Il17* promoter transactivation. The clinical relevance of these findings is emphasized by (1) increased Tiam1 and Rac1 expression in $CD4^+$ T cells in MS patients, (2) pharmacological targeting of Rac1 using two distinct compounds induced suppression of both murine and human Th17 cell development and (3) amelioration of clinical outcome of EAE. Our findings highlight a novel pathway in Th17 cells and suggest that Tiam1/Rac1 signaling may be a therapeutic target for Th17 cells in multiple sclerosis and other autoimmune diseases.

W.70. Dimethyl Fumarate (DMF) Treatment Mediates an Anti-Inflammatory Shift in B Cell Subsets of Patients with Multiple Sclerosis

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Recently, we and others have demonstrated that antibody-independent functions of distinct B cell subsets are important in mediating multiple sclerosis (MS) inflammatory disease activity. DMF has been approved for treating MS patients, yet the mode of action is not fully understood. The aim of this study is to test the impact of DMF treatment on B cell subsets in patients with MS. Peripheral blood mononuclear cells from 13 patients with RRMS were obtained pre-treatment and then at 3,6 and 12months after initiation of DMF treatment. Multi-color flow cytometry was used to define and quantify different B cell sub-populations and their cytokine response profiles. Total B cell counts decreased following initiation of DMF treatment. This decrease largely reflected loss of circulating differentiated but not of immature transitional B cells, suggesting that DMF does not impact the output of B cells from bone marrow, but preferentially impacts more mature B cell subsets. Further analysis within the mature B cells revealed that DMF has greater impact on memory than naïve B cells. Consistently, disease-implicated memory B cell subsets (including $CD80^+$and $CD11c^+$B cells) were significantly reduced with DMF treatment. Functional analysis further revealed that treatment with DMF enhanced B cell IL-10 responses, while limiting B cell GM-CSF, IL-6 and TNFα expression, overall resulting in a significant anti-inflammatory shift of B cell response. In conclusion, the capacity of DMF to limit new MS inflammatory activity may, in part, relate to its ability to mediate an anti-inflammatory shift in the balance of phenotypically and functionally distinct B cell subsets.

W.71. Novel Myasthenia Gravis Microarray Panel to Determine Specific Epitope Targets of Disease Subtypes

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Myasthenia gravis (MG), a chronic neuromuscular autoimmune disease, is primarily characterized by fluctuating muscle weakness in various skeletal muscle groups that are generally ocular, bulbar, proximal extremity, or respiratory in nature. Patients with MG are broadly clustered into five subtypes based on the location and severity of muscle weakness. Although the disease mechanisms are not fully understood, autoantibodies against neuromuscular proteins such as acetylcholine receptor (AChR), muscle specific kinase (MuSK), and low-density related lipoprotein 4 (LRP4) have been shown to be involved in the pathogenic process. Our goal was to characterize the antibody profile of patients with MG to identify neuromuscular proteins as well as specific epitopes within the previously studied proteins (AChR, MuSK, and LRP4) that are potential targets of autoantibody-mediated disease pathogenicity. Using a novel in-house microarray panel probed with MG patient sera, we found that the most highly targeted epitopes of antibodies from MG patients are within the AChR gamma subunit extracellular region and the MuSK extracellular region. Specifically, we also found that patients with oculobulbar manifestations have increased antibodies to the AChR epsilon subunit. Patients with respiratory presentations display elevated levels of antibodies to receptor-associated protein of the synapse (RAPSYN) and potassium channel 4.1 (KIR4.1) epitopes. Taken together, our results show that the subtypes of MG vary in the expression of antibodies targeting different proteins and epitopes. Our work has the potential to develop a novel predictive panel to better distinguish between the disease subtypes in order to provide more a tailored, effective treatment for MG.

W.72. Clinical Application of Frozen Vitamin D3-Tolerogenic Dendritic Cells

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Background: Vitamin-D3-induced tolerogenic dendritic cells (tolDC-VitD3) loaded with myelin autoantigens are able to abrogate disease progression in mouse experimental autoimmune encephalomyelitis (EAE). *In vitro* studies, tolDC-VitD3 of multiple sclerosis (MS) patients have demonstrated specific inhibition of lymphocyte proliferation and of IFN-g production, increasing IL-10 levels in co-culture experiments. With the aim to translate this cell-based therapy to the clinic, GMP grade tolDC-VitD3 have been developed and a multicentric phase I/IIa clinical trial in MS patients is going to start imminently in Europe. Frozen human tolDC-VitD3 have shown the same functionality as fresh ones, but so far, no *in vivo* experiments with frozen tolDC-VitD3 have been performed.

Objective: To demonstrate the *in vivo* functionality of frozen tolDC-VitD3 in EAE.

Methods: tolDC-VitD3 were differentiated and cryopreserved in medium with 50% FBS+10% DMSO. Thawed tolDC-VitD3-MOG cells (1·10^6 cells) or PBS (sham) were administrated to C57BL/6-EAE induced mice after the onset of clinical signs. Mice were monitored daily for 74 days.

Results: Treatment with frozen tolDC-VitD3 abrogated clinical progression of the disease ($p<0.001$) and reduced MOG-specific proliferative response ($p=0.004$) compared to control mice, and similarly to fresh tolDCVitD3 cells. Treatment was well tolerated. Interestingly, the therapeutic effect of the cells after each administration was progressively increased, extending the interval dosing required. Treated mice had an increase of regulatory T and B cells ($p<0.05$) and a reduction of NK cells ($p<0.05$).

Conclusion: These results show that frozen tolDC-VitD3 keep their *in vivo* beneficial clinical effect, increasing their feasibility for its translation to the clinic.

W.73. CREB Binding Protein Regulates Interferon **β** Signaling and Interferon **β**-mediated Regulation of Tumor Necrosis Factor **α** Production by Human Monocytes

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Background: Interferon (IFN) β is widely used as a first-line treatment for multiple sclerosis (MS), but both its therapeutic mechanism and the reason that some patients are unresponsive to treatment are not clearly understood. In this study, we analyzed the effect of IFNβ on tumor necrosis factor (TNF) α production by monocytes, as well as the role of CREB binding protein (CBP), in IFNβ signaling and TNFα regulation by IFNβ. Subjects and methods: Venous blood samples were obtained from 12 healthy subjects, 13 untreated MS patients, and 9 MS patients treated with IFNβ. Monocytes isolated by magnetic beads were stimulated with lipopolysaccharide (LPS) for 24 h, and TNFα in the supernatant was quantified by enzyme-linked immunosorbent assay. In some experiments, monocytes were pre-incubated with recombinant IFNβ and C646, a small molecule inhibitor of CBP, before stimulation, and the effects on STAT1 phosphorylation, the induction of IFN-responsive gene, and the production of TNFα were analyzed. Results: Monocytes from IFNβ-treated MS patients produced more TNFα compared with monocytes from untreated MS patients. IFNβ enhanced TNFα production by monocytes from healthy subjects *in vitro*. C646 suppressed IFNβ-induced phosphorylation of STAT1, and the induction of MxA, IRF2, and BAFF mRNA in monocytes. The IFNβ-mediated augmentation of TNFα production by monocytes was further enhanced by C646. Conclusion: IFNβ enhanced LPS-induced TNFα production by human monocytes. CBP was involved in the regulation of IFNβ signaling and IFNβ-mediated augmentation of TNFα production by monocytes. Responsiveness to IFNβ therapy in MS might be regulated by CBP.

W.74. New Insights Into the Contributions Made by B Cells to Myasthenia Gravis Immune Dysregulation

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Myasthenia gravis (MG) is an autoimmune condition in which neurotransmission is impaired by the binding of disease-causing autoantibodies to acetylcholine receptors. The mechanisms underlying autoantibody production are not well understood. We investigated whether the autoimmune mechanisms contributing to MG include compromised B cell tolerance and distorted B cell repertoires; both of which have not been explored in MG. Validated assays to assess B cell tolerance checkpoint fidelity were performed. The frequency, phenotype and repertoire of B cell subsets were determined using flow cytometry and next-generation B cell repertoire sequencing. Abnormally high frequencies of self-reactive naïve B cells accumulate in MG implicating a breach in tolerance. Consistent with these tolerance defects was a distorted naïve B cell repertoire revealed by sequencing. Circulating, antibody-producing plasmablasts were present in MG subjects and positively associated with more severe disease activity. B cell sequencing identified abnormal clonal expansions of both memory B cells and plasmablasts that may directly contribute to the production of circulating pathogenic autoantibodies (currently under investigation). In summary, first we demonstrate that the naïve B cell repertoire in MG is abnormally formed as a consequence of B cell tolerance checkpoint defects. This represents a fundamental component of autoimmunity contributing to the initiation of this disease, which develops prior to antigen exposure. Secondly, autoantibodies that affect disease may be produced by high frequency circulating peripheral blood plasmablasts, which are more easily targetable by therapeutics than autoantibody-producing long-lived plasma cells residing in the bone marrow. These newly described mechanistic components of MG autoimmunity are of particular importance when considering the durability of MG treatment modalities.

W.76. Interactions of B Cell and CD8 T Cell Subsets and their Implication in Multiple Sclerosis

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Antibody-independent functions of B cells are now understood to play key roles in modulating immune responses in both health and disease. Demonstrated functions of distinct B cell subsets includes their capacity to secrete different cytokines thereby modulating both $CD4^+$ T cell and myeloid cell functions. B cell:$CD4^+$ T cell and B cell: myeloid cell interactions have both been implicated in multiple sclerosis (MS) disease activity, and can be targeted therapeutically with B cell depletion treatments. Little however is known about the interaction between distinct B cell subsets and $CD8^+$ T cell subsets that are also thought to be involved in MS. We investigated whether distinct disease implicated B cell subsets may engage in bi-directional communication with $CD8^+$ T cell subsets, and how such cross talk may impact their respective functions. We found that human CpG-activated B cells suppressed both proliferation and secretion of the pro-inflammatory cytokines GM-CSF and TNF from total $CD8^+$ T cells. While the overall $CD8^+$ T cell responses were suppressed by the activated B cells, proliferative responses of mucosal-associated invariant T (MAIT) $CD8^+$ T cells were substantially increased, as was secretion of IFNγ, IL-6 and IL-10 in the same cultures. These findings highlight contrasting effects of activated human B cells on distinct $CD8^+$ T cell subsets. Further investigation is warranted to elucidate the significance of such interactions between distinct $CD8^+$ T cell and B cell subsets, and potential relevance of such interactions to the benefit of B cell targeted therapy in immune-mediated conditions such as MS.

W.77. Impact of Microglia Depletion on Neuroinflammation

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Microglia are highly specialized and long-lived macrophages that comprise 20% of all glial cells in the central nervous system (CNS). Recent studies have demonstrated that depleting microglia leads to early impairment of post-synaptic pruning and a subsequent rapid repopulation program from brain resident progenitors. However, the impact of microglia ablation on the long-term state of the CNS inflammatory environment has yet to be determined. Here, we specifically depleted microglia using the CX3CR1-CreERT2 mouse system to assess their functional role in controlling both homeostatic and autoimmune-driven neuroinflammation. Notably, microglia depletion triggered a severe ataxia phenotype starting at day 10 days post depletion, which was characterized by loss of motor coordination and mortality. Moreover, ataxic mice demonstrated atypical grey matter microgliosis and associated neuronal apoptosis in the somatosensory region of the cortex. Transcriptomic and mass cytometry analysis of microglia isolated from ataxic mice revealed acquisition of an atypical activation state and loss of homeostatic gene signature. Blunting CNS inflammation and restoring microglia homeostasis reduced ataxic symptoms and protected mice from mortality. Finally, depletion of microglia during experimental autoimmune encephalomyelitis (EAE) induced worsening of disease and mortality, however spinal cord lesions and CNS T cell responses were unaffected. Instead, depletion triggered pronounced recruitment of Ly6C$^+$ inflammatory monocytes, atypical grey matter microgliosis and neuronal loss in the brain. Collectively, we identified and characterized a novel neurodegenerative activation state of grey matter microglia that occurs independently of autoimmune neuroinflammation.

W.78. Chemokine Receptor Expressions on CD4$^+$T Cells in Chronic Inflammatory Demyelinating Polyneuropathy

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Background: Chronic inflammatory demyelinating polyneuropathy (CIDP) is a chronic disease of the peripheral nervous system presumably mediated by pathogenic Th1 or Th17 cells. The purpose of this study is to elucidate the disease-relevant T cell subset of CIDP.

Methods: CIDP patients fulfilled EFNS/PNS criteria (n=27) and age- and sex-matched control subjects (n=22) were enrolled. Peripheral blood T cells were analyzed by flow cytometry to detect a disease-relevant T cell subset defined by chemokine receptor expression. Cytokine-producing capacity and transcription factors of the subset were measured by flow cytometry, enzyme-linked immunosorbent assay (ELISA) and real-time PCR. Expression of adhesion molecules and the p-glycoprotein (p-gp) were analyzed by flow cytometry. Finally, the correlation between immunological data and clinical parameters was evaluated.

Results: CCR5$^+$CCR6$^+$CD4$^+$ T cells in the peripheral blood were selectively decreased in the CIDP patients. T cells of this subset exhibited features of both Th1 and Th17 cells, high melanoma cell adhesion molecule (MCAM) expression, and high frequency of p-gp$^+$ cells among helper T cells.

Discussion: The decreased frequency of CCR5$^+$CCR6$^+$ CD4$^+$ T cells in CIDP PBMC may reflect the accumulation of this subset in demyelinating lesions. The expression of MCAM, implicated in the pathogenesis of multiple sclerosis, may relate to the invading capacity of this subset into the CIDP lesions. As P-gp is involved in drug-effluxing machinery, this subset may constitute a drug resistant population. Conclusion: CCR5$^+$CCR6$^+$ Th cells are candidate pathogenic Th17 cells in CIDP.

W.79. AD Genes: Perturbing Neuroimmunity Leads to Pathology

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Background: Chronic brain inflammation, characterized by microglia activation and macrophage infiltration, is described as one of the features of Alzheimer's disease (AD). In addition to the long established role of APOE in AD, many genes identified in AD genome-wide association studies (GWAS) are implicated in inflammation, including CD33, PTK2B, and TREM2. Recently, our group has reported that the CD33 risk variant rs3865444 modulates TREM2 expression and that the NME8 risk variant rs2718058 modulates PTK2B expression in human monocytes.

Hypothesis: The level of protein expression of CD33, PTK2B, TREM2 and NME8 and the type of cells in which they are expressed may be related to AD pathophysiology.

Materials and Methods: Using human post-mortem tissue of AD and non-AD patients from two prospective studies (N=150), we performed immunohistochemical staining for CD33, TREM2, PTK2B and NME8 protein in frontal cortex and co-stained for cell-specific markers for neurons (NeuN), microglia (IBA1) and astrocytes (GFAP). Protein expression was analyzed according to the type of cells and to the clinical status of the patient.

Results: The immunohistochemical co-staining revealed that CD33 and PTK2B are expressed predominantly by microglia/macrophages whereas NME8 is mostly expressed by neurons in the cortex of AD patients.

Discussion: This study has begun to uncover the interconnections among AD susceptibility genes in microglia/neurons and to evaluate how this emerging immune molecular network relates to various cognitive and pathologic features of AD.

W.80. Autoimmune Recognition of Sulfatide by Human Invariant Natural Killer T Cells

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Natural Killer T (NKT) cells are innate-like lymphocytes that include cells that recognize lipid antigens presented by CD1d. The prototypical antigen, alpha galactosylceramide (αGalCer) defines a subset termed invariant NKT cells (iNKT). The physiological antigen(s) recognized by these cells remain to be determined, although a number of candidates have been described. Here we report the surprising finding that human, but not mouse, iNKT cells recognize myelin-derived sulfatide as well as sulfatide isolated from cerebrospinal fluid (CSF) apolipoprotein E (apoE). Sulfatide is not recognized by mouse iNKT cells, we propose, due to unique features of the human invariant NKT cell receptor, thus demonstrating an important physiologic distinction between mouse and human NKT cell biology. The activity of sulfatide in CSF is associated with apoE, and is enhanced upon exposure to serum lipoproteins, components which cross the blood brain barrier (BBB) in early multiple sclerosis (MS) lesions. Thus, we have identified myelin-derived sulfatide as a self-lipid recognized by human iNKT cells, and propose that sulfatide exposure in the context of CSF apoE or serum lipoproteins may be an important pathologic feature of MS or other neurologic diseases involving BBB breakdown.

W.81. Cytomegalovirus Infection Exacerbates Autoimmune Mediated Neuroinflammation

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Cytotoxic $CD4^{+}CD28^{null}$ T cells arise during chronic activation of the immune system and are present in a subset of patients with multiple sclerosis (MS), a disabling autoimmune disease of the central nervous system (CNS). In this

study, we focused on cytomegalovirus (CMV) as a potential driver for $CD4^+CD28^{null}$ T cell expansion and its potential role in aggravating MS.

Our association study between CMV serology and $CD4^+CD28^{null}$ T cells in MS patients and healthy controls (HC), demonstrated that $CD4^+CD28^{null}$ T cell expansions are predominantly present in CMV+ donors ($p<0.0001$) and that CMV-specific IgG titers correlate with the percentage of these cells ($\rho_s=0.6$, $p<0.0001$). Chronic stimulation of peripheral blood mononuclear cells (PBMCs) with CMV peptide *in vitro* resulted in expansion of pre-existing $CD4^+CD28^{null}$ T cells. In murine CMV (MCMV) infected mice, we observed the formation and expansion of $CD4^+CD28^{null}$ T cells over time ($p<0.0001$). In an experimental autoimmune encephalomyelitis (EAE=mouse model for MS) model, an increased percentage of peripheral $CD4^+CD28^{null}$ T cells was found to correlate with a worsening of clinical symptoms ($\rho_s=0.6$, $p=0.0002$). Pre-exposure to MCMV further aggravated EAE disease (mean cumulative score: $p<0.01$), which was paralleled by augmented peripheral expansion of $CD4^+CD28^{null}$ T cells, increased splenocyte reactivity to myelin oligodendrocyte protein (MOG, $p<0.01$) and higher levels of demyelination in the spinal cord ($p<0.05$). Cytotoxic $CD4^+$ T cells were identified in these demyelinated regions, suggesting that peripherally expanded $CD4^+CD28^{null}$ T cells migrate towards the CNS to inflict damage.

Taken together, we demonstrated that CMV enhances the expansion of $CD4^+CD28^{null}$ T cells, thereby boosting the activation of disease-specific $CD4^+$ T cells and aggravating autoimmune mediated inflammation and demyelination.

W.82. Synaptic Autoantibody Accompaniments and Neurological Manifestations of Thymoma

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Thymoma is recognized in association with paraneoplastic autoimmune myasthenia gravis (MG), an IgG-mediated impairment of synaptic transmission targeting the nicotinic acetylcholine receptor (AChR) of muscle. To investigate the frequency of potentially pathogenic synaptic autoantibodies with thymoma, we identified 193 patients in the Mayo Clinic Neuroimmunology Laboratory database with serum available to test for autoantibodies reactive with molecularly-defined synaptic proteins of muscle, peripheral and central nervous systems.

Patients were classified in four groups: I) lacking neurological autoimmunity (n=43); II) isolated MG (n=98); III) MG plus additional neurological manifestations (n=27); IV) neurological autoimmunity other than MG (n=25). The mean age (52 years) did not differ significantly by sex or group. MG was the most prevalent clinical manifestation (65%) followed by dysautonomia (8%) and encephalopathy (5%). Synaptic autoantibodies were more frequent in patients with neurological autoimmunity, and most frequent in patients with neurological manifestations other than or in addition to MG. Overall, 159 patients (82%) had at least one synaptic autoantibody, muscle AChR being most frequent (78%). Next most frequent were autoantibodies specific for ganglionic AChR (20%), voltage-gated Kv1 potassium channel-complex (VGKC-complex) (13%) and alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor (5%). Less frequent specificities were aquaporin-4, VGKC-complex-related proteins (leucine-rich glioma-inactivated 1 and contactin-associated protein-like 2), glycine and γ-aminobutyric acid-A ($GABA_A$) receptors.

Synaptic autoantibodies, particularly those specific for ion channels of the ligand-gated nicotinic AChR superfamily (including AMPA, $GABA_A$ and glycine receptors), are highly prevalent in patients with thymoma, with or without neurological manifestations. Evaluating an extended synaptic autoantibody profile may aid preoperative diagnosis of thymoma.

T.83. A Rare Case of Stiff Person Syndrome Manifesting in a Patient with CVID

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Background: Stiff Person Syndrome (SPS) is a rare disease affecting less than 1 in one million individuals which is easily confused with neurological and psychiatric disorders before being diagnosed. It usually presents between the third and seventh decade of life, with a rare incidence in adolescents and is associated with anti-glutamic acid decarboxylase antibodies. Case report:This case is a 26-year-old woman with evidence of immune deficiency since birth with numerous unusual childhood infections and hospitalizations, who was not diagnosed with common variable immune deficiency (CVID) until it progressed to disseminated Mycobacterium fortuitum infection at age 18. She concurrently exhibited symptoms of neuropathy, Bell's palsy and muscle spasms since age 11 which progressed to worsening dystonia and gait disturbance rendering her unable to ambulate at age 16. EMG results corresponded with her symptoms and a diagnosis of a rare neurological condition known as SPS was made at age 24. Conclusion: SPS symptoms include painful, sporadic muscle spasms of the extremities, abdomen, face and neck which manifest on EMG as continuous low frequency motor unit activity occurring in agonist and antagonist muscles simultaneously. Her SPS symptoms improved greatly after initiation of low dose IVIG twice monthly for the CVID. She slowly regained the ability to walk with this therapy. CVID is a heterogeneous immunodeficiency disease with decrease in antibody production due to unknown etiology. Patients present with recurrent encapsulated bacterial infections in the respiratory tract, skin, urinary tract and central nervous system.

Autoimmune Rheumatologic Disease

OR.15. Sequencing the Plasmablast Antibody Repertoire in Systemic Lupus Erythematosus to Discover Pathogenic Complement Factor Autoantibodies

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Thrombotic Microangiopathy (TMA) is the triad of microangiopathic hemolytic anemia, thrombocytopenia, and organ dysfunction caused by abnormalities in vascular endothelium with resultant thrombosis. TMA is seen in up to four percent of patients with systemic lupus erythematous (SLE) and can be autoantibody mediated. The hallmark antibody mediated TMAs are thrombotic thrombocytopenic purpura (TTP) and atypical hemolytic uremic syndrome (aHUS). Although they share laboratory findings and symptoms, their pathophysiology differs. In aHUS, antibodies to complement factor H (CFH) inhibit normal protein function leading to endothelial damage, and increased alternative complement pathway activation on cellular surfaces resulting in membrane attack complex (MAC) formation and cytotoxiticy. We hypothesize that aHUS/SLE patients generate additional complement factor antibodies that drive disease through complement dysregulation. To test this, we are seqeuncing the plasmablast antibody repertoires of patients with TMA and SLE using a DNA barcoding method that sequences the cognate heavy-and light chain pairs of antibodies expressed by individual blood plasmablasts. The data sets are bioinformatically analyzed to generate phylogenetic trees that identify clonal families of antibodies sharing heavy- and light-chain VJ sequences. Representative antibodies are expressed and their binding properties analyzed by (i) complement factor ELISAs and (ii) a complement factor antigen array being developed in our laboratory. The functional properties of recombinant antibodies are being assessed by (i) a MAC activation ELISA and (ii) a sheep red blood cell lysis assay. We are working to demonstrate that TMA in SLE and aHUS is driven by both anti-CFH and other complement factor autoantibodies.

OR.21. Dysregulation of Adaptive Immunity Appears Prior to and Concurrent with Autoantibody Accrual in Early SLE Pathogenesis and Predicts Transition to Classified Disease

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Systemic lupus erythematosus (SLE) is a complex autoimmune disease stemming from a poorly understood preclinical stage of immune dysregulation that leads to clinical disease. Autoantibodies and inflammatory mediators are associated with SLE, but how these factors influence disease development is unknown. This study evaluates timing and changes in SLE-linked autoantibodies and soluble mediator pathways in the years preceding SLE classification. Serial sera from 84 SLE cases spanning pre- and post-SLE classification (average time = 5.98 years) and matched healthy controls (HC) were obtained from the Department of Defense Serum Repository. Adjusting for multiple comparison, a number of soluble mediators, including IL-5 ($q=4.35x10^{-6}$), IL-6 ($q=8.26x10^{-6}$), and IFN-γ ($q=0.037$), were significantly elevated in cases vs. HC >3.5 years pre-classification, prior to (IL-5 and IL-6) and concurrent with (IFN-γ) the earliest SLE-specific autoantibody specificity, anti-Ro/SSA. Innate (IFN-α), Th_1-type (IL-12, IFN-γ), Th_{17}-type (IL-23, IL-21), chemokines (MIG, IP-10) and TNF superfamily (BLyS, APRIL) soluble mediators increased longitudinally in cases approaching SLE classification, but not in HC ($q\leq0.04$). In particular, serum levels of BLyS ($q=0.003$) and APRIL ($q=0.019$) were comparable in cases and HC until <10 months pre-classification. Random forest models incorporating IL-5, IL-6, and IFN-γ levels (79-82% accuracy) identified future SLE patients better than models with ANA alone (58% accuracy) >3.5 years before classification. These results reveal a progression of immune dysregulation leading to SLE classification. Immunological profiles identifying individuals who develop clinical SLE may be useful for delineating early pathogenesis, discovering therapeutic targets, and designing prevention trials.

OR.22. Autoantibodies Targeting TLR and SMAD Pathways Define New Subgroups in Systemic Lupus Erythematosus

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The molecular targets of the vast majority of autoantibodies in systemic lupus erythematosus (SLE) are unknown. Using a baculovirus-insect cell expression system to create an advanced 1545 full-length protein microarray with improved protein folding and epitope conservation, we assayed sera from three independent cohorts of SLE individuals (total n=277) and age, gender and ancestry-matched controls (n=280). We identified 103 novel autoantigens in SLE sera (FDR<0.01) and showed that SLE autoantigens are distinctly clustered into four functionally related groups. The original SLE autoantigens Ro60, La, and SMN1/Sm complex formed a distinct antigen cluster (SLE1a), extended by a second cluster (SLE1b) of proteins involved in RNA/DNA/chromatin processing. The largest two clusters of novel autoantigens revealed two networks of interconnected proteins: the receptor-regulated SMAD2, SMAD5 and proteins linked to TGF-β signalling (SLE2); and the TLR adaptor MyD88 and multiple key proteins involved in TLR signalling, regulation of NF-κB, and lymphocyte development (SLE3). SLE patients clustered into four groups with autoantibody responses against networks of proteins with related cellular or immunological functions, suggesting that different pathogenic mechanisms underlie the four SLE subgroups. ROC curve analysis of antibody biomarker panels showed improved diagnostic sensitivity/specificity compared to anti-nuclear and anti-dsDNA antibody tests. Of particular clinical importance, autoantibodies from cluster SLE1b and SLE3 enabled detection of SLE individuals who were negative for conventional anti-nuclear or anti-dsDNA antibody tests. The novel autoantibody clusters identified in this study represent a major advance in SLE diagnostics, and define new subgroups in SLE, each of which may require different therapeutic strategies.

OR.23. Circulating miR-483-5p Can Regulate Fibroblasts Activation and Constitutes a Potential Biomarker for the Diagnosis of Systemic Sclerosis

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Systemic sclerosis (SSc) is a severe autoimmune disorder characterized by skin and internal organ fibrosis, vascular dysfunction and immune dysregulation. MicroRNAs are small non-coding RNAs that represent crucial regulators of gene expression and their abnormal levels are implicated in several disease conditions, including autoimmunity. The presence of microRNAs in biological fluids, their stability and association with specific diseases, candidate microRNAs as good potential biomarkers. With the aim to diagnose early-stage SSc and study the implication of circulating microRNAs in the pathogenesis of the disease, this project evaluated the levels of 750 microRNAs in the serum of 27 SSc patients, as compared to 10 healthy subjects, by using an RT-qPCR-based array. Among 33 differentially expressed microRNAs, miR-483-5p was further validated as the strongest up-regulated microRNA in the serum of additional 130 SSc patients and subjects showing preclinical SSc symptoms (early-SSc) (Kruskal-Wallis, $p \leq 0.0001$). Interestingly, miR-483-5p was not differentially abundant in patients with SLE or Sjögren's syndrome, indicating a specific association of this microRNA with SSc. Furthermore, we demonstrated that the circulating miR-483-5p was embedded in extracellular vesicles, suggesting that it can be released by activated cells, possibly mediate cell-to-cell communication between affected tissues, and therefore be implicated in SSc pathogenesis. Supporting this hypothesis, we showed that the overexpression of miR-483-5p in fibroblasts can induce markers of fibrosis and myofibroblast differentiation, pathological hallmarks of SSc. Overall, miR-483-5p could provide both a potential biomarker for the identification of SSc at early stages and also represent a potential novel therapeutic target for this disease.

OR.24. Reduced Modulation of GATA-3 Activity by T-bet in $CD8^+$ T Cells from Patients with Systemic Sclerosis Leads to IL-13 Over-production and Cutaneous Fibrosis

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The type-2 cytokine interleukin (IL)-13 is a main pro-fibrotic factor in systemic sclerosis (SSc), a connective tissue disease characterized by vasculopathy, inflammation and fibrosis. In previous studies we demonstrated that IL-13-producing $CD8^+$ T cells play a critical role in cutaneous fibrosis, the most characteristic feature of SSc, and we showed that IL-13 over-production by SSc $CD8^+$ T cells is caused by up-regulation of the Th2-specific transcription factor GATA-3. Here we investigated the molecular mechanism underlying GATA-3 up-regulation by SSc $CD8^+$ T cells. We focused on the role played by the Th1-specific transcription factor T-bet, which induces IFN-g production and inhibits type-2 cytokines by antagonizing GATA-3 expression and/or function. We found that patient $CD8^+$ T cells express high levels of IL-13 and GATA-3 but similar levels of IFN-g and T-bet compared to controls. However, the levels of the active phosphorylated form of T-bet as well as the physical interaction between T-bet and GATA-3 are reduced in the nucleus of SSc $CD8^+$ T cells, allowing more GATA-3 to bind to the IL-13 promoter and induce IL-13 expression. We established that this defect results from the binding of T-bet to the adaptor protein 14-3-3 in the cytosol of $CD8^+$ T lymphocytes from patients, which reduces T-bet translocation into the nucleus and its modulation of GATA-3 activity. Our new insights into disease pathogenesis will establish novel biomarkers of immune dysfunction in SSc patients that can be used as therapeutic targets.

OR.26. Single Cell Gene Expression Studies in Lupus Patient Monocytes Reveal Novel Patterns Reflecting Disease Activity, Interferon, and Medical Treatment

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Background: Our previous studies have shown that different cell types from the same sample demonstrate diverse gene expression, and important findings can be masked in mixed cell populations. In this study, we examine single cell gene expression in SLE patient monocytes and determine correlations with clinical features.

Methods: $CD14^{++}CD16^{-}$ classical monocytes (CLs) and $CD14^{dim}CD16^{+}$ non-classical monocytes (NCLs) from SLE patients were purified by magnetic separation. The Fluidigm single cell capture and Rt-PCR system was used to quantify expression of 87 monocyte-related genes.

Results: Both CLs and NCLs demonstrated a wide range of expression of IFN-induced genes, and NCL monocytes had higher IFN scores than CL monocytes. Unsupervised hierarchical clustering of the entire data set demonstrated two unique clusters found only in SLE patients, one related to high disease activity and one related to prednisone use. Independent clusters in the SLE patients were related to disease activity (SLEDAI 10 or greater), interferon signature, and medication use, indicating that each of these factors exerted a different impact on monocyte gene expression that could be separately identified. A subset of anti-inflammatory gene set expressing NCLs was inversely correlated with anti-dsDNA titers (rho = -0.77, p=0.0051) and positively correlated with C3 complement (rho = 0.68, p=0.030) in the SLE patient group.

Conclusion: Using single cell gene expression, we have identified a unique gene expression patterns that reflect the major clinical and immunologic characteristics of the SLE patients which are not evident in bulk cell data, supporting the critical importance of the single cell technique.

OR.27. The Interface Between the Skin Microenvironment and Systemic Immunity Shapes the Inflammatory Immunome in Systemic Sclerosis: a Multidimensional, High Throughput Analysis

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Pathogenic immune responses can be profoundly shaped by the interplay between the periphery and the microenvironment. In this work we aimed at defining the immune mechanisms at the interface between the skin microenvironment and the periphery that are relevant for the disease pathogenesis in Systemic sclerosis (SSc), an autoimmune disorder of the connective tissue. We integrated in the same experimental flow: mass cytometry (CyTOF) for the identification of disease-specific immune signatures (35 T cell specific markers), next generation RNA seq to characterize molecular patterns of antigen-specific T cells, Nanostring to understand the molecular characteristics of the skin microenvironment and various functional assays to validate the findings. Using this approach we interrogated peripheral blood (n=23) and lesional skin derived T cells (n=5) from SSc subjects and compared them with healthy controls (n=13). Pathogenic Th17/Treg cells were enriched in SSc subjects both in the periphery and in the skin microenvironment. RNA seq analysis revealed skin derived T cells had elevated expression of IL-11 receptor (IL-11RA). Skin T cells expressing IL-17 induced the expression of IL-11 in skin fibroblasts. We discovered that at the interface between the systemic and the skin microenvironment a self reverberating loop centered on the IL-17, IL-11 and IL11-RA triad expands and maintains disease-specific and probably pathogenic Th17 T cell subsets. Our findings have a dual translational valency both for targeted therapies and for understanding immune pathogenesis of SSc.

OR.31. Downregulation of CD3ζ in NK and NKT Cells Contributes to SLE Pathogenesis

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NK and NKT cells have an essential role in the regulation of autoimmunity through their cytotoxic and cytokine production functions both of which are altered in systemic lupus erythematosus (SLE). CD3ζ is an important signaling molecule in T, NK and NKT cells. CD3ζ has been described diminished in SLE T cells, resulting in their dysfunction. Whether CD3ζ is decreased in NK and NKT cells from SLE patients and whether decreased levels alter their function is unknown.

We hypothesized that CD3ζ is downregulated in NK and NKT cells from SLE patients and accounts for their decreased cytotoxic function, increased IFNγ production and increased ability to infiltrate tissues.

CD3ζ expression levels, cytotoxicity and cytokine production of NK and NKT cells were analyzed in peripheral blood from 16 SLE patients and 10 healthy donors as well as in CD3ζ-deficient mice using flow cytometry, co-cultures with target cells and different stimulations. Tissue infiltration of NK and NKT cells was analyzed in CD3ζ-deficient mice.

CD3ζ was found decreased in NK and NKT cells from SLE patients. Decreased CD3ζ levels in SLE NK cells correlated inversely with natural cytotoxicity and directly with CD16, an important protein in antibody-dependent cell-mediated cytotoxicity. Additionally, NK and NKT cells from CD3ζ-deficient mice were found to infiltrate target organs including the kidney and produce more IFNγ contributing to tissue inflammation in these mice.

CD3ζ downregulation is a common feature in SLE T, NK AND NKT cells and compromises their function by conferring a proinflammatory phenotype characterized by IFNγ production and tissue infiltration.

OR.38. Early-onset Severe Arthritis Associated with a *De Novo* Gain-of-function Germline Mutation in MYD88

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We evaluated a 9-year old female with progressively deforming polyarticular juvenile idiopathic arthritis (JIA) and an intermittent erythematous rash since age 2. Synovial biopsy revealed a prominent neutrophilic infiltrate with scant mononuclear cells. We further evaluated the patient and family members by whole exome sequencing (WES), peripheral blood phenotyping, and functional studies of monocytes and dermal fibroblasts. WES revealed a *de novo* heterozygous missense mutation in MYD88 (c.666T>G, p.Ser222Arg), which was confirmed by Sanger sequencing and determined to be germline. Immunophenotyping showed an absence of $CD16^+$ monocytes, an expansion of $CD4^+$ Th17 T cells, and the presence of a previously uncharacterized $CD123^+CD11c^+$ dendritic cell population, as well as markedly increased basal and stimulated p-STAT3 in monocytes and T lymphocytes in the patient. Monocytes exhibited an interferon gene expression signature and increased expression of neutrophil and monocyte chemokines at baseline and with LPS-stimulation. Whole blood secretion of TNF-α, IL-8, IL-6, CXCL1, and MCP-1 was also increased at baseline and with LPS-stimulation. Fibroblasts exhibited ~10-fold greater baseline CXCL1 and IL-8 expression over controls. We generated MYD88-knockout THP-1 cells expressing wild type MYD88 or S222R-MYD88 GFP fusion proteins, and found increased NF-κB activation at baseline and with stimulation in S222R-MYD88-expressing cells. Loss-of-function MYD88 mutations result in immunodeficiency, and gain-of-function (GoF) somatic mutations have been reported in B cell lymphoma. This is the first description of a *de novo* germline MYD88 GoF mutation associated with severe arthritis and suggests the mutation causes a significant increase in innate immune activation through this critical adapter molecule.

W.12. Alpha(v) Integrins Engage Autophagy Components to Regulate B Cell Immune Response

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Aberrant activation of B cells by self-antigens is a key feature of autoimmune diseases like SLE. Signaling through Toll-like receptors (TLR) such as TLR7 and TLR9 has been implicated in B cell activation by self-antigens and development of autoimmunity. Our recent work based on murine models has identified a novel mechanism by which a family of adhesion molecules called alpha(v) integrins and the autophagy pathway limit excessive B cell responses to antigens containing TLR ligands including self-antigens.

Specifically, we have found that alpha(v)beta3 integrin regulates B cell TLR signaling by directing trafficking and maturation of TLR containing endosomes and activating autophagy components which down-regulate TLR signaling. When B cells lack either alpha(v) integrins or autophagy components LC3 and Atg5 , endosomal trafficking of the TLRs is disrupted and this leads to enhanced TLR signaling and increased B cell responses. We have used alpha(v) conditional knockout mice to determine the consequences of loss of this alpha(v)beta3-mediated immune regulation. Mice lacking B cell alpha(v) show increased antibody responses to antigens containing TLR-ligands and develop increased levels of autoantibodies.

We see a similar regulation of TLR signaling by the autophagy components in human B cell subsets and therefore propose that alpha(v) and autophagy components limit potential pathological self-reactive B cell responses that lead to autoimmune diseases. Polymorphisms in some of the autophagy components have also recently been linked to development of autoimmune diseases and we are investigating how the disruption of the regulatory mechanism we describe may contribute to human autoimmune diseases.

W.13. BANK1 Regulates IgG Production in a Lupus Model by Controlling STAT1 Activation

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The purpose of our study was to investigate the effects of the adaptor Bank1 in TLR7 signaling using the B6.*Sle1.yaa* mouse, a lupus model that develops disease through exacerbated TLR7 expression. Crosses of B6.*Sle1.yaa* with *Bank1*$^{-/-}$ mice maintained several B and myeloid cell phenotypes close to normal wild-type levels. Most striking was the reduction in total serum IgG antibodies, but not of IgM, and reduced serum levels of autoantibodies, IL-6, and BAFF. *Bank1* deficiency did modify numbers of MZ B cells and total B cell numbers, as well as expression of CXCR4 by follicular helper T cells. Other T cell changes were not observed. *Bank1* deficiency did not modify numbers of germinal center B cells or plasma cells or clinical disease outcomes. Purified B cells from *Bank1* deficient mice had strongly reduced *Ifnb*, *Ifna4, Irf7*, *Aicda* and *Stat1* gene expression following TLR7 agonist stimulation. Interestingly, phosphorylation of Tyr701, but not of Ser727 of STAT1, was impaired in splenic B cells from *B6.Sle1.yaa.Bank1-/-* mice, as was the nuclear translocation of IRF7 in response to TLR7 agonist stimulation. Further, *Bank1* deficiency in B6.*Sle1.yaa* mice reduced the production of IgG2c after *in vitro* TLR7 agonist stimulation. Our results demonstrate that *Bank1* controls TLR7-mediated type I interferon production. Combined with the control of the nuclear translocation of IRF7, and the modulation of transcription and STAT1 phosphorylation, *Bank1* contributes to IgG production during development of autoimmune disease.

W.14. Btk Inhibition Treats TLR7/IFN Driven Murine Lupus

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Bruton's tyrosine kinase (Btk) is expressed in a wide variety of immune cell types and previous work using Btk inhibitors and Btk deficient mice has demonstrated that blocking Btk is a promising therapeutic strategy for treating autoimmune diseases such as systemic lupus erythematosus (SLE). Btk has been demonstrated to regulate signaling downstream of both the B cell receptor (BCR) and Fc receptors (FcR).Thus, Btk inhibition may be a highly

efficacious therapeutic approach as it can inhibit disease through multiple mechanisms of action. Herein, we utilized a tool Btk inhibitor, M7583, to determine the therapeutic efficacy of Btk inhibition in two mouse lupus models driven by TLR7 activation and type I interferon. In BXSB-*Yaa* lupus mice, Btk inhibition reduced autoantibodies, decreased nephritis, and improved survival. By comparison, in the pristane-induced DBA/1 lupus model, Btk inhibition completely suppressed arthritis, but autoantibodies were only modestly impacted and the IFN gene signature was unaffected; suggesting efficacy was mediated primarily through effects on myeloid cells and inhibition of FcRs. *In vitro* studies using primary human immune cells revealed that Btk inhibition can block activation of macrophages by immune complexes and TLR7 which contributes to tissue damage in SLE. Overall, our results provide translational insight into how Btk inhibition may provide therapeutic benefit to a variety of SLE patients by affecting both BCR and FcR signaling.

W.15. Mechanisms Underlying the Activation of MYD88 by L265P and S222R Mutations

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MYD88 is an adaptor protein that mediates signaling through the interleukin-1 and certain toll-like receptors (TLRs), leading to NF-κB activation and transcription of pro-inflammatory mediators. Somatic mutations in *MYD88*, including L265P and S222R, have been demonstrated to constitutively activate NF-κB and contribute to the development of certain B cell lymphomas. We identified a patient with severe destructive arthritis and a *de novo* germline S222R mutation in *MYD88*. This prompted us to investigate the mechanism(s) by which S222R-MYD88 leads to NF-κB activation in monocytic cells, using the strongly activating, and better characterized, L265P-MYD88 for comparison. GFP fusion proteins of wild type (WT)-MYD88 and MYD88 mutants were re-expressed in MYD88-knockout THP-1 cells. After confirming constitutive activation by both mutations through an NF-κB reporter and expression of NF-κB regulated proteins, we used co-immunoprecipitation (co-IP) to identify MYD88 interacting partners. Consistent with previously published data in B cells, L265P-MYD88, but not WT or S222R-MYD88, forms a stable complex with both IRAK1 and phospho-IRAK1. Additionally, we found that L265P-MYD88 spontaneously nucleates a stable complex with IRAK4 and phospho-IRAK4, as well as the negative regulator IRAK-M, while S222R does not. These data reveal that while S222R and L265P mutations both activate NF-κB in monocytic cells, they apparently operate through distinct mechanisms. The exact mechanism of S222R activation remains elusive, though other protein interactions are being investigated. Nonetheless, these data suggest that the biological significance of activating MYD88 mutations extends beyond the realm of B cell malignancies and may be relevant to other myeloid cell mediated pathologies.

W.16. Functional Genetics of PTPN2 in Rheumatoid Arthritis: Haploinsufficiency of PTPN2 Promotes Severity of CD4$^+$ T Cell Mediated Autoimmune Arthritis

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Several genome-wide associations studies have in recent years linked polymorphisms in the *PTPN2* locus to rheumatoid arthritis (RA) and other autoimmune diseases. *PTPN2* encodes for the tyrosine phosphatase TC-PTP, an important regulator of cytokine signaling in hematopoietic cells. Disease-associated variants of *PTPN2* are believed to result in a parial loss of protein expression. Since TC-PTP plays an important role in T cell regulation, we hypothesized that reduced levels of TC-PTP in T cells can lower the threshold for autoimmunity in genetically-predisposed individuals. To model the functional genetics of RA-associated *PTPN2* variants in mice, we studied the effect of *PTPN2* haploinsufficiency in the SKG mouse, a spontaneous CD4$^+$ T cell-driven model of autoimmune arthritis.

Mice homozygous for the SKG Zap-70^{W163C} mutation develops IL-17 dependent spontaneus arthritis, which is due to reduced TCR signaling resulting in altered thymic selection and emergence of autoreactive CD4$^+$ T cells. In the SKG mouse, haploinsufficiency of *PTPN2* was sufficient to significantly increase the severity of arthritis. Importantly, transfer of *PTPN2*$^{+/-}$ CD4$^+$ T cells to RAG2$^{-/-}$ mice was sufficent to increase severity of arthritis when compared to transfer of *PTPN2*$^{+/+}$ CD4$^+$ T cells. Further investigation into the effect of *PTPN2* haploinsufficiency in T cell function revealed no alterations in thymocyte development or selection; however, arthritic *PTPN2*$^{+/-}$ SKG mice showed an increased accumulation of pathogenic Th17 cells in arthritic joints.

Together these results indicate that haploinsufficiency of *PTPN2* increases severity of autoimmune arthritis in mice likely by promoting the expansion of arthritogenic Th17 cells.

W.17. Dysregulated mTOR-dependent Signaling in Lymphocytes from Lupus Patients

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Mammalian/mechanistic target of rapamycin (mTOR) is a serine/threonine kinase that plays a central role in regulating metabolism, protein translation, autophagy, proliferation and survival. Two mTOR complexes have been described with distinct substrates. These pathways have been shown to regulate T cell differentiation. The mTORC1 complex is required for TH1 and TH17 differentiation, whereas the mTORC2 complex regulates TH2 differentiation. Lupus patients were shown to have higher mTORC1 activity as measured by phosphorylation of the ribosomal S6 protein and lower mTORC2 activity as monitored by AKT phosphorylation when compared to healthy controls. We have reproduced and extended these findings. In this report we describe the comprehensive phenotyping of a cohort of lupus patients. This phenotyping included monitoring phosphorylation of S6 ribosomal protein and AKT in B and T cell subsets. Consistent with published reports, B and T cells subsets from lupus patients had elevated basal S6 phosphorylation and lower basal AKT phosphorylation as compared to cells from healthy controls. Within the lupus cohort, basal phosphorylation of S6 in B cells, CD4 cells and CD8 cells was higher in patients with nephritis as compared to patients without renal involvement. Basal phosphorylation of AKT was also significantly lower in naïve B cells from patients with nephritis. Patients with elevated basal S6 phosphorylation in CD4 T cells also had elevated levels of IL-17A positive and IFNγ positive CD4 cells, consistent with the role of mTORC1 in regulating TH1 and TH17 differentiation. Additional biomarkers associated with dysregulated mTOR signaling in this cohort will be described.

W.18. Lupus-susceptibility Gene *CLEC16A* Represses Starvation-induced Autophagy by Enhancing mTOR Activity

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CLEC16A is genetically-linked with multiple autoimmune disorders but its functional relevance in autoimmunity remains obscure. It encodes a novel protein with a putative C-type lectin domain but likely it may not function like other C-type lectin receptors. By ectopic expression and siRNA silencing, we show that CLEC16A has an inhibitory role in starvation-induced autophagy in human epithelial cells. CLEC16A was expressed in cytoplasmic vesicles which, however, did not co-localize with LC3$^+$ autophagosomes upon autophagy induction. CLEC16A was found partially residing in the Golgi in steady state, and nutrient removal promoted peri-nuclear clustering of CLEC16A vesicles with increasing Golgi co-localization. Overexpression of CLEC16A was found to upregulate basal mTOR activity, which in turn diminished LC3 autophagic flux following nutrient deprivation. CLEC16A deficiency, on the

other hand, delayed mTOR activity in response to nutrient sensing, thereby resulted in an augmented autophagic response. We previously reported a reduced expression of CLEC16A in the peripheral leukocytes of systemic lupus erythematosus (SLE) patients. Interestingly, we also observed a negative correlation between CLEC16A expression and autophagic flux in SLE T cells. These findings suggest that Golgi-associated CLEC16A negatively regulates autophagy via modulation of mTOR activity, and may provide support for a functional link between CLEC16A expression and SLE pathogenesis.

W.19. Activation Status of Mucosal-Associated Invariant T Cells Reflects Disease Activity of Systemic Lupus Erythematosus

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Objective: Mucosal-associated invariant T (MAIT) cells are innate-like lymphocytes that express a semi-invariant TCRα chain: Vα7.2-Jα33 in humans and Vα19-Jα33 in mice. MAIT cells are restricted by the MHC-related molecule-1 (MR-1) and recognize microbial-derived vitamin B metabolites presented by MR1. MAIT cells are abundant in human peripheral blood, suggesting possible roles of these cells in various types of immune responses. In this study, we investigated whether MAIT cells are involved in the pathogenesis of systemic lupus erythematosus (SLE).

Methods: The frequency, activation status, cell proliferation, and cell death of MAIT cells were evaluated by flow cytometry. Antigen presenting cells were isolated from peripheral blood mononuclear cells (PBMCs) and co-cultured with MAIT cells in the presence of a MR1 ligand, and the expression of CD69 on stimulated MAIT cells was evaluated. PBMCs were cultured in the presence of various cytokines, and CD69 expression on MAIT cells was analyzed by flow cytometry.

Results: The frequency of MAIT cells was markedly reduced in SLE. However, the remaining MAIT cells were activated and their CD69 expression levels correlated with disease activity. Lupus monocytes exhibited increased ability to induce MAIT cell activation, compared with monocytes from healthy controls. MAIT cells were also activated by cytokines in the absence of exogenous antigens.

Conclusion: The activated status of MAIT cells reflects the disease activity of SLE. The capacity of lupus monocytes to activate MAIT cells and the elevated cytokine levels may contribute to the hyperactivated state of MAIT cells in SLE.

W.20. Engagement of SLAMF3 Enhances $CD4^+$ T Cells Sensitivity to IL-2 And Redirects Regulatory T Cell Defects in Systemic Lupus Erythematosus Patients

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Objective: Systemic lupus erythematosus (SLE) is characterized by aberrant T cell activation and a compromised IL-2 production leading to abnormal regulatory T cells (Tregs) development/function. Signaling lymphocytic activation molecule family 3 (SLAMF3) is a co-regulatory molecule implicated in T cell activation and differentiation. Here, we sought to determine how SLAMF3 functions as a co-stimulator of $CD4^+$ T cells, influences T helper differentiation and is implicated in SLE.

Methods: Naïve $CD4^+$T cells from SLE patients and control subjects were co-stimulated with SLAMF3 mAb. Surface markers, cytokines production and protein phosphorylation were assessed by flow cytometry. Smad3 and STAT5

protein-levels were determined by western blot. CD25 gene expression was analyzed by qPCR. SLAMF3 gene knockout was obtained by using a CRISPR-Cas9 system. $CD4^+$ T cells differentiation was performed using naïve $CD4^+$T cells cultured under Th1, Th2, Th17 or Treg polarizing conditions. Suppression capacity was determined by assessing the proliferation of autologous CFSE-labeled T cells.

Results: SLAMF3 ligation promotes T cell responses to IL-2 via up-regulation of CD25 in a Smad3-dependent mechanism. This promotes the activation of the IL-2/IL-2R/STAT5 pathway and enhances cell proliferation in response to exogenous IL-2. SLAMF3 co-stimulation promotes Treg cell differentiation from naïve $CD4^+$ T cells, while reducing Th1, Th2 and Th17 cytokines production. Ligation of SLAMF3 receptors on SLE naïve $CD4^+$ T cells restores IL-2 responses to levels comparable to healthy controls and promotes functional Treg cell generation.

Conclusions: These results suggest that SLAMF3 promotes may be a promising therapeutic target in SLE, through an increasing $CD4^+$ T cells sensitivity to IL-2.

W.21. Moderate Ethanol Drinking Delays the Progression of Systemic Lupus Erythematosus by Inhibiting Lipid Raft Clustering

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Objectives: Ethanol has been elaborated to have a beneficial effect on destructive arthritis. Nevertheless, the effect of ethanol on the development of systemic lupus erythematosus (SLE) remains controversial. This study was performed to determine the potential role of moderate ethanol consumption in SLE pathological progression and its specific mechanism.

Methods: We chose to use MRL/lpr mice to assess whether ethanol drinking has any impact on the development of SLE and investigated whether ethanol regulates pathologic progression of SLE through inhibiting lipid rafts.

Results: We found that 10% ethanol *in vivo* delayed disease progression and organ damage and prolonged survival. *In vitro* ethanol treatment not only inhibited the aggregation, proliferation, and activation of adhesion molecule expression and cytokine secretion of T cells but also decreased lipid raft clustering on T cells. In addition, ethanol inhibited SLE serum-induced skin inflammation and monocyte differentiation into dendritic cells (DCs). Furthermore, ethanol treatment of monocytes that were in the process of differentiating into DCs decreased lipid raft clustering.

Conclusions: These data strongly support the viewpoint that ethanol delays the disease progression of SLE by inhibiting lipid raft clustering and suggest that moderate drinking of ethanol may have a therapeutic benefit for patients with SLE.

W.22. Characterization of Intradermal Lupus IgG-induced Skin Inflammation

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Objective: Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by high levels of autoantibodies and multiorgan tissue damage. Skin injury is the second common clinical manifestation in SLE patients, but its pathogenesis remains unclear. Here, we characterize skin inflammation induced by intradermal lupus IgG.

Methods: We injected intradermally lupus serum IgG from SLE patients or lupus-prone mice and analyzed the impact of immune cells, cytokines and chemokines using gene deficient mice or normal mice.

Results: We found skin inflammation appears 3h and peaks 3d after intradermal injection and is related to the dose of injected IgG but not related to systemic disease activity. Severity of skin inflammation induced by lupus IgG is significantly decreased in mice with depletion of monocytes and in mice with TNF-a deficiency but not in mice without mature lymphocytes. Furthermore, lupus IgG can promote process of monocytes differentiation into dendritic cells (DCs) and increase the expression of proinflammatory cytokines such as TNF-α. TNF-α was found to stimulate IgG-induced DCs' maturation, and plays a major role in keratinocyte proliferation and activation.

Conclusion: These results reveal that IgG and monocytes play critical role in the development of skin lesions in SLE, and TNF-α can accelerate the process. It also indicates that skin IgG deposition exerts important role in the pathogenesis of skin injury in patients with SLE and blocking of IgG/FcR signaling pathway is a therapeutic target in skin lesions of patients with SLE.

W.23. The Features of Skin Inflammation Induced by Lupus Serum

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Objective: We recently developed a model of lupus serum-induced skin inflammation, which was used to study pathogenesis of skin injury in systemic lupus erythematosus. We further characterized the features of lupus serum-induced skin inflammation.

Methods: We characterized skin inflammation induced by lupus serum by using histopathology, immunochemistry and ELISA methods.

Results: Histopathological signs of this skin inflammation were evident within 3 hours and lasted for at least 2 weeks. The skin inflammation was characterized by an influx of monocytic, $CD11b^+$ cells and by a scarcity of T and B lymphocyte. Depletion of IgG from serum abrogated skin inflammatory response. The skin inflammation was related to lupus patients skin history but not to SLE disease activity and kind of autoantibody. Expression of TNFR1, NF-kB and MCP-1 was increased locally in skin lesion.

Conclusion: These findings suggest that this novel model has valuable in studying of pathogenesis and therapy of skin injury of SLE.

W.24. TRAF1 in Regulation of T Cell Responses and Inflammation

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TNF receptor associated factor one (TRAF1) is a signaling adaptor that links a subset of TNFRs to downstream survival signaling through NF-kB and MAP kinases. TRAF1 is critical for survival signaling downstream of 4-1BB but its role downstream of TNFR2 has been controversial. Genome-wide association studies have identified a single nucleotide polymorphism (SNP), rs3761847, in an intronic segment of the *TRAF1* gene as contributing to susceptibility to and severity of Rheumatoid arthritis. However, the effect of this SNP on TRAF1 expression or T cell biology have not been examined to date. To avoid the complications of chronic inflammation and treatment we chose to examine the effect of this SNP on TRAF1 levels and function in healthy donors. Samples were collected from 80 donors and TRAF1 levels and cytokines measured by intracellular flow cytometry on T cells from resting and anti-CD3/CD28 treated PBMC. Individuals homozygous for the risk polymorphism exhibited significantly lower TRAF1 protein levels in T cells than donors with the disease resistant genotype. T cells from the disease susceptible donor T cells produced less IFNγ, TNFα and IL-2 upon stimulation. Work is in progress to investigate how TRAF1 specifically impacts TNFR2 signaling in T cells. Taken together, our data suggest that the TRAF1 SNP results in lower TRAF1 protein and lower cytokine production by T cells, supporting the evidence that TRAF1 has a net positive role in T cell

cytokine production. However, these data also present a paradox of how lower TRAF1 protein levels contribute to increased inflammatory disease.

W.25. Characterizing the Evolution of the Anti-citrullinated Protein Autoantibody Repertoire in Rheumatoid Arthritis

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Although an adaptive autoimmune B cell response against post-translationally modified citrullinated antigens is known to be involved in rheumatoid arthritis (RA), the etiology, fine specificity, and functional properties of the anti-citrullinated protein antibodies (ACPAs) in RA remain poorly understood. We hypothesize that evaluating the antibody repertoire in the peripheral blood at different time points, as well as in paired blood and synovial tissue samples from a subset of patients, will reveal a more complete picture of the adaptive autoimmune response and elucidate the key autoantibodies, thereby providing insight into the pathogenic mechanisms underlying RA. To characterize the autoantibody response in RA, we applied a high-throughput single-cell barcoding technology to sequence paired heavy (HC) and light chain (LC) antibody genes expressed by individual B cells. Specifically, we sequenced plasmablasts from peripheral blood sampled at serial time points as well as matched synovial tissue and peripheral blood from multiple individuals with RA. We also used a citrullinated peptide-based sort reagent to detect B cells producing ACPAs. We identified lineages that persisted across the serial time points, and characterized the expansion and contraction of clonal lineages. Furthermore, we observed substantial clonal expansion in the synovial tissue and identified shared clonal lineages between the synovium and peripheral blood. Recombinant antibodies representative of the bioinformatically identified B cell clonal families are being expressed and characterized with *in vitro* assays to further define their antigen targets and the mechanisms by which they mediate synovitis and joint destruction.

W.26. Treatment Refractory Rheumatoid Arthritis: Is Repository Corticotropin Injection (RCI) an Effective Option in Patients Resistant to Biologic Therapies? (A Pilot Study)

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Despite availability of DMARDs and biologic agents, there are many patients with RA who are resistant to therapy and remain inadequately controlled. RCI may provide relief of RA and exacerbations in patients refractory to treatment. A total of 9 patients were treated for 12 weeks. Patients were on DMARDs, biologics, and a minimum of 7.5 mg prednisone daily. RCI 40 U was given daily for 7 days. If there was adequate response in DAS28-CRP, patients were given 40 U twice weekly through Week 12. For inadequate responses, patients received 80 U daily for 7 days, followed by 80 U twice weekly through Week 12. The primary endpoint was >1.2 point reduction in DAS28-CRP at Week 12. Secondary endpoints were improvements in ACR20, 50 and 70, HAQ-DI, FACIT and HRQOL scores. Six of 9 patients met the primary endpoint. Mean DAS improvement on 40 U was greater than on 80 U. Four of 9 patients had improved HAQ-DI and FACIT scores at Week 12; one had improved HAQ-DI only. FACIT and HAQ-DI improved as early as Week 1 and the improvements remained throughout treatment. There did not appear to be an association between cortisol levels and RCI response. Mild AEs included Cushingoid features (n=2), mild hyperglycemia (n=1), herpes zoster (n=1), and pedal edema (n=1). RCI produced a clinically meaningful reduction in markers of disease activity, improved QOL, and demonstrated a favorable safety profile. The response rate to RCI was substantial and shows great promise in this difficult to treat population.

W.27. The Role of Tim-3 in Promoting Profibrotic Functions in Immune Cells from Scleroderma Patients

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Scleroderma (SSc) is a systemic autoimmune disorder that causes fibrosis of the skin and internal organs leading to decreased organ function which can cause severe morbidity and mortality with roughly fifty percent of patients succumbing to the disease within 10 years. Using a novel 16 color flow cytometry panel we identified an increased expression of several inhibitory receptors (PD-1, LAG-3, Tim-3) on PBMCs from SSc patients, compared to age-matched healthy controls. *In vitro* functional assays with blocking antibodies revealed that these receptors regulate pro- and anti-fibrotic cytokine expression in immune cells from SSc patients. In particular, blocking the inhibitory receptor Tim-3 expressed on T cells and Natural killer cells (NK cells) of SSc patients modulates cytokine production in SSc PBMCs, leading to a decrease in the production of the pro-fibrotic cytokine IL-13. This result thus suggests a role for Tim-3 in modulating fibrosis. Natural Killer cells express the highest levels of Tim-3 of all lymphocytes in healthy subjects, and we found that SSc patients show an additional increase in Tim-3 on the surface of their NK cells. Interestingly, NK cell function is reduced in SSc patients, as demonstrated by decreased cytoxicity and IFNγ production *in vitro*, and loss of these functions may promote fibrosis. We are currently investigating whether Tim-3 engagement underlies the reduced functionality of NK cells in SSc and if blocking Tim-3 may restore anti-fibrotic activities in SSc NK cells.

W.28. Gene Expression Analysis of Tissues from Lupus Patients Documents Common Immune Cell Pathogenic Pathways

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Immunologic mechanisms are the basis of tissue damage in SLE. However, the specific pathways and mechanisms involved are not understood. To gain additional insight, gene expression profiles obtained from lupus affected skin, synovium and kidney were obtained and compared to meta-analyzed data obtained from active lupus B, T and myeloid cells. More than 300 arrays from lupus patients and appropriate controls were analyzed to determine differentially expressed (DE) genes (8279 discoid lupus skin, 5465 synovium, 6381 glomerulus, 5587 tubulointerstitum). The majority of lupus affected tissue DE genes were detected in more than one tissue and 439 were differentially expressed in all tissues. The molecular pathways accounting for these common lupus affected tissue DE genes were assessed. Curated STRING-based interaction analysis identified a number of pathways including co-stimulation of T cells, activation of B and myeloid cells, APCs, TLR signaling and p38 activation; from a total of 193 IPA-documented pathways, 59 were common to all tissues including p38 signaling, TLR signaling, maturation of dendritic cells, B cell activation and ICOS-ICOSL in T cells. Novel bioinformatics approaches documented that more than 50% of the DE genes in the tissues were associated with immune cell function and 11-18% were unique to the immune system. Further analysis using LINCS and additional novel software highlighted specific molecular pathways of immune cell activation including the JAK/STAT and the IL12 pathways. These results demonstrate the value of comprehensive application of orthogonal curated bioinformatics tools in identifying the role of immune cells in lupus pathogenesis and tissue damage.

W.29. Novel Targets of Intervention in Disease Pathogenesis is Revealed by Gene Expression Analysis of Tissues from Lupus Patients

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The hypothesis to be tested is that gene expression analysis of lupus affected tissues will generate novel insights into targets of immunological intervention. To test this, gene expression profiles obtained from lupus affected skin, synovium and kidney were obtained and cross-referenced various pathway analytic tools including the Library

Integrated Network of Cellular Signatures (LINCS). More than 300 arrays from lupus patients and appropriate controls were analyzed to determine differentially expressed (DE) genes [8279 discoid lupus skin, 5465 synovium, 6381 glomerulus, 5587 tubulointerstitum (TI)]. Genes upregulated in all lupus tissues cross-referenced to molecular pathway and drug interaction databases revealed unique targets of therapeutic intervention. For example, the IL12 pathway (but not IL23), the JAK, S1P1, IL21, IL17 and IL13 pathways were identified in all tissues. In addition, IL6 was only found in the skin, synovium and TI of the kidney; CD154-CD40 was found in the skin, syovium and glomerulus of the kidney; C5a was involved in synovium and the TI region of the kidney; ROCK2 was involved in the skin and synovium; whereas PDE4 was only identified in the skin. This approach has demonstrated that there are pathways common to all lupus tissue involvement and those involved in inflammatory response of some but not all tissues. This approach promises to identify therapies that may be useful in all lupus patients versus those with involvement of specific tissues.

W.30. PAD4-deficiency and Abrogated NETosis Do Not Ameliorate Murine Lupus

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Though recent reports suggest that neutrophil extracellular traps (NETs) and associated neutrophil death by NETosis are a source of antigenic nucleic acids in Systemic Lupus Erythematosus (SLE), we recently showed that inhibition of classical NETs by targeting the NADPH oxidase complex via Nox2-deletion exacerbated rather than ameliorated disease in the MRL.Fas^{lpr} lupus mouse model. While these data challenge the paradigm that NETs promote lupus, it is conceivable that global regulatory properties of Nox2 confound these findings. Furthermore, recent reports indicate that an inhibitor of peptidyl arginine deiminase 4 (PAD4), a distal mediator of NET formation, ameliorates lupus in several murine models. Here, to clarify the contribution of NETs to SLE, we employed a genetic approach to delete PAD4 in both F2 and fully backcrossed MRL.Fas^{lpr} cohorts. In contrast to inhibitor studies, we found that PAD4-deficiency did not significantly ameliorate any aspect of lupus or immune activation. Strikingly, *Pad4*-genotype had no impact on proteinuria, interstitial and glomerular nephritis, or skin disease. PAD4-deficiency did not alter autoantibody responses nor had any effect on the myeloid, DC, B cell, and T cell compartments. Thus, two independent genetic approaches to blocking NETosis failed to reduce any aspect of murine lupus, which prompts a reevaluation of the concept that NETs promote SLE. Furthermore, these data suggest that Nox2 plays a NET-independent regulatory role in lupus, a finding that likely applies to human disease as suggested by autoimmunity in Nox2-deficient boys and carrier mothers, as well as murine models.

W.31. Human Ro60-positive Lupus Patients Mount Adaptive Immune Responses to Commensal Ro60 Orthologs

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The earliest autoantibodies in lupus are directed against the autoantigen Ro60, an RNA binding protein with orthologs that we identified in a subset of skin, oral, and gut commensal species. Thus we hypothesized that commensal Ro60 orthologs may trigger autoimmunity via epitope cross-reactivity in genetically susceptible individuals. Ro60-producing gut commensals were prevalent in healthy controls and lupus patients. However, when human serum was used to co-immmunoprecipitate Ro60 and its bound Y RNA from a skin commensal, *P. propionicum*, only antibodies from human Ro60-positive lupus patients bound commensal Ro60. Lack of reactivity in Ro60-negative patients or healthy controls suggested antibody cross-reactivity between human and commensal Ro60. Next, Ro60 autoantigen-specific CCR6^{+} and CCR6^{-} CD4 memory T cells clones from lupus patients were isolated and expanded using a T cell library assay. Ro60 T cell clones positive for the tissue-homing marker CCR6 proliferated in response to *P. propionicum*, demonstrating T cell cross-reactivity with commensal Ro60. Finally, germ-free mice produced fecal anti-human Ro60 IgA antibodies (n=4, p=0.04) after monocolonization with the Ro60

ortholog-containing gut commensal, *B. thetaiotaomicron*, associating anti-Ro60 commensal responses *in vivo* with the production of human Ro60 autoantibodies. In summary, Ro60 autoimmune T and B cells from human lupus patients responded to commensal Ro60 *in vitro* and commensal Ro60 triggered anti-Ro60 antibodies *in vivo*. Together these data suggest that colonization with autoantigen ortholog-producing species may sustain chronic autoimmunity in patients. This concept may apply more broadly to human autoimmune disease and could lead to development of novel microbiota-targeted approaches to treat autoimmunity.

W.32. Modulating FOXO1 in HUT102 T Cells to Query Human Treg Function

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Despite a strong suggestion for their involvement in autoimmunity, no absolute proof of regulatory T cell (Treg) dysregulation or dysfunction has been demonstrated in human rheumatologic disease (HRD). A key pathway in T cell biology, the PI3K/Akt/mTOR pathway has been shown to be down-regulated in Tregs, and Akt inhibition is necessary for Treg suppressive function. FOXO1, a transcription factor (TF) downstream of the Akt/mTOR pathway, also appears to play a role in tolerance. It has been shown that active Akt phosphorylation of FOXO1 sequesters the TF in the cytoplasm. We hypothesize that FOXO1 is involved in dysfunction and/or dysregulation of Tregs in HRD, in particular conditions like systemic lupus erythematous (SLE) where Treg numbers have been shown to be normal or even elevated in patients with more active disease. We are testing the hypothesis that a differential FOXO1 phosphorylation and nuclear localization profile as compared to effector or naïve T cells is necessary to maintain normal Treg function and that this may be dysregulated in SLE. To test this hypothesis, we developed an assay to consistently modulate FOXO1 in the HUT102 T cell lymphoma line, which displays a Treg-like phenotype at baseline. We observed a kinetic difference in nuclear FOXO1 protein 30 and 60 minutes with PMA/I stimulation as compared to un-stimulated controls that returned to baseline at 4 and 6 hours. This reproducible assay will allow us to block or stimulate various signaling pathways that feed into FOXO1 to assess its role in human Treg dysregulation in SLE.

W.33. Novel Cytokines and Mechanisms in Lupus Lung Disease

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Systemic lupus erythematosus (SLE) is a chronic autoimmune disease that can affect multiple organs including skin, lung, heart, and kidney. Pulmonary inflammation is relatively common among SLE patients and can result in hemorrhaging when severe. Our observations have an association between a novel pro-inflammatory cytokine and lung involvement in Lupus patients. To further analyse this association and uncover the potential role of this cytokine in the pathology of lung inflammation in SLE, we have used an *in vivo* model of inducible Lupus, whereby a single intraperitoneal injection of pristane drives a rapid recruitment of inflammatory Ly6C^{hi}, IFN-α-producing monocytes in the peritoneum, which in turn is followed by lung inflammation and hemorrhaging within two weeks. Using this model, the influx od neutrophils and inflammatory monocytes into lung could be inhibited by blocking the cytokine using antibody specific for i and protected the lung from pristane-associated pathology.recruitment. Interestingly we observed a shift in phenotype of lung neutrophils, suggestive of low-density neutrophils, which are known to be proinflammatory and critical in pathogenesis of lupus. Staining for anti-MPO and neutrophil elastase in pristane-treated lung revealed that recruited neutrophils underwent NETosis, potentially driving the pathology observed. Taken together these results reveal that neutrophils play an important role in the pathology of lung inflammation in a mouse model of lupus and that strategies to target NETosis, as has been suggested by for other organ-specific manifestations of Lupus, may be important may be useful as novel therapeutics for treatment of patients with lung involvement in lupus.

W.34. Deciphering the Immunome of Clinically Effective Immune Tolerization in Rheumatoid Arthritis

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We have previously described (Nature Medicine, PNAS, A&R, Nature Rheum, Lancet) how oral treatment with the pro-inflammatory, heat shock protein-derived dnaJP1 peptide induces detectable clinical amelioration in Phase I and IIa clinical studies in rheumatoid arthritis. In previous work, we have also identified T effector (Teff) immune deviation and clinically synergistic effect with hydroxychloroquine (HCQ) treatment. Our previous studies, however, did not capture, yet, the complexity of the dynamic interactions among subsets of immune cells. Here, we apply a novel software clustering approach capable of extracting immune populations unique to responder populations from flow cytometric data.

T cell subsets which were significantly enriched in dnaJP1 responders were activated antigen experienced T cells which displayed tolerogenic/regulatory characteristics ($CD4^+CD45RO^+TGF\text{-}B^{int}CD69^{int}$, $p<0.05$) and ($CD4^+HLA\text{-}DR^{hi}GITR^{int/hi}$, $p<0.05$). Analysis of the antigen presenting cells (APCs) compartment revealed two functionally distinct subsets which are significantly elevated in dnaJP1 clinical responders, both subsets probably the outcome of cross talk between tolerized/regulatory T cells and APC: (a) $CD14^+CD16^+$ ($p<0.05$) monocytes that exhibit $CD86^{low/hi}$ expression, which has been described to relate to and augment Treg function, (b) two subsets of CD19 B cells which express (i) $PDL\text{-}1^{hi}$, $ILT4^{int}$, $HLA\text{-}G^{int}$ that may down-regulate activated Teff, or (ii) CD83 indicating matured B cells.

This holistic approach to deciphering big data has dual translational value, as it provides mechanistic knowledge and also potential biomarkers directly related to the therapeutic intervention.

W.35. High Prevalence of a Variety of Autoreactive IgA Antibodies Associated with IgG Autoantibodies and Clinical Manifestations in Systemic Lupus Erythematosus, Systemic Scleroderma and Idiopathic Inflammatory Myositis

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Systemic lupus erythematosus (SLE), systemic scleroderma (SSc) and idiopathic inflammatory myositis (IIM) are systemic autoimmune diseases characterized by autoantibodies (AutoAbs) against various self-antigens. In this study, we measured the serum IgA AutoAbs against 124 self-antigens in a cohort of 128 SLE, 73 SSc, 75 IIM and 140 healthy controls (HC) using autoantigen microarrays. Our result indicated that IgA AutoAbs were highly prevalent in SLE and SSc compared with IIM and NC. 58.4% SLE and 41.7% SSc have 5 or more IgA AutoAbs compared with 16% in IIM and 14.1% in NC. 9 IgA AutoAbs most significantly elevated in SLE were anti-DNA antigens including dsDNA (54.7%), ssDNA (51.1%), and chromatin (46%). 32 IgA AutoAbs were significantly increased in SSC with highest reactivity to Scl-70 (55.6%). IIM showed lower IgA prevalence of IgA AutoAbs with highest in anti- Jo-1 (20%), anti-muscarinic receptor (8%) and anti-B2-microglubulin (6.7%). Significant correlation was observed between IgA and IgG isotypes on most AutoAbs. 3 IgA autoAbs (anit-gDNA, anti-C1q and anti-dsDNA) positively correlated with SLEDAI Score, but only anti-C1q was significantly elevated in lupus nephritis. 14 anti-nuclear IgA AutoAbs negatively correlated with serum complement C3 and/or C4 levels. Our study indicated that autoreactive IgA AutoAbs against nuclear components, in parallel with IgG AutoAbs, are highly prevalent in SLE and SSC patients. IgA AutoAbs could be used as biomarkers for diagnosis and prognosis of systemic autoimmune diseases, and the molecular mechanisms underlining IgA AutoAbs production in systemic autoimmune diseases warrant further study.

W.36. Checkpoints for Autoreactive B Cells in Peripheral Blood of Lupus Patients Assessed by Flow Cytometry

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Antibodies to nuclear antigens (ANA) are diagnostic in several autoimmune disorders, yet how B cell tolerance is broken in these diseases remains poorly understood. Although secreted ANA measured by an indirect immunofluorescence assay are the gold standard for autoreactivity, there has been no convenient methodology to measure the frequency of circulating B cells that recognize nuclear antigens (ANA^+ B cells). We generated and validated a novel flow cytometry-based assay that easily identifies ANA^+ B cells using biotinylated nuclear extracts, and used it to examine B cell tolerance checkpoints in peripheral blood mononuclear cells obtained from patients with systemic lupus erythematosus (SLE) and healthy controls. Both SLE patients and healthy subjects show progressive selection against autoreactive ANA^+ B cells as they mature from transitional to naïve to $CD27^+IgD^-$ memory cells, but SLE patients demonstrated an impairment of anergy induction of ANA^+ naïve B cells. Analysis of B cells from SLE patients treated with belimumab, an inhibitor of B cell-activating factor (BAFF), showed that BAFF blockade restored anergy of ANA^+ naïve B cells. This assay will facilitate studying B cell tolerance in large populations in health and disease and be useful in monitoring B cell responses to therapeutics.

W.37. African American SLE Patients Present with an Activated B cell Phenotype

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Systemic Lupus Erythematosus (SLE) is a complex systemic autoimmune disease driven by both innate and adaptive immune cells. African Americans tend to present with more severe disease at an earlier age compared to patients of European ancestry. In order to better understand the immunological differences between African American and European American patients, we analyzed the frequencies of B cell subsets, as well as activation markers on B cells from 72 SLE patients and 69 normal healthy volunteers. We found that $CD19^+$CD27-IgD- double negative B cells were particularly enriched in African American patients vs. patients of European ancestry. In addition, frequencies of $CD86^+$, $CD80^+$, $PD1^+$ and $CD40L^+$ B cells were higher in African American patients, while expression of surface CD40 on B cells was lower, suggesting the engagement of the CD40 pathway. *In vitro* experiments confirmed that CD40L expressed by B cells could indeed lead to CD40 activation and internalization on adjacent B cells. To conclude, these results indicate that compared to European American patients, SLE in African American patients is characterized by a particularly active B cell component, possibly via the activation of the CD40-CD40L pathway. These data may help guide the development of novel therapies.

W.38. T Cell Restricted Deletion of Serine/Arginine-rich Splicing Factor 1 (SRSF1) in Mice Causes Immune Cell Dysfunction, Autoimmunity and Lupus-like Nephritis

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T cells from patients with systemic lupus erythematosus (SLE) express reduced amounts of the critical CD3 zeta signaling chain, and are poor producers of interleukin (IL)-2. Using a discovery approach (protein “pull-down” and mass spectrometry analysis), we identified the splicing protein serine arginine-rich splicing factor 1 (SRSF1) binding to the 3`UTR of CD3 zeta. We showed that SRSF1 regulates alternative splicing of CD3 zeta mRNA to generate a full-length transcript rather than a short unstable isoform, to promote normal expression of CD3 zeta chain in human T cells. We also showed that SRSF1 expression levels are decreased in T cells from SLE patients, and overexpression of SRSF1 into SLE T cells rescues IL-2 production. To define the role of SRSF1 in T cell function and development of immune-mediated disease *in vivo*, we used the Cre-lox strategy to generate T cell restricted *Srsf1*

conditional knockout (*Srsf1*-cko) mice. *Srsf1*-cko mice exhibit T cell lymphopenia in the peripheral lymphoid compartment, with a striking reduction in the CD8 subset. The CD4 T cells exhibit increased proportions of activated (CD69hi) cells, and produce increased amounts of IFN-γ and IL-17 upon e*x vivo* stimulation. These mice develop autoantibodies, exhibit proteinuria and show evidence of kidney histopathology with features of lupus nephritis. Our results reveal that SRSF1 is a novel regulator of T cell function, and its deficiency leads to autoimmunity and kidney disease. Therefore, deficiency of SRSF1 may represent a molecular defect that contributes to the pathogenesis of systemic autoimmune disease.

W.39. Patients Who Develop Nephritis as They Transition to SLE Classification Demonstrate Preclinical Dysregulation of Distinct Adaptive and Renal-associated Mediators

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Systemic lupus erythematosus (SLE) is a heterogeneous autoimmune disease marked by immune dysregulation and a spectrum of pathogenic autoantibodies. Why some patients have only moderate symptoms and others develop organ-threatening manifestations is unclear. This study evaluates the temporal expression of autoantibodies and cytokines in sera from the Department of Defense Serum Repository during transition from preclinical lupus to SLE in patients (n=83) who do or do not present with nephritis. Patients who met renal criteria (n=30; 36%) did so within 5.2 (± 5.5) months of SLE classification (range -5.2 to +12 months) and were less likely than non-renal cases to have a history of hydroxychloroquine use prior to SLE classification (*p<0.0001*). Renal cases experienced earlier onset of autoantibody positivity vs. non-renal cases (mean -3.8 vs -2.6 years relative to SLE classification, *p=0.0442*), although no difference in number or type of autoantibody specificity was detected. Prior to developing nephritis, renal cases exhibited elevated levels of distinct soluble mediators compared to matched non-renal cases and healthy controls, including adaptive mediators IL-4, IL-5, IL-12, and IFN-γ, chemokines IP-10 and MCP-3, and nephritis-associated mediators SCF and shed TNFRII (all *p<0.05*), increasing again at the time of nephritis (all *p<0.02* compared to pre-nephritis levels). These same mediators were significantly increased at SLE classification in patients with nephritis vs. non-renal patients (all *p<0.0001*). These data indicate that perturbations in distinct immune mediated inflammatory processes may help identify individuals at high risk of renal involvement for early and continued monitoring and intervention.

W.40. Methotrexate is Metabolized by Human Gut Bacteria

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Methotrexate is a first-line therapy used in the treatment of rheumatoid arthritis. About 30-40% of patients respond to methotrexate treatment while others are non-responders. Human genetics explains part of this variation, but not all of it. We hypothesize that a patient's response to methotrexate is influenced by their gut microbiome. Here, we examined whether bacterial growth is affected by methotrexate and whether bacteria can metabolize methotrexate, a drug that targets a conserved DNA synthesis pathway found in both human and bacteria cells. To examine growth, we investigated the minimal inhibitory concentration (MIC) of methotrexate for the following organisms: *Flavobacterium spp* ATCC 25012, *E. coli* BW25113, *Prevotella copri* DSMZ 18205, *Bacteroides thetaiotaomicron* DSMZ 2079, *Clostridium scindens* DSMZ 5676, *Clostridium asparagiforme* DSMZ 15981, and *Bacillus subtilis* 168. Using an HPLC assay, we asked whether these bacteria could metabolize methotrexate. We found that some gut bacteria are resistant to methotrexate, including *Flavobacterium*, *E. coli*, and *C. asparagiforme* (MIC > 900 ug/ml). Some bacteria are intermediate in their sensitivity, such as *P. copri*. Others are relatively sensitive such as *B.*

thetaiotaomicron and *C. scindens*. Interestingly, *C. asparagiforme*, which is resistant, metabolizes methotrexate into DAMPA and an unknown metabolite. These findings suggest that methotrexate is an antimicrobial in addition to being a chemotherapeutic agent and an immunosuppressant. Additionally, gut bacteria can metabolize methotrexate, perhaps even before this drug enters a patient's bloodstream. This raises the possibility the variability in a patient's response to methotrexate may be determined by their gut microbiome.

W.41. Young Adults with Quiescent Juvenile Dermatomyositis (JDM) have Diminished Brachial Artery Reactivity (BAR) and Reduced miRNA-10a in White Blood Cells (WBC) in Childhood Active Disease

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Objective: To test JDM BAR (endothelial cell dysfunction) & miRNA-10a in WBCs.

Background: miRNA-10a inversely regulates NFkB and VCAM-1 in JDM (J Rheum 43:161, 2016).

Methods: 20 adults, 14 F, mean age 21.8± 4.2 SD yrs; duration of untreated disease (DUD) at diagnosis 5.4 ± 4.8 months; total disease duration 14.2 ±3.9 years. JDM Total Disease Activity Score (DAS) = 1.9 ± 2.5 (max=20). JDM and 20 healthy controls, matched (age, race, sex, BMI, age 23.8 ± 4.2 years), were tested for: BAR, nailfold end row capillary loop # (ERL), height and weight. miRNA-10a levels (standard qRT-PCR methods) were determined in JDM WBC @: 1) active disease (childhood); 2) BAR testing (quiescent JDM adult) vs age-matched controls.

Results: Compare with adult controls, JDM had: 1) fewer ERL, 6.35 ± 1.29 vs 7.4±0.58 (p=0.003); 2) were shorter: JDM women, 159.7 ±8.8cm vs. 165.6 ±5.4cm (p=0.048); JDM men, 172.7±3.6cm vs. 181.5 ±4.6cm (p=0.0046). JDM BAR = 3.35±3.06% vs control= 5.22±3.07% (p=0.068). After adjustment (height age, sex, BA diameter), JDM brachial artery reactivity was less than controls, 2.31% (p=0.04). BAR: no associations with ERL, DUD, or DAS. miRNA-10a was similar in young adults: JDM, adult controls (p=0.27). In contrast, miRNA-10a was higher in pediatric controls than adult controls (p<0.01), and was decreased in childhood active JDM (p=0.03).

Conclusions: 1) Young adult JDM have impaired endothelial cell function (BAR); 2) miRNA-10a is decreased in childhood JDM vs pediatric controls. We speculate: early in JDM disease, miRNA-10a may contribute to persistent endothelial cell damage.

W.42. Remission by DAS28 Score with Current Therapies Does Not Eliminate Disease Associated Anti-Citrullinated Protein Memory B Cells in Rheumatoid Arthritis

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Background: Anti-citrullinated protein antibodies (ACPA) document breaches in tolerance in RA. However, the effect of agents on this central disease driver is not understood. We therefore examined serum ACPA, frequency and epitope reactivity of ACPA-producing memory B cells, and DAS28 score after treatment.

Methods: 26 seropositive RA-patients were enrolled at NYU and University of Pittsburgh. We cultured FACS-sorted B cells [$CD19^+/CD27^+/IgD^-$] with CD40L/CpG2006/IL-21 for 12 days. Controls were seronegatives and healthy adults. Supernatants were analyzed for ACPA-IgG reactivity and for 8 citrullinated and native peptide/protein multiplexed pairs, as well as diagnostic peptide CCP3 (Inova Diagnostics) and glutamine-containing CQP3.

Results: Autoantibodies in serum and supernatants from matched cultured cells showed similar patterns of ACPA fine-specificities. 18/26 RA patients had detectable ACPA-positive well(s). Switched memory B cells ($CD19^+/CD27^+/IgD^-$) were the main source of ACPA-IgG. A subset of RA patients had high frequencies of circulating ACPA memory-B cells, including patients in DAS28 remission and patients treated with methotrexate and TNFi. High levels of serum anti-CCP3-IgG significantly correlated with high ACPA-producing switched-memory B cells ($p<0.01$**). Individuals in clinical remission by DAS28 often had substantial levels of circulating ACPA memory-B cells.

Conclusions: In this cross-sectional study, disease-associated anti-Cit memory B cells persisted despite treatment with methotrexate and/or TNFi, demonstrating that the adaptive immune cellular disease process in RA was not eliminated. DAS28 clinical remission was not a good predictor for reduced or absent autoimmune lymphocyte burden. Our platform may be useful for informing future treatment decisions in individual RA patients, and evaluating new agents.

W.43. Using the American College of Rheumatology and Systemic Lupus International Collaborating Clinics Criteria to Measure Disease Severity in Discoid Lupus Erythematosus

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Discoid lupus erythematosus (DLE) progresses to systemic lupus erythematosus (SLE) in up to 28% of cases. The Systemic Lupus International Collaborating Clinics (SLICC) criteria were developed to improve the American College of Rheumatology (ACR) criteria, but the SLICC criteria have not been evaluated in DLE. This is a case-control study comparing patients with DLE who meet ACR and/or SLICC SLE criteria against patients with DLE-only disease. The data was obtained from a database of cutaneous LE patients at Penn and from their respective medical records. Using the ACR criteria, 74 (52%) patients were classified as DLE/SLE and 68 (48%) as DLE-only, compared with 66 (46%) DLE/SLE and 76 (54%) DLE-only patients using the SLICC criteria ($p=0.08$). This net increase of eight patients meeting ACR criteria was due to the presence of the photosensitivity criterion and fewer immunologic criteria under ACR. Overall, DLE/SLE patients were more likely than DLE-only patients to exhibit hematologic and immunologic criteria with respect to leukopenia (ACR $p<0.0001$; SLICC $p<0.0001$), + anti-dsDNA (ACR $p<0.0001$; SLICC $p<0.0001$), and + ANA (ACR $p<0.0001$; SLICC $p<0.0001$) under both criteria. Furthermore, DLE/SLE patients were more likely than DLE-only patients to exhibit significant systemic symptoms with regard to arthritis (ACR $p<0.0001$; SLICC $p<0.0001$), serositis (ACR $p<0.0001$; SLICC $p<0.0001$), and renal disorder (ACR $p<0.0001$; SLICC $p<0.0001$) using both criteria. These findings suggest that DLE patients who meet SLE criteria are more likely than DLE-only patients to have significant internal disease. Both criteria are useful in distinguishing DLE patients with internal organ involvement from those without.

W.44. Unique Transcriptome Signatures Identified in Systemic Lupus Erythematosus Patients with Distinct Autoantibody Specificities Using RNA-seq Analysis

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Systemic lupus erythematosus (SLE) patients exhibit immense heterogeneity which is challenging from the diagnostic perspective. SLE patients categorised based on autoantibody specificities are reported to have differential immuno-regulatory mechanisms. Therefore, we performed RNA-seq analysis to identify transcriptomics of SLE patients with distinguished autoantibody specificities and to understand the underlying complex and dynamic disease processes. The SLE patients were segregated into three subsets based on the type of autoantibodies present in their sera (anti-$dsDNA^+$ group with anti-dsDNA autoantibody alone; anti-ENA^+ group having autoantibodies against ENA only, and anti-$dsDNA^+ENA^+$ group having autoantibodies to both dsDNA and ENA). In this study, we observed dysregulation of 715 transcripts in anti-$dsDNA^+$ SLE patients, 541 and 331 transcripts in anti-ENA^+ and anti-

dsDNA^{+}ENA^{+} SLE subsets, respectively that were clearly distinct in each patient's subsets. Analysis of transcripts uniquely expressed in different SLE groups by using ingenuity pathway analysis software revealed specific biological pathways to be affected in each SLE subsets. Multiple *cytokine signaling* pathways were specifically dysregulated in anti-dsDNA^{+} patients whereas *Interferon signaling* was predominantly dysregulated in anti-ENA^{+} patients. In anti-dsDNA^{+}ENA^{+} patients *regulation of actin based motility by Rho* pathway was significantly affected. The granulocyte gene signature was a common feature to all SLE subsets; however, these genes predominantly express in anti-dsDNA^{+} subset. Association of specific canonical pathways with the uniquely expressed transcripts in each SLE subgroup indicates towards the subsets specific disease events in each SLE patients. This 'sub-grouping' approach could further be useful for clinical evaluation of SLE patients and interventional studies.

W.45. PD-1^{hi} Peripheral Helper CD4^{+} T Cells Promote B Cell Responses in Rheumatoid Arthritis

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Chronically inflamed tissues commonly accumulate plasma cells. B cells can differentiate into plasma cells within tissues and secrete autoantibodies locally in autoimmune disease. However, the helper T cell population that promotes plasma cell differentiation in non-lymphoid tissues has not been defined.

Follicular helper CD4^{+} T (Tfh) cells, identified as CXCR5^{+} PD-1^{+}, are prototypical B cell-helpers; however, Tfh cell localization is largely restricted to secondary lymphoid organs. We sought to identify tissue-homing B cell-helper T cells in blood and synovial fluid from patients with rheumatoid arthritis (RA), a disease characterized by synovial plasma cell accumulation and autoantibody production.

We report that PD-1^{hi} CXCR5^{-} CD4^{+} T cells are expanded in the circulation of seropositive, but not seronegative, RA patients and are strikingly enriched in seropositive RA synovial fluid, constituting ~30% of synovial fluid CD4^{+} T cells. Mass cytometry and transcriptomics demonstrate numerous similarities between PD-1^{hi} CXCR5^{-} T cells and PD-1^{hi} CXCR5^{+} Tfh cells, including high expression of IL-21, CXCL13, MAF, and ICOS. *In vitro*, both PD-1^{hi} CXCR5^{-} cells and Tfh cells induce plasmablast differentiation via IL-21 and SLAMF5-interactions. However, PD-1^{hi} CXCR5^{-} cells express a unique transcriptional signature distinct from Tfh cells that includes increased expression of Blimp1 and the inflammatory chemokine receptors CCR2, CCR5, and CX3CR1. We propose that PD-1^{hi} CXCR5^{-} CD4^{+} T cells represent a previously undefined 'peripheral helper' T (Tph) cell population uniquely poised to support B cell responses in inflamed tissues. Given their marked expansion in seropositive RA, Tph cells may represent an important population driving pathologic autoantibody production.

W.46. Next-generation Sequencing of TCR Repertoires Identifies Synovial Treg Cells Recirculating Into The Bloodstream During Active Inflammation in Human Arthritis

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The imbalance between effector and regulatory T (Treg) cells is crucial in the pathogenesis of autoimmune arthritis. Immune responses are often investigated in the blood because of its accessibility, but circulating lymphocytes are not

representative of those found in inflamed tissues. This disconnect hinders our understanding of the mechanisms underlying disease. Our goal was to identify Treg cells implicated in autoimmunity at the inflamed joints, but also readily detectable in the blood upon recirculation. To find immune features dysregulated in disease, we compared JIA patients responding or not to therapy. We took advantage of TCR sequencing to identify Treg clonotypes shared between blood and synovial fluid. Treg cell stability was interrogated by inspecting DNA methylation at the FOXP3 TSDR. Flow cytometry and suppression assays were used to probe the tolerogenic functions of Treg cells. We found a subset of synovial Treg cells that recirculated into the bloodstream of juvenile idiopathic and adult rheumatoid arthritis patients. These inflammation-associated (ia)Treg cells, but not other blood Treg cells, expanded during active disease and proliferated in response to their cognate antigens. Despite the typical inflammatory-skewed balance of immune mechanisms in arthritis, iaTreg cells were both stably committed to the regulatory lineage and fully suppressive. Using an innovative antigen-agnostic approach, we uncovered a population of synovial Treg cells readily accessible from the blood and selectively expanding during active disease, paving the way to non-invasive diagnostics and better understanding of the pathogenesis of autoimmunity.

W.47. A Dose Response Study of Hydrocarbon Oil Induced Inflammation and Lipogranuloma Formation in Multiple Organs

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Accidental exposure or administration during cosmetic surgery of hydrocarbon oil has been associated with autoimmunity and lipogranuloma formation in humans. An intraperitoneal injection of hydrocarbon oil 2,6,10,14-tetramethylpentadecane (TMPD) at high doses (500µl) elicits multi-organ inflammation, including pneumonitis, vasculitis, and pulmonary hemorrhage in C57BL/6 mice. However, there is a lack of information about hydrocarbon exposure levels and autoimmunity. Here, we investigated the dose-dependent effect of TMPD on inflammation. C57BL/6 mice were injected with different doses of TMPD intraperitoneally and observed over two months. All animals at all doses (125µ, 250µl, and 500µl) developed peritonitis with granulomas. 66% animals at 500µl and 14% at 250µl dose developed >20% weight loss and lung hemorrhage within three weeks. >50% animals at all doses developed varying degrees of inflammation and lipogranulomas in various organs including lungs, liver, spleen or kidneys. Mechanism of TMPD-induced lipogranuloma formation and inflammation in remote organs is unclear. The engulfment of TMPD by macrophages, resulting in the activation of these cells and release of cytokines/chemokines, is considered to be initial events. We found oil droplets in all four organs, and the lungs were affected at the earliest timepoint compared to other organs. There was a positive correlation between the TMPD dose and the number of organs displaying oil droplets. Ongoing studies will test the hypothesis that TMPD translocates through the peritoneal lymphatic drainage to the subdiaphragmatic lymphatics, thoracic duct, superior vena cava, heart, pulmonary artery, and then to the lungs. From there, TMPD may translocate to other organs through blood circulation.

W.48. A Novel ELANE Mutation Associated with Inflammatory Arthritis, Decreased NET-Osis, and Recurrent Parvoviral Infection

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Introduction: We describe a 38-year-old woman presenting with a 2-year history of inflammatory arthritis, rash and daily fevers. She was found to have a chronic parvoviral infection with persistently detectable viral titers, of unclear etiology. Ultimately, she was found to a novel mutation in the ELANE gene.

Background: The ELANE gene encodes neutrophil elastase, a neutrophil serine protease whose functions include generation of reactive oxygen species intracellularly, and association with NETs (neutrophil extracellular traps).

Pathogenic ELANE mutations have been described in forms of hereditary neutropenia. However, our patient was never neutropenic. We sought to better understand the biological relevance her novel mutation.

Findings: Three-dimensional mapping revealed that the mutation could alter surface charge distribution of the protein, potentially perturbing protein trafficking. The patient's neutrophils did not demonstrate any functional deficiency by oxidative burst assay. However, by immunohistochemistry, the patient's neutrophils did not demonstrate expected spontaneous NETosis. Further, the patient's activated neutrophils demonstrated qualitatively decreased NETosis by scanning electron microscopy. Compared to activated neutrophils from normal controls the patient's neutrophils demonstrated altered levels of IL-12 and IL-8 - key cytokines for antiviral immunity and neutrophil chemotaxis.

Conclusions: This is the first description of an ELANE mutation in a non-neutropenic yet symptomatic patient with recurrent parvoviral infection. Our observations suggest that the mutation impacts neutrophil function by decreasing NETosis and antiviral and immune cytokine production. Further studies are underway to better characterize patient's neutrophils and the mutations impact on neutrophil viability, and ultimately to outline an appropriate treatment course.

W.49. High Levels of Serum IFN-alpha Mark a Subgroup of SLE Patients with Distinct Immunophenotypic Features, Hyper-Responsiveness to TLR4 Stimulation, and Elevated Circulating and Stimulated Levels of IL-1RA

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Background: High serum interferon activity (IFN-high) marks a subgroup of SLE patients associated with increased disease severity and autoantibody formation. Differences in the cellular immune system between IFN-high and IFN-low patients remain largely unknown. We sought to better characterize these subgroups in human SLE by studying the stimulated immune response.

Methods: Serum IFN-activity scores were calculated using the WISH assay, and used to bin SLE patients as IFN-high and IFN-$_{low}$. Immune cell subsets were quantitated by flow cytometry. Whole blood was dispensed into tubes coated with the TLR agonists LPS, CpG, and R848 (Tru-Culture.) Stimulated IFN-alpha and cytokine production was measured by WISH and multiplex assay, respectively.

Results: The frequency of circulating pDCs was significantly lower in IFN-high patients compared to controls ($p=0.00095$). After exposure to LPS, induced IFN-alpha production was greater for IFN-high patients than IFN-$_{low}$ ($p<0.05$), and controls ($p=0.05$). Compared to IFN-$_{low}$, IL-1 receptor antagonist (IL-1RA) levels were significantly higher in the sera of IFN-high ($p=0.020$), and after stimulation with R848 ($p=0.050$) and IFN-alpha ($p=0.014$).

Conclusions: We have observed novel biologic differences between IFN-high and low SLE subgroups. That pDC counts were only reduced in the high IFN patients would suggest trafficking out of the circulation and into inflamed tissue prior to IFN production. Additionally, that IFN-high patients are significantly more responsive to TLR4 stimulation is novel, and may shed light on differential responses to SLE therapies. Finally, IL-1RA may represent a novel biomarker differentiating IFN-^{high} versus IFN-$_{low}$. Further studies of the stimulated inflammatory cytokine response are ongoing.

W.50. Th17/TfH Cells Correlate with Disease Activity in Rheumatoid Arthritis Patients and Are Not Changed By TNF Inhibition

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Th17 and TfH cells are thought to promote tissue inflammation (Th17) and autoantibody production (TfH) in rheumatoid arthritis (RA) and other autoimmune diseases. TfH cells that co-express Th17 markers (Th17/TfH) correlate with disease activity in juvenile dermatomyositis. However, Th17/TfH cells have not been detailed in RA subjects in context of therapy response. Blood T cell and B cell subsets were analyzed by flow cytometry in 16 subjects with active RA, before and after TNF inhibitor therapy. Proportions of $CXCR5^+$ TfH cells that co-expressed the Th17 marker CCR6 (Th17/TfH cells) directly correlated with disease activity at baseline. In contrast, there were no correlations between disease activity and other T cell subsets including total Th17 ($CCR6^+$), TfH ($CXCR5^+$) or Th1 ($CXCR3^+$) cells, or CXCR3-expressing TfH cells. Th17/TfH cell frequencies were unchanged by TNF inhibition, fitting with no effect of TNF on stimulation of IL-17 or IL-21 *in vitro*. In fact, Th17/TfH cell proportions showed remarkable stability within individuals over time despite changes in disease activity. Activated class-switched B cells were increased in subjects with high autoantibody, but B cell subsets did not correlate with disease activity or with Th17/TfH cells. Finally, baseline Th17/TfH cells correlated with numbers of swollen joints at baseline and following one year of stable therapy. These data demonstrate the stability of chemokine receptors as markers of blood T cell phenotypes within individuals, and point to differences in underlying T cell phenotypes as drivers of autoimmune joint disease activity and response to TNF inhibitor therapy.

W.51. Suppression of IL-1 Signaling Ameliorates Dermatitis in a Murine Model of Systemic Lupus Erythematosus (SLE)

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Our laboratory previously showed that MyD88, a downstream signaling component of IL-1, is a central mediator of SLE pathogenesis. MyD88 signals downstream of numerous receptors, including Toll-like receptors (TLR) 7 and 9 which are clearly implicated in SLE. However, evidence in humans and mouse models suggest a pathogenic role for IL-1, the receptor for which also signals via MyD88, particularly in cutaneous lupus. Further, data from our laboratory suggest that dermatitis in murine SLE is MyD88 dependent but TLR7/9 independent, differing from systemic manifestations of the disease.

To examine the role of IL-1 we evaluated the MRL.Fas^{lpr}(MRL/lpr) mouse model of SLE. The potential to produce IL-1 was evaluated in target organs, skin and kidney, by qPCR. IL-1β mRNA was increased in kidney in MRL/lpr mice with proteinuria nearly 5-fold compared to non-diseased young MRL/lpr mice ($p=0.05$). While in inflamed skin, IL-1β mRNA was elevated nearly 50 fold compared to controls ($p<0.005$).

To test for causative roles in disease, IL-1R1 deficient mice were crossed with MRL/lpr mice and disease was evaluated in an F2 intercross cohort. F2 cohorts are a validated and rapid method for screening for genetic control of autoimmunity in the context of an autoimmune-predisposing genetic background. While there was no significant difference in proteinuria, glomerulonephritis, ANA production, or spleen size, dermatitis was significantly reduced in the IL-1R1 deficient F2 Fas^{lpr} mice ($p<0.008$). Thus, IL-1 signaling is a significant contributor to autoimmune dermatitis in the MRL model and may be a potential target for treating cutaneous lupus manifestations.

W.52. Citrullinated Aggrecan Peptides as Targets of Auto-reactive $CD4^+$ T Cells in Rheumatoid Arthritis

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T cell frequencies against citrullinated epitopes derived from major auto-antibody targets (vimentin, fibrinogen, α-enolase, etc.) are increased in rheumatoid arthritis (RA). Emerging serologic data suggests antibody reactivity against additional citrullinated proteins, including histones and aggrecan, in RA. Among these potential targets, aggrecan has been previously reported as being immunogenic in RA (Boots AM *et al.,* 1997). In this study we undertook a systematic approach to identify cit-aggrecan epitopes and to verify the relevance of aggrecan specific

$CD4^+$ T cell responses in RA. Starting with 28 cit-aggrecan peptides predicted to bind DR04:01 we identified 6 epitopes capable of activating and expanding $CD4^+$ T cells from RA patients with DR04:01 haplotypes. Using the corresponding tetramers we isolated cit-aggrecan specific T cell clones specific for these peptides. These clones selectively recognized citrullinated peptide and largely exhibited a Th1-like functional phenotype. *Ex vivo* tetramer analysis of PBMC revealed that RA patients had significantly increased frequencies of cit-aggrecan specific T cells in comparison to healthy controls. Ongoing studies will determine whether cit-aggrecan specific T cells arise early or late in disease, and whether they have a distinct transcriptional signature. The ability to characterize T cells that recognize cit-aggrecan and other relevant epitopes has the potential to improve our understanding of their role in disease progression and pathogenesis and to inform the selection of appropriate antigens and epitopes for targeted antigen specific tolerance in RA.

W.53. Single Cell Gene Expression in Monocyte Subsets Correlates with Treatment Response Groups to TNF-alpha Inhibition in Rheumatoid Arthritis

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Background: In rheumatoid arthritis (RA), it is critical to initiate effective treatment as soon as possible, but we cannot predict which patients will respond to a given medication. Anti-tumor necrosis factor-α (anti-TNF-α) agents are commonly used drugs, and recent work from our group demonstrated that high pre-treatment serum type I IFN-β/α activity ratio can predict non-response to anti-TNF-α therapy in RA patients. The cellular mechanisms that underlie this ratio are not known.

Methods: We used single cell expression analysis to investigate whether monocyte gene expression differs significantly between RA patients according to their pre-treatment serum IFN-β/α activity ratio. Single classical (CL) and single non-classical (NCL) blood-derived monocytes were isolated from 15 RA subjects, grouped by high versus low serum IFN-β/α activity ratio.

Results: Hierarchical clustering revealed striking differences in CL monocytes between patients with high vs. low IFN-β/α activity ratio. This same clustering was not observed in NCL monocytes. Two major gene sets that differentiated subjects with high IFN-β/α ratio in CL monocytes included TLR and IFN pathway genes, cell surface markers and cytokines: cluster 1 (GMCSF, TLR7, STAT2, ILT7, MYD88) and cluster 2 (TLR2, CD16, JAK1, IFI27, IL1A, and MAVS).

Conclusion: Within-cell expression patterns demonstrate biological differences in CL monocytes of RA patients with high IFN-β/α activity ratio predictive of non-response to anti-TNF-α therapy. This work illuminates molecular differences that determine treatment response to anti-TNF-α in RA, and will allow for more individualized therapy.

W.54. A Novel Method for Assaying Human Treg Direct Suppression of B Cell Effector Function

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We have established a highly reproducible and reliable protocol for testing human regulatory T cell function in suppressing IgM production from an immature human B cell line. Magnetic bead-purified $CD4^+$ Tregs are confirmed to be >90% $FoxP3^+$ as well as >65% $Helios^+$. The Ramos B cell line provides a stable reporter of B cell effector function that can be tested by a straight-forward IgM ELISA. Tregs from healthy volunteers display a range of ability for suppressing baseline IgM production as well as higher IgM production stimulated by the addition of CD40L and IL-

21. Having established the normal range for human Treg direct suppression of B cell effector function, it will now be possible to efficiently test Tregs from various autoimmune conditions in which B cell hyperactivity and secretion of auto-antibodies are a hallmark of disease.

W.55. Examining Granzyme A in Ankylosing Spondylitis

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Ankylosing Spondylitis (AS) is a severe inflammatory disease with an unknown pathological mechanism. Despite 85% of patients presenting with the MHCI allele, HLA-B27, no clear role for pathogenic $CD8^+$ T cells has been identified. In a recent microarray study, we found the expression of granzyme A (GZMA) to be lower in AS patients compared to controls. While GZMB is well known for its perforin-dependent cell lysis function, recent literature suggests GZMA has a perforin-independent novel inflammatory role. We therefore decided to further examine GZMA in samples from AS, osteoarthritis and rheumatoid arthritis patients with healthy controls. By applying flow cytometry to mononuclear cells from blood and synovia fluid (SF), we have confirmed a recruitment of $GZMA^+/perfor^{in-}$ $CD8^+$ T cell population to the inflamed joint, specifically in AS patient. Such observation was further supported by ELISA and immunobead assay on serum and SF of AS patients, which showed a significant increase of GZMA and decrease of perforin protein concentration in SF compare to blood. The experiment outcome offered intimation of GZMA's potential inflammatory role in AS development, while further investigation is still needed to fully understand the molecule mechanism of GZMA's action in such relationship. The next stage of this study includes vitro test of GZMA function on multiple human cell-lines by directly applying GZMA treatment followed by flow cytometry analysis. GZMA blockage on SKG mice by GZMA inhibitor will also be tested and its effect on inflammation condition of treated individual will be analyzed.

W.56. Myocarditis and Fibrosis Detected by Cardiac Magnetic Resonance Imaging Correlates with Pro-Inflammatory Cytokine Expression in NZM2410 Mice with Non-end Stage Lupus Nephritis

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While the primary clinical manifestations of systemic lupus erythematosus (SLE) are observed in the skin, joints, and kidneys, there is also a high risk of cardiovascular disease (CVD). Accordingly, epidemiological data shows that CVD is the primary cause of morbidity and mortality subsequent to the first 10 years of SLE diagnosis. Previously, we used cardiac magnetic resonance imaging (CMR) with quantitative T2 mapping in a human cohort to show that subclinical myocarditis is common in SLE and observed enhanced T2 signals in active lupus patients during flares, which suggested subclinical levels of cardiac inflammation. Here, we examined the NZM2410 mouse model of lupus nephritis (LN), which develops severe lupus-like glomerulonephritis. CMR T2 mapping was performed and mice were monitored bi-weekly for cardiac abnormalities by echocardiogram (EC). When ECs showed significantly decreased ejection fractions relative to baseline, CMR with T2 mapping was performed again and mice were euthanized. While these mice did not show signs of advanced renal disease, as measured by blood urea nitrogen levels and weight loss, T2 signals were elevated, fibrosis was significantly induced, and the myocardium was thickened. Furthermore, serum cytokine levels of IL-10, IL-2, IL-6, CXCL1, and TNF-α were significantly up-regulated and immunohistochemical staining for $CD3^+$ T cells and IL-17 was observed in cardiac infiltrates. Collectively, these data indicate that myocarditis is associated with LN in NZM2410 mice and establish CMR T2 mapping as a non-invasive technique to longitudinally examine the contribution of SLE to CVD and to monitor experimental therapeutic strategies to pharmacologically inhibit cardiac damage.

W.57. Expression of T-box Transcription Factor T-bet Identifies a Novel Population of Inflammatory B Cells in

Systemic Lupus Erythematosus

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We demonstrated that development of murine plasma cells (PCs) is controlled by IFNγ-signaling and the transcription factor, T-bet. However, little is known about the role of IFNγ-dependent signaling or T-bet in the development of human PCs. We hypothesized that inflammatory cytokines, produced by autoimmune patients, control the expression of T-bet and regulate the fate of autoreactive B cells. To address this hypothesis, we developed a novel human T_H/B allogenic co-culture system. A significant fraction of the Be1 cells (Beff cells from Th1 co-cultures), but not the Be2 cells (Beff cells from T_H2 co-cultures), expressed high levels of FcRL5, CD11c and T-bet. By contrast, the Be2 cells expressed high levels of CD23. Interestingly, higher frequencies of PCs were found in T_{H1}/B co-cultures compared to T_{H2}/B co-cultures. Next, using biomarkers identified in the *in vitro* generated Be1 and Be2 populations, we asked whether similar Beff subsets were present in the blood of healthy controls (HCs) or autoimmune patients. Be1-like cells which express high levels of FcRL5, CD11c and T-bet, and low levels of CD23 were present in low numbers in HCs. Interestingly, this population was expanded in SLE patients and correlated with the levels of systemic IFNγ, IP10 and anti-Smith antibodies in plasma. In addition, upon co-culturing allogenic T_H1 cells with highly enriched T-bet$^+$ B cells sorted from SLE patients, we observed that a significant fraction of the T-bet$^+$ B cells differentiated into PCs, suggesting that T-bet expressing B cells represent a pool of pre-PCs that are expanded in lupus patients.

W.58. Eosinophilic Granulomatosis with Polyangiitis Presenting as Aortic and Mitral Regurgitation

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Eosinophilic granulomatosis with polyangiitis (EGPA) is a multisystemic small-to-medium sized artery vasculitis with the lungs most commonly affected. We describe a less common but more serious case of EGPA presenting with cardiac involvement.

An 86-year-old man presented with a four-day history of worsening dyspnea and wheezing. He had a past medical history of heart failure with preserved ejection fraction (68%) and aortic regurgitation treated with bioprosthetic valve replacement ten-years prior. He was on 5mg prednisone alternate days for hypereosinophilic syndrome diagnosed by his primary physician. Cardiac ascultation revealed an ejection-systolic murmur (2/6) right-upper sternal border and pansystolic murmur (3/6) at the apex. Chest ascultation revealed bilateral expiratory wheezes and fine bibasilar crackles. A complete blood count showed leukocytosis with 12% eosinophilia. A chest radiograph showed diffuse interstitial opacities. A transesophageal echocardiogram revealed severe perivalvular aortic regurgitation and severe mitral regurgitation with moderate diastolic dysfunction.The pulmonary findings and eosinophilia in the presence of new-onset valvular disease raised suspicion of eosinophilic myocarditis which was confirmed on endomyocardial biopsy. Patient's symptoms resolved after three days on prednisone 80mg daily, and he was discharged on oral cyclophosphamide and prednisone taper with plan to undergo repair of the peri-aortic valvular leak.

Cardiac involvement is a rare but serious manifestation of the disease attributing to one-half of disease-related deaths. Patients with cardiac involvement demonstrate signs of heart failure, pericarditis or arrhythmias. Such patients have a mortality of 60% greater than those without cardiac involvement over a 78 month span from diagnosis in the absence of treatment.

T.82. Role of Biologic Therapy in SLE: Current and Future Trends

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Background: Systemic Lupus Erythematous (SLE) is a chronic inflammatory autoimmune disease, with autoantibodies produced against healthy body organs. Deeper knowledge and studies on the immunopathology of SLE has led to identification of multiple new immunotherapeutic agents targeting immune cells and cytokines.

Discussion: Despite ongoing researches in SLE there are still unmet needs in this field especially in long term management of lupus nephritis. Current treatment is limited to azathioprine, mycophenolate, hydroxychloroquine, cyclophosphamide and corticosteroids in varying doses for lupus nephritis and other complications. However, practice defined toxicity of these drugs including serious infections, infertility, and malignancy limits their use especially in young population .The new area of SLE treatment started with biological therapies in the last decade. Rituximab has been investigated in several trials including EXPLORER and LUNAR, but showed non-significant differences especially in moderate to severe proliferative nephritis. FDA approved Belimumab, a human IgG λ1 monoclonal antibody which acts on B-lymphocyte stimulator, for reduction in flares, and possible early remission. It has fewer side effects when used in addition to standard care, however is not approved for nephritis.

Conclusion: Biologics have transformed the treatment of many patients with Rheumatoid Arthritis and Psoriatic Arthritis but not so in SLE. Clinical trials on T cell blockade (Abatacept), anti-IL6R (Tocilizumab), anti IFNα (Sifalimumab), anti-C5a (Eculizumab), anti CD22 (Eculizumab) among others are ongoing, with hope for additional agents for SLE treatment.

Bone Marrow or Stem Cell Transplantation

OR.52. Non-human Primate Model for CMV Viremia: Immunologic Impact and Treatment Approaches

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Cytomegalovirus (CMV) infection is a serious complication in patients with an impaired immune system. We have studied the effects of CMV disease and immunological reconstitution in cynomolgus macaques (cyno) (n=10) undergoing non-myeloablative MHC-mismatched allogeneic bone marrow transplantation (BMT) with two types of post-transplant monotherapy. Animals received 2.5-3Gy total body and 7Gy thymic irradiation, anti-CD40L and anti-thymocyte globulin. Six animals received a 28-day course of cyclosporine A (CyA) and four received rapamycin (Rapa). 9/10 animals were CMV seropositive before BMT. Immune reconstitution was monitored via FACS and viremia via qPCR. All animals developed CMV viremia 10-34 days post-BMT. When viremia surpassed 10,000 copies/mL, CMV disease was observed. High doses of gancyclovir (GCV) (12.5mg/kg IV BID) usually resolved viremia. Two animals died of uncontrollable CMV disease. Successful resolution of CMV disease required early treatment before the viremia reached 10,000 copies/mL. Anti-virals induced BM suppression causing severe cytopenias, graft loss and delayed T cell recovery. Control of CMV (without continued IV GCV) was achieved when CD4, CD8 and CD4CD8 DP counts were above 312, 465 and 52 cells/uL respectively. Unlike CyA, Rapa controlled CMV viremia in 3/4 animals during the 28 days of treatment post-BMT without requiring antivirals. In conclusion, cyno CMV reactivation is induced by lymphodepletion in 100% of seropositive animals. Like humans, T cell responses are likely required to clear the infection. GCV is protective albeit at high (myelosuppressive) doses. We also demonstrated that CMV viremia can be better controlled with Rapa during the most vulnerable lymphopenic phase.

OR.60. Control of Graft-versus-host Disease by Regulatory T Cells Depends on TNF Produced by Effector T Cells and TNFR2 Expressed by Regulatory T Cells

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Therapeutic $CD4^+Foxp3^+$ natural regulatory T (T reg) cells can fully control graft-versus-host disease (GVHD) after allogeneic hematopoietic stem cell transplantation (alloHCT) by suppressing T conv cells. Previous findings in the setting of autoimmunity revealed a feedback mechanism that renders T conv cells able to stimulate T reg cells by TNF. We tested this phenomenon in a GVHD setting in our previously described model of GVHD protection using T reg cells specific for the HY antigen. Using different experimental approaches to prevent TNF/TNFR2 reaction, we observed that control of GVHD by Treg cells was fully abolished by blocking TNF or by using TNF-deficient donor T cells or T reg cells deficient for the TNF receptor type 2 (TNFR2). Thus, T conv cells exert a powerful modulatory activity on Treg cells and, the sole defect of TNF production by donor T cells was sufficient to completely abolish the T reg suppressive effect in GVHD. Our results suggest that targeting TNF/TNFR2 interaction represents an opportunity to efficiently modulate alloreactivity in alloHCT to either exacerbate it for a powerful anti-leukemic effect or to reduce it to control GVHD.

F.01. Inducing Hematopoietic Microchimerism by Gene-modified Bone Marrow Transplantation Elicits Transgene-specific Immunological Tolerance for Factor IX Gene Therapy

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Background and objective: Gene therapy is a promising treatment option for hemophilia. Inasmuch as replacement therapy is hampered by the risk of immunization towards recombinant coagulation factor, immune response against the transgene product is also an obstacle to gene therapy. Induction of a hematopoietic chimerism is a potent means for inducing immunological tolerance in solid organ transplantation settings, including grafting of alloantigen expressing gene-modified autologous bone marrow. The objective of this study was to elicit transgene-specific immunological tolerance by inducing hematopoietic microchimerism in a gene therapy setting.

Methods: We developed a lentiviral vector (LV) encoding a strong immunogene composed of an immunodominant peptide of ovalbumin (OVA) linked to a MHC class I – beta-2-microglobulin fusion protein. Another LV included factor IX coding sequences under a pgk promoter (LV-FIX). C57BL/6 Ly5.2 mice were lethally (10 Gy) or sub-lethally (5 Gy) irradiated and reconstituted with LV-transduced congeneic C57BL/6 Ly5.1 bone marrow cells.

Results: Inducing less than 1% bone marrow microchimerism was sufficient to tolerize the $CD8^+$ T cell compartment towards OVA and to allow persistent transgene expression after *in vivo* gene transfer. We could abolish the humoral immune response against FIX and maintain persistent production of circulating FIX after LV-mediated gene transfer *in vivo* in contrast to controls.

Conclusion: These results provide the proof of concept that inducing microchimerism by autologous gene-modified bone marrow provides tolerance to a gene therapy product using the same vector and opens perspectives for further preclinical evaluation.

F.02. Differential Impact of Acute Graft-vs-Host-Disease on New B and T cell Production after Allogeneic Hematopoietic Stem Cell Transplantation

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One of the main problems in allogeneic Hematopoietic Stem Cell Transplantation (allo-HSCT) is the long-lasting immunodeficiency that leave patients highly susceptible to infections. Acute chronic Graft-versus-Host Disease (aGvHD) have been shown to delay both B and T cell reconstitution. Here, we compared the production of new B and T cell after allo-HSCT in a cohort of 399 patients treated in a single center.

Patients were treated mainly for hematological malignancies (n=337) using myeloablative (n=172) or reduced intensity (n=227) conditioning. New B and T cell production was assessed at 3, 6, 12 and 24 months after graft using quantification of Kappa Chain (sjKREC) or TCR (sjTREC) excision circles, respectively.

We confirmed, in a multivariate analysis, the delay of sjKREC and sjTREC recovery in case of aGVHD. Moreover, when we examined the effect of aGVHD according to the grade (0, 1, 2 or 2-3), we saw a difference between B and T cell reconstitution. At month 6, aGVHD had a progressive effect on sjKREC production, being significant only beyond a grade 2. Inversely, sjTREC were already significantly reduced with an aGVHD of grade 1 with no further difference with increasing grade. Moreover, sjKREC production recovered faster with only grade 3-4 aGVHD being significantly lower at month 12 and nothing at month 24.

So, in this large cohort of patients, sjKREC and sjTREC quantification provided a new insight in the kinetics of B^- and T cell reconstitution after allo-HSCT indicating a potential differential impact of aGVHD on thymic and bone marrow lymphoid production.

F.03. Induction of the Anti-Leukemia Immunomodulation Effects by Iron-Chelation Therapy after Allogeneic Hematopoietic Stem Cell Transplantation: a Role of Regulatory T Cells and/or NK-cells?

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Background: The clinical benefits of iron-chelation therapy (ICT) for AML with hyperferritinemia after Allo-HSCT have not been evaluated.

Materials and methods: All of 276 patients were analyzed for impact of ICT were monitored from initial diagnosis to the several time points during the post-transplantation period in both the ICT group (n=128) and the non-treated group (NT, n=148). As a validation cohort for monitoring the immunobiologic effects of ICT, we serially examined the subsets of regulatory T (Treg) and/or NK cells in another representative subgroup of transplanted recipients.

Results: The overall median follow-up for total survivors (ICT) was 58 months (range: 48~66). The group of high serum ferritin with ICT showed superior overall survival (72% vs. 51%, HR 0.596, p=0.007), good disease free survival (71 vs. 48%, HR 0.576, p=0.003), low cumulative incidence of relapse (11% vs. 28%, HR 0.591, p<0.001), and high incidence of chronic graft-versus-host disease (cGVHD) (64% vs. 38%, HR 1.87, p<0.001). Treg cells both pre-HSCT (*P*=0.002) and post-HSCT (*P* <0.001) periods after ICT showed a very close correlation with various clinical outcomes, specifically with lower rates of relapse in patients with lower levels of Treg cells as well as higher levels of $CD16^+$ NK cells compared to those who did not after at least 60 days in total use of deferasirox after HSCT.

Conclusion: Our data suggest the independent association of ICT with increased incidence of cGVHD and decreased relapse rate. Iron chelation may modulate the immunobiologic properties during the period of immune reconstitution.

F.04. Differential Development of Early Progenitors and Antigen Presenting Cells from Adult vs Fetal Hematopoietic Stem Cells in Humanized Mice

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Humanized mice generated from adult human bone marrow (BM) $CD34^+$ have promise as a powerful tool for preclinical human research. However, humanized mice generated from BM $CD34^+$ cells but not fetal liver (FL) derived $CD34^+$ cells have reduced multilineage human chimerism at late time points, with T cells dominating and antigen-presenting cells (APCs) declining. We hypothesized that inherent differences between BM HSC and FL HSC differentiation potential may contribute to this phenomenon. To understand the differences between humanized mice derived from BM and FL $CD34^+$ cells, we performed a thorough analysis of APC populations and their progenitors in humanized mice derived from these two HSC sources. We found that humanized mice generated from BM $CD34^+$ cells consistently had reduced APC populations, including macrophages, plasmacytoid DCs and $CD11c^+$ DCs, than those generated from FL $CD34^+$ cells. A thorough analysis of hematopoietic progenitors in these animals revealed only minor differences, except for HSCs and multipotent progenitors, which were significantly enriched in BM $CD34^+$ cells of humanized mice derived from FL compared to BM $CD34^+$ cells. Together, our data suggest that BM HSCs have a reduced ability to maintain antigen presenting cell populations long-term compared to FL HSCs, and this may reflect a lower number of long-term repopulating HSCs in BM vs FL $CD34^+$ cell populations.

F.05. Increased Newly-Generated B Cell Output And Regulatory B Cells After Autologous Hematopoietic Stem Cell Transplantation In Systemic Sclerosis Patients

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Autologous Hematopoietic Stem Cell Transplantation (AHSCT) has shown its efficacy for treating rapidly progressive diffuse systemic sclerosis (SSc). Here, peripheral blood was collected from 31 SSc patients before and every 6 months until 36 months after AHSCT and from 15 SSc patients treated with other immunosuppressive drugs (IS). Newly-generated B cells were quantified by RT-qPCR on genomic DNA of coding joint (Cj) and signal joint-kappa-deleting recombination excision circles (sjKREC). Mean number of B cell divisions (n) was calculated using the following formula: n=LOG(Cj/sjKREC)/LOG2. No change in the sjKREC expression was observed in SSc patients treated by IS, while in AHSCT group its expression increased significantly ($P<0.05$) from 12 until 24 months post-transplant compared to baseline. No change in the Cj expression was observed in IS patients, while for the aHSCT group, Cj expression remained higher than in the non-transplanted group ($P<0.05$). Cj and SjKREC expression resulted in reduced B cell divisions in the peripheral blood from 6 until 18 months as compared to the non-AHSCT group ($P<0.05$). $CD19^+CD24^{hi}CD38^{hi}$ immature/regulatory B cells increased transiently within the total pool of $CD19^+$ B cells from 6 until 12 months after aHSCT ($P<0.05$). In conclusion, our data indicate that the renewal of the B cell pool through increased newly-generated B cells, may contribute to reset of self-tolerance in SSc patients after aHSCT.

Cytokines/Chemokines

OR.32. IL-13 and IL-33 Pathways Influence Inflammation in Metabolic Tissues: Contribution of IL-**13Rα2**

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In obesity, adipose tissue macrophages take on a pro-inflammatory (M1) phenotype and promote insulin resistance. IL-13 can restore homeostasis by driving M2 macrophage differentiation through the IL-13Rα1/IL-4R signaling receptor. These responses can be modulated by the “decoy” receptor, IL-13Rα2, which binds with high-affinity to inactivate and deplete IL-13, and the alarmin, IL-33, which triggers release of IL-13 and IL-5. Strategies to elevate IL-13 levels and activity, including blocking IL-13Rα2 and/or administration of IL-33, are predicted to preserve metabolic

function. We found that IL-13Rα2 expression was elevated by approximately 10-fold in adipose tissue under conditions of obesity. To examine its role, mice deficient in IL-13Rα2 were fed a high fat diet (HFD). Expression of adipose M2 macrophage markers was increased in IL-13Rα2-deficient mice, consistent with higher endogenous IL-13 activity, but glucose tolerance was not affected. Because IL-33 promoted IL-13 production, and IL-33 deficiency exacerbated HFD-induced metabolic dysregulation, we examined the consequences of IL-33 administration to wild-type and IL-13Rα2-deficient mice. IL-33 increased serum and tissue IL-5 and IL-13, produced an enlarged spleen, elevated peritoneal eosinophils and macrophages, and expanded ILC2 and eosinophil numbers in adipose tissue. Induction of cytokines and cytokine response genes by IL-33 was attenuated in livers of IL-13Rα2-deficient mice. These findings show that IL-13Rα2 deficiency can skew immune responses toward an M2/Th2 bias *in vivo* under conditions of metabolic stress, including HFD or administration of IL-33. IL-13Rα2 deficiency also reduced tissue inflammation with HFD and decreased expression of inflammatory markers in metabolic tissues, but did not affect glucose homeostasis.

OR.34. IL-33 Exerts Immunoregulatory Effects on Mesenchymal Stem Cells

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IL-33 has been previously described as an *alarmin*, but it is currently associated with the induction of $Foxp3^+$ Treg cells. Based on this, we decided to assess its potential modulatory properties on mesenchymal stem cells (MSCs). To accomplish this, bone marrow-derived MSCs were incubated for 24 hours with IFNγ in presence or absence of IL-33. Then, MSCs were harvested for phenotype analysis using flow cytometry, and total RNA was isolated to evaluate gene expression by qRT-PCR. Additionally, supernatants were collected for cytokine quantification by ELISA test. Our results suggest that both cytokines did not have any effect on MSCs typical surface markers, but IFNγ increases MHC-II expression in these cells. Regarding cytokine production, only IL-33 increases IL-6 secretion. In addition, our study included the expression analysis of key immune-regulatory genes: induced nitric oxide synthase (iNOS), arginase-1 (Arg1), plus two metalloproteinases (MMP2 and MMP9). By qRT-PCR assay, we observed that IFNγ up-regulates the expression of these genes, but IL-33 exerts a more potent effect. Lastly, in co-culture assays using MSCs pre-treated with the described cytokines and cultured with $CD4^+$ T cells, we show that CD^{4+} T cells up-regulates the expression of CD25 and Nrp-1 when cultured with IFNγ-treated MSCs, affecting the activation and potential functional activity of T cells. Altogether, our study suggests that IL-33 may affect the expression levels of various immunoregulatory molecules, which are part of the mechanism by which MSCs alter T cell biology, proposing IL-33 as a potential immunomodulator factor.

OR.48. S1P Kinases Axis Promotes Th17 Polarization in Human Lymphocytes

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Sphingosine-1-phosphate (S1P) is a bioactive phospholipid involved in many cellular processes and has also a wide array of effects on immune system. S1P has been long known to regulate trafficking of cells of the immune system, although increasing evidences suggest that S1P could be also involved in determining cell functions as production of cytokines and chemokines. S1P is formed upon phosphorilation of a cellular pool of Sphingosine by two kinases, SphKI and SphKII, several phosphatases dephosphorilate S1P to Sphingosine, and a Sphingosine lyase cleaves S1P into aldehydes and phosphoethanolamine. The two kinases that regulate S1P formation have a different distribution within the cell, SphKI is localized in cytosol of eukaryotic cells while SphKII is found in the nucleus. We wanted to test the hypothesis that S1P balance in human lymphocytes could be involved in T cell polarization and we found that S1P is critical to differentiate human T lymphocytes towards Th17 subset. Indeed, we first found that pharmacological inhibition of SphKI and SphKII was able to inhibit the differentiation of Th17 from peripheral T cells. Then, we confirmed the role of these two kinases by specific siRNA experiments. Finally, we showed that

transfection of the two kinases in T cells upregulated the production of IL-17. No effects on Th1 and Th2 polarization was found in these experiments. These data indicate that SphKI and SphKII are able to regulate IL-17 production in human T cells, thus determining the functional polarization of T lymphocytes.

T.01. CCR7$^+$ T-helper Lymphocytes Detection in Periodontal Disease

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Formerly, the appearance of periodontal lymphoid clusters has been postulated in chronic periodontitis patients, promoting alveolar bone resorption in a periodontal site-specific manner. Molecules such as CCR7 have been widely associated with the formation of ectopic lymphoid-like structures in inflammatory diseases. This study aimed to analyze the detection of CCR7$^+$ naïve and memory T-helper lymphocytes, as well as the expression of CCR7, CXCR4, CXCL12, CCL19, and CCL21 in periodontal tissues from healthy and periodontitis diseased individuals.

Methods: Gingival samples were obtained from healthy or moderate-to-severe chronic periodontitis individuals. Total cells were analyzed by flow cytometry using the following monoclonal-antibodies: anti-CD4, CD25, CD45RA, CD45RO, RORC2, Foxp3, T-bet, and CD197. In addition, mRNA expression for CCR7, CXCR4, CXCL12, CCL19, and CCL21 was quantified by qRT-PCR. CCL19, CCL21, and CXCL12 secretion was quantified from gingival crevicular fluid samples by ELISA. Finally, CCR7 was detected by western-blot and immuno-fluorescence in periodontitis-biopsies.

Results: Higher levels of CCR7, CXCR4, CCL19, CCL21, and CXL12 were detected in periodontitis patients compared with healthy individuals. In addition, the number of CCR7$^+$ naïve T cells and CCR7$^+$RORC2$^+$ memory T cells were greater in periodontitis patients. CCR7 was broadly localized in the gingival connective tissue, and particularly clustered around blood vessels.

Conclusions: An increment in the number of infiltrating CCR7$^+$ naïve and memory T-lymphocytes was detected in periodontal tissues from periodontitis versus healthy individuals, and this increment was associated with local CCL19, CCL21, and CXCL12 production suggesting that CCR7 could play a role in periodontitis pathogenesis through a periodontal ectopic-lymphoid cluster formation

T.02. Renal Macrophage Infiltration and Expression of TNF-**α and MCP**-1 is Exacerbated by Social Stress and Mitigated with Exercise in a Murine Model of Lupus Nephritis

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Physical exercise and psychosocial stressors have been shown to induce opposing immunomodulatory effects. Numerous studies investigating regular exercise or stress modification have been efficacious in influencing outcomes of various autoimmune diseases, including systemic lupus erythematosus (SLE). Lupus nephritis (LN) is a debilitating manifestation of SLE characterized by inflammation and progressive renal failure; however, daily moderate exercise (DME) and stress management are underemphasized to patients with LN due to a poor comprehensive understanding. To investigate these effects on murine LN, we monitored disease progression with either DME or social disruption stress (SDR) induction in LN-prone NZM2410 mice. Histopathological examination of renal disease showed significant enhancement with SDR and improvement with DME, as indicated by both IgG and C3 complex deposition and macrophage infiltration. To better understand these responses mechanistically, serum was examined for pro-inflammatory cytokine expression. IL-5, IL-6, IL-10, and TNF-α levels were increased in NZM2410 mice with disease progression and induced more rapidly with SDR. While DME had no effect on IL-6, expression of IL-5, IL-10, and TNF-α was suppressed with this regimen. Since monocyte chemoattractant protein-1 (MCP-1) is known to be

induced by TNF-α, we measured serum MCP-1 expression and observed a decrease with DME and an increase with SDR. This suggests that macrophage-mediated inflammation of kidneys in LN can be modified via exercise and stress reduction by suppressing TNF-α-mediated induction of MCP-1. Future investigation will provide further pathway characterization to elucidate the precise effects of DME and stress reduction in LN and identify potential targets to exploit therapeutically.

T.03. CCR2 Upregulated on T Cell Populations in Peripheral Circulation Among Patients with Osteoarthritis Compared to Bone Marrow and to Healthy Control

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A large portion of lymphocytes reside in bone marrow and early development of all leukocytes occurs in this locality. Although being the main primary lymphoid organ and although many questions on leukocyte maturation remain unanswered, relatively few studies have been conducted on bone marrow material if compared to peripheral blood. We have devised a protocol for extracting seemingly healthy bone marrow from patients undergoing orthopaedic surgery in large quantities without additional discomfort for the patients. Chemokine receptors reveal migratory characteristics of lymphoid cells and can also contribute in phenotyping cell populations into functional subcategories. This study show a difference in chemokine receptor 2 expression on T cell populations in peripheral blood among osteoarthrosis patients compared with heathy controls which could prove useful as a screening tool for osteoarthritis patients.

The difference in chemokine receptor 2 expression between peripheral circulation and bone marrow also suggest a mechanism for T cell bone marrow egression.

T.04. The TNF Superfamily Member LIGHT Induces Distinct Proinflammatory Responses in Dermal Fibroblasts and Keratinocytes Relevant to Systemic Sclerosis Via Activation of MAP3K8

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Systemic sclerosis (SSc) is a severe autoimmune connective tissue disorder leading to fibrosis of skin and internal organs. LIGHT/TNFSF14 can influence both structural and inflammatory cells to promote fibrosis. This study sought to investigate the effects of LIGHT on normal human dermal fibroblasts (n=5) and keratinocytes (n=5) and compare them with SSc dermal fibroblasts (SSc-fib) generated from lesional skin (n=7) and SSc keratinocytes (SSc-keras) generated from hair follicles (n=7). Using NanoString gene expression platform we could show that LIGHT induced a distinct set of genes in both fibroblasts and keratinocytes such as the the chemokines CCL2, CCL5, CCL11, CCL20, CXCL1, CXCL2, CXCL3, CXCL5 and CXCL6; the cytokines IL-6, IL-8, IL-15 and TSLP; metalloproteinase MMP- 9 and the kinases MAP3K8 and MEKK1/2. In comparison to normal fibroblasts and keratinocytes, SSc-fib and SSc-keras secreted significantly higher ($p<0.05$) amounts of CCL20, IL-6, TSLP and MMP-9. LIGHT induced a dose dependent upregulation of MMP-9 in keratinocytes. Using a coculture system of keratinocytes and T cells we could show that LIGHT induced MMP-9 mediated migration of T cells through an artificial basement membrane. Through western blot and by using specific inhibitor (TC-S7006) we could show that LIGHT mediated proinflammatory effects were contingent upon MAP3K8 mediated MEKK1/2 and ERK1/2 activation. Therefore, therapeutic targeting of LIGHT is a treatment option for diseases with skin infiltration of T cells such as SSc and atopic dermatitis.

T.05. Ultra-sensitive Detection of Interferon Alpha Protein in Human Disease by Digital ELISA

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Type I interferons (IFNs) have long been recognized as essential mediators of the anti-viral response, and have also been implicated in the pathogenesis of autoimmune and Mendelian disease states including systemic lupus erythematosus, dermatomyositis and the monogenic interferonopathies. Despite this fundamental role in both human health and disease, the direct quantification of type I IFN protein in biological samples has remained elusive. Harnessing the recent innovation of digital ELISA technology, we developed an ultra-sensitive, quantitative assay that measures type I IFN protein at attomolar (femtogram/ml) concentrations in human serum, plasma and cerebrospinal fluid. In the context of a group of single gene disease, complex-disease and infectious phenotypes, we were able to quantify elevated levels of IFNα, which were below the limit of detection of currently available assays. For all patient groups tested we observed a positive correlation of IFN protein levels with anti-viral activity, as measured by a cytopathic protection assay. Interestingly, a comparison of IFN protein with expression of IFN stimulated gene transcripts revealed a similarly strong correlative association in lupus and dermatomyositis, but not in the monogenic interferonopathies. These differences are likely indicative of the dynamic relationship between IFN receptor engagement and subsequent ISG expression in the different diseases examined. These data demonstrate the potential of digital ELISA technology in the diagnosis and stratification of pathologies associated with an upregulation of IFN, and in enhancing our understanding of the regulation of type I IFN responses.

T.06. Levels of Inflammatory Markers in Gingival Crevicular Fluid from Newly Diagnosed Type 2 Diabetes Patients with Chronic Periodontitis

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Introduction: The immune system of diabetic subject is altered due to diabetes mellitus, this affects the periodontal tissue.

Objective: To evaluate the inflammatory markers IL-2, IL-6 and IL-8 in gingival crevicular fluid of patients with Type 2 Diabetes Mellitus (T2DM).

Methodology: Participants were grouped into four groups: 1) healthy (n = 20), 2) with periodontitis (PCM, n = 20), 3) ≤1 year with diabetes and periodontitis (DMT2≤1+PCM, n = 20) and 4) ≥10 years diabetic and periodontitis (DMT2≥10+PCM, n = 20). Cytokines were quantified by flow cytometry.

Results: The DMT2≥10+PCM group showed higher levels of IL-2 respect to the other groups, as elevated levels of IL-6 respect to the PCM. The levels of IL-8 were higher in the healthy group compared to other groups.

Conclusion: DMT2≥10+PCM subjects showed a destructive periodontal tissue adaptive immune response compared to DMT2≤1+PCM which show an immunological disturbance.

T.07. Selective Inhibitors as Tools to Dissect the Role of JAKs in Cytokine Signaling

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Janus Kinases (JAKs) play a central role in cytokine signaling. In particular, the restricted expression of JAK3 in hematopoietic cells makes it an attractive therapeutic target. In fact, inhibition of JAKs with first-generation pan-JAK inhibitors has shown therapeutic efficacy in autoimmune, autoinflammatory, and hematopoietic diseases. More recently, the development of JAK isoform-selective inhibitors has the potential to elucidate the distinct mechanistic roles of JAKs. JAK1 has been postulated to have a dominant role in IL-2 signaling, based on inhibitors data, but the issue remains controversial. To dissect the roles of JAK1 and JAK3 in IL-2 signaling, we utilized new JAK isoform-selective inhibitors and assessed the activation of Signal Transducer and Activator of Transcription 5 (STAT5) in $CD4^+$ T cells. Interestingly, we found that both the JAK1-specific and the JAK3-specific inhibitors completely abrogated STAT5 phosphorylation and nuclear translocation, confirming that both JAK1 and JAK3 are critical enzymes for IL-2 signaling. Furthermore, we compared the pharmacological effects of these inhibitors to tofacitinib, which blocks both JAK1 and JAK3, and to a lesser extent JAK2. Interestingly, JAK1- and JAK3-selective inhibitors have additive actions in inhibiting the IL-2 signaling cascade. These results indicate that blockade of JAK1 or JAK3 is sufficient to inhibit IL-2 signaling. Our work provides mechanistic basis to test this class of inhibitors in diseases, which may benefit more from JAK isoform-selective inhibition.

T.08. Homing Capacity of Thymic Regulatory T Cells Can Be Tailored by Expansion in Cytokine-enriched Culture Conditions

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Regulatory T cell (Treg)-based therapy is a promising approach to treat allograft rejection. We have previously found that thymuses, routinely removed during pediatric cardiac surgery, are a potential source of therapeutic Tregs. To be effective, Tregs must express homing receptors and migrate to inflammatory sites. For example, expression of the chemokine receptor CXCR3 on Tregs is essential for homing to and suppression of Th1-mediated inflammation. We hypothesized that homing capacity of thymic Tregs could be tailored by including cytokines in the expansion protocol.

$CD4^+CD25^+$ Tregs were isolated from pediatric thymuses and expanded with artificial antigen-presenting cells, rapamycin and IL-2. IL-12 and IFN-gamma were included for Th1-polarizing conditions. Addition of Th1-inducing cytokines significantly increased CXCR3 expression and resulted in 3-fold higher Treg expansion compared to neutral conditions. Importantly, Tregs cultured under Th1-polarizing conditions maintained a stable phenotype, including high FOXP3 expression, did not acquire the ability to produce Th1-cell associated cytokines and potently suppressed the proliferation of conventional T cells *in vitro*. Moreover, expansion with Th1-inducing cytokines increased *in vitro* migration of Tregs towards the CXCR3-specific chemokine CXCL10 and migration could be blocked using a CXCR3-neutralizing antibody.

Thus, expansion conditions can be manipulated to specifically tailor the homing capacity of thymic Tregs. The ability to direct Tregs towards specific tissues or sites of inflammation may enable optimal targeting as a therapeutic *in vivo*. Future investigations will focus on testing the ability of Th1-polarized thymic Tregs to specifically suppress Th1 cells *in vitro* and in a pancreatic islet transplant model *in vivo*.

T.09. Reduced Response to IL-2 in Memory Teff of T1D is Cell Intrinsic and Due to Increased Expression of Negative Regulators

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IL-2 based therapies hold promise for the treatment of T1D. Yet, there is substantial variability in IL-2 responsiveness across subjects and cell types that may result in a narrow dosing range. Thus, we need to better understand the sources and causes of this variability. Using large (n>75 subjects/group), age and gender matched, cross-sectional control and T1D cohorts, we measured response to IL-2 using an optimized pSTAT5 assay. Response to IL-2 in T1D subjects was reduced in CD4 memory Treg (p=0.0058) and Teff (p=0.0162) populations. *IL-2RA*rs2104286 and *PTPN2*rs1893217 risk genotypes conferred a significant reduction in pSTAT5 in memory Treg and Teff of controls. Yet, when *IL2RA* and *PTPN2* genetics were held constant, T1D subjects still displayed reduced pSTAT5. IL2RA and phosphatases have been shown to regulate IL-2 responses in Treg. To determine additional factors underlying reduced IL-2R signaling in Teff, we compared control and T1D subjects held constant for known risk alleles. IL-2 signaling in CD4 memory Teff of T1D subjects remained significantly lower than controls (n=8/group) even after *in vitro* activation and proliferation, suggesting a role for intrinsic factors. Further, when comparing the ratio of positive and negative regulators of IL-2 signaling by qPCR, we found enhanced negative regulation, including SOCS1, SOCS3, PIM3 and PTPN11 (SHP-2), in T1D low IL-2 responders (n=10/group). This enhanced negative regulation was also found in *PTPN2*rs1893217 risk individuals. Thus, IL-2 responsiveness is reduced by enhanced negative regulation, influenced by genetics and disease. These findings have implications for targeted therapies and stratification for IL-2 based therapies.

T.10. Effect of Vitamin A Supplementation on Th-1 to Th-2 Balance in Patients with Atherosclerosis

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Background: Atherosclerosis (AS) is the major cause of death worldwide due to imbalance of immune responses and higher levels of proatherogenic T helper 1 (Th-1) cytokines. Vitamin A biases immune response in to Th-2 direction. We examined how vitamin A supplementation modified Th-1 to Th-2 cytokines responses in AS patients compared to controls.

Design: we enrolled thirty five patients with stable coronary artery disease and thirty eight healthy controls in this randomized controlled trial study. Patients and controls were divided into two groups based on receiving 25000 IU/ day oral supplement of vitamin A or placebo for 3 months. We measured specific cytokines of Th-1 (IL-2, IL-12, IFN-γ) and Th-2 (IL-10, IL-4) in plasma, unstimulated or stimulated supernatants of peripheral blood mononuclear cells (PBMCs) by oxidized LDL (ox-LDL) or PHA. Th-1 to Th-2 ratio was calculated by using cytokine of PHA treatment.

Results: The basement levels of each cytokine were not significantly different between patients and controls. Vitamin A decreased plasma levels of IL-2, IL4 in patients (P=0.022, P=0.01) and plasma levels of IFN- γ and IL-12 in controls (p=0.005) and (P=0.017) respectively. While, it increased IL-2 supernatants levels in controls (p= 0.002). Diminished levels of IL-10 in plasma and PBMCs' unstimulated supernatant were observed following intervention in both groups, while Th1 to Th2 ratio remained constant.

Conclusion: Vitamin A supplementation decreased cytokine levels of Th-1 and Th-2, so that pro-inflammatory to inflammatory cytokines ratios remained unchanged. Beneficial effects of vitamin A supplementation are more in patients rather than controls.

T.11. Phenotyping Corticosteroid-induced IL-17^{+} Cells

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Asthma affects 300 million people worldwide but in 5-10% of cases, the disease is unresponsive to steroids, a cornerstone treatment for asthma. Steroid refractory asthma is characterised by high levels of IL-17A, which we have previously reported to be upregulated by the synthetic steroid dexamethasone in PBMC cultures. Here we aim to further phenotype steroid-induced IL-17A$^+$ cells.

Dexamethasone increased the synthesis of IL-17A, IL-17F and IL-22 by proliferating memory CD4$^+$ T cells. Dexamethasone-induced IL-17A$^+$ cells were RORc$^+$CCR6$^+$MDR1$^-$, with 2/3rds of cells expressing CD161. Notably, the proportion of immunoregulatory IL-17A$^+$IL-10$^+$ cells was significantly increased following dexamethasone treatment ($p<0.0001$). In contrast a subgroup of the IL-17A$^+$ cells co-expressed Th1-associated markers CCR4, Tbet, IFNγ and GM-CSF, which is indicative of pathogenic Th17.1 cells; the proportion of these cells was not modulated by dexamethasone.

Induction of IL-17A by dexamethasone was dependent upon anti-CD3 and low dose IL-2 stimulation, and was blocked by neutralising IL-2 or signalling via CD28. By modulating the IL-2 signalling pathway (IL-2, CD25, c-maf, phospho-STAT5), dexamethasone is thought to enable the preferential outgrowth and *de-novo* differentiation of Th17 cells via mechanisms currently under investigation.

Steroid refractory asthma and various autoimmune diseases that are typically treated with steroids are associated with elevated levels of IL-17A. We identify that *in vitro*, steroids promote primarily immunoregulatory Th17 cells (IL-10$^+$) alongside a sub group of Th17.1-like cells (IFNγ$^+$). We are now performing transcriptional profiling and functional assays on these Th17 subpopulations in the hope of generating more targeted therapeutics.

T.13. Ultrasensitive Measurement of IL-21 in Autoimmune Serum and Plasma

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Interleukin-21 (IL-21) is a pleiotropic cytokine affecting key cell types like B cells and T follicular helper cells likely to be involved in the pathogenesis of seropositive autoimmune diseases like systemic lupus erythematosus (SLE) and Sjögren's Syndrome (SS). Several groups have reported elevated circulating levels of IL-21 in SLE and SS compared with healthy control samples as measured by ELISA. As part of our validation of commercial assays we found matrix and specificity effects in the most commonly used commercial assay in published data. For example, endogenous IL-21 immunoreactivity could not be immunoprecipitated (IP) out using anti-IL-21 monoclonal antibodies. This prompted an effort to develop a more specific and sensitive assay to measure IL-21 in autoimmune plasma using Lilly anti-IL-21 antibodies in the Quanterix Simoa™ platform.

We performed IL-21 spike recovery, dilutional linearity, IP depletion, in replicate samples in 3 different matrices: serum and both EDTA and heparin plasma. The IL-21 assay can detect sub-pg/ml levels (LLOQ 6 fg/mL; ULOQ 35,000 fg/mL), irrespective of rheumatoid factors.

Amounts of IL-21 in autoimmune serum and plasma using the ultrasensitive assays are 100-500 times lower that previously published data. In addition, we are now able to accurately measure IL-21 in healthy control samples, previously not possible using existing assays.

T.14. An Optimized Approach for Isolating Viable Human Cytokine-defined B Cells

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Distinct cytokine-expressing B cell subsets can differentially modulate other immune-cell functions, with implications to both host immunity and disease pathogenesis. A current limitation to detailed characterization of such B cells is a lack of specific cell-surface markers with which viable cells can be FACS-sorted for functional analysis. Intracellular cytokine staining (ICS) kills cells of interest, and the 'MACS® cytokine-secretion assay' was developed as an alternative approach. It uses a bi-specific antibody targeting both cell-surface CD45 and the cytokine of interest. While enabling preservation of cell integrity and viability, this approach works best for isolating relatively high-frequency cytokine-expressing subsets and has not been applied to concurrent isolation of multiple distinct low-frequency subsets. In optimizing such an assay, we first individually sorted high-frequency (TNF^+) and low-frequency ($GM\text{-}CSF^+$ and $IL\text{-}10^+$) human B cells and confirmed their respective unique expression of *TNF, CSF2* and *IL-10*, respectively, as well as their selective secretion of the appropriate cytokines upon further activation. We then demonstrate successful dual concurrent staining and sorting of high-(TNF) and low-(GM-CSF or IL-10)-frequency B cell subsets. We further define parameters that minimize competition between cytokine stains during multi-parametric staining, with minimal signal-loss and finally demonstrate for the first time successful concurrent triple-staining and sorting of viable and purified TNF^+, $GM\text{-}CSF^+$ and $IL\text{-}10^+$ B cell subsets. We conclude that the cytokine secretion assay can be adapted to simultaneous multi-parametric FACS-sorting of viable, distinct (both high- and low-frequency) B cell subsets, enabling concurrent assessment of their detailed phenotypic and functional properties.

T.15. Quantitative Phosphoproteomic Analysis of Diverse Signaling Networks Activated by IL-23 and IL-12 Stimulation

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The heterodimeric IL-12 family cytokines, IL-12 and IL-23, initiate distinct cellular processes (e.g., Th1 vs Th17 biology) despite sharing a common component in both their ligand (p40) and receptor (IL-12Rb1) complexes. Targeting the subunits of these proteins has important therapeutic potential; therefore, as new medicines are developed, it is imperative to understand how these two closely related signaling proteins mediate vastly different immunogenic responses. While it is known that IL-23 and IL-12 stimulation can induce phosphorylation of STAT3 and STAT4, respectively, an unbiased, global assessment of the unique intracellular signaling processes propagated by these two cytokines had not been performed. Therefore, we implemented phosphopeptide enrichment methodology to first identify robust and reproducible phosphorylation modifications from whole cell lysates of a cell culture model that endogenously responds in a selective manner to IL-23 or IL-12 stimulation. Our work in whole cell lysates identified a signature of 623 IL-23-specific phosphorylated peptides, 999 IL-12-specific phosphorylated peptides, and, through additional theoretical analysis of the surrounding motifs, potential kinases responsible for these events. Next, we enacted a deeper analytical approach to interrogate various enriched cellular fractions at two time points post-cytokine stimulation. We also present novel methodology that may provide a path forward in the quantitative analysis of the phosphoproteome of human peripheral blood mononuclear cells (PBMCs). In conclusion, this study represents the most diverse set of phosphorylation modifications reported for IL-12 and IL-23-mediated signaling in a T cell-like cell line, and provides key insights into the differential regulation of these events.

T.16. Characterization of the Piperazino-enaminone Compounds as Novel Anti-inflammatory Agents

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Pro-inflammatory mediators including TNF-alpha, IL-6, and nitric oxide are important for the regulation of the immune response when an infection is present, but when overproduced can be responsible for the development of tissue and organ injury seen in sepsis, as well as severe asthma, and autoimmune diseases such as Crohn's disease and Rheumatoid arthritis. Data from our lab to characterize the novel compound enaminone E121 have suggested that macrophages stimulated with lipopolysaccharide (LPS) release significantly decreased levels of TNF-alpha and IL-6

as measured by enzyme-linked immunosorbent assay (ELISA) as compared to the DMSO control group. Further data have suggested that E121 may exert its immunosuppressive effect on the TLR4 pathway by upregulating SOCS1 (suppressor of cytokine signaling). Additionally, functional experiments in an animal model of asthma have shown that the enaminones are efficacious. The JODI series compounds are analogous to E121 in that an N-arylpiperazino motif is incorporated on the aromatic side of the enaminone pharmacophore. This may enhance their immunosuppressive activity as anti-inflammatory agents by also acting as a chemokine receptor antagonist. These piperazino enaminones appear to suppress TNF-alpha in a dose-dependent manner and also have an effect on NF-kB as seen by Western Blot of phospho-p65. Lastly, the JODI compounds were found to be more effective in reducing TNF-alpha after LPS stimulation as compared to dexamethasone. If found to block TLR4 signaling, E121 and its corresponding piperazino-analogs could act as strong anti-inflammatory agents in asthma or other autoimmunities where efficacious therapeutic options are needed.

T.17. Altered Soluble Mediator Levels and Systemic Lupus Erythematosus (SLE)-Specific Connective Tissue Disease Screening Questionnaire (CSQ) Scores Differentiate Unaffected Relatives of Lupus Patients from Healthy Individuals with No Family History of SLE

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Identifying populations at risk of SLE who remain unaffected would provide insights for potential disease prevention. Using a unique resource of SLE patient family members, first degree relatives (FDRs) of SLE patients (n = 154) with plasma samples available from previous genetic studies and who remained unaffected at follow-up evaluation (mean time = 6.8 years) were matched to healthy individuals unrelated to SLE patients (Controls). FDRs and Controls provided clinical and demographic information, and completed screening questionnaires at baseline (BL) and follow-up (FU). BL and FU plasma samples were assessed for autoantibody production and soluble mediators. FDRs had significantly higher BL and FU CSQ scores than Controls ($p<0.0001$). Although no significant difference in the number of positive autoantibody specificities were noted between FDRs and Controls, FDRs had significant alterations in 38 (of 52) soluble mediators, with APRIL and BLyS, IFN-associated chemokines IP-10, MIG and MIP-1α, as well as the regulatory mediators IL-10 and TGF-β, being significantly higher in FDRs vs. Controls ($p<0.002$). A number of mediators (14 at BL and 18 at FU) found to best separate FDRs from Controls by Random Forest strongly correlated with CSQ scores ($p<0.0002$). Of these, levels of MIP-1α ($p=0.008$), MIG ($p=0.019$), GROα ($p=0.001$), ICAM-1 ($p=0.007$), and VEGF ($p=0.004$), along with CSQ scores ($p=0.010$), best distinguished FDRs from Controls in logistic regression models. These alterations are present despite lack of progression to classified SLE, suggesting that multiple perturbations in immune-mediated inflammatory processes present in FDRs of SLE patients may be offset by inhibitory factors.

Diabetes and Other Autoimmune Endocrine Diseases

OR.10. Lack of Central Tolerance Facilitates the Emergence of High Avidity Antigen-Experienced ZnT8-reactive $CD8^+$ T Cells in Type 1 Diabetic Patients

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$CD8^+$ T cells are the final mediators of beta-cell destruction in type 1 diabetes (T1D). However, the epitopes whose recognition mediates such destruction are heterogeneous among patients. We recently reported a notable exception for an HLA-A2-restricted zinc transporter (ZnT)8 185-194 epitope, which is recognized in more than 70% of HLA-$A2^+$ new-onset T1D patients. We therefore set forth to understand the reasons for this unprecedented immune prevalence.

Using HLA-A2 multimers, ZnT8 185-194-reactive $CD8^+$ T cells were detected *ex vivo* at similar frequencies in T1D and healthy subjects, but harbored signatures of *in vivo* priming predominantly in T1D patients. Further characterization revealed higher antigen avidity, sensitivity, polyfunctionality and cytotoxicity in clonal ZnT8 185-194-reactive $CD8^+$ T cells isolated from T1D patients versus healthy subjects. A skewed yet private T cell receptor repertoire was observed. These features were unexpected, since high avidity autoreactive T cells are usually eliminated in the thymus. Indeed, these findings were associated with virtual absence of ZnT8 expression in human thymic medullary epithelial cells, which likely favors the escape of ZnT8-reactive $CD8^+$ T cells from thymic deletion and their subsequent autoimmune activation in T1D patients.

We here provide a first example of how incomplete central tolerance towards a beta-cell antigen results in an unusually high prevalence of autoreactive $CD8^+$ T cells. ZnT8 185-194 may be the prototype of a more generalized autoimmune mechanism, leading to the emergence of immunodominant T cells amenable to biomarker development and therapeutic targeting.

OR.14. Detection of Islet Antigen Expanded CD4 T Cell TCR Pairs and Transcriptomes by Single Cell RNAseq

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Islet-reactive CD4 T cells contribute to the development of type 1 diabetes (T1D) and may provide a window into β-cell destruction. To identify unique characteristics of islet reactive T cells from T1D subjects, we coupled CD154 enrichment of antigen specific T cells with novel single-cell RNAseq methods. PBMC from three DR4 T1D subjects and three matched healthy controls (HC) were stimulated with a pool of DR4 restricted islet peptides. Activated $CD154^+$ cells were enriched using magnetic beads and $CD154^+CD69^+$ memory CD4 T cells were sorted directly into a Fluidigm C1 Single-Cell chip. Nextera sequencing libraries were sequenced on an Illumina HiSeq sequencer. RNAseq reads were aligned to the human reference genome, mapped to ENSEMBLE gene models, and tabulated to enumerate transcript levels. TCR chains were assembled into *de novo* contigs using Trinity and productively rearranged V, (D), J and CDR3 regions were identified using IMGT/HIGHV-QUEST. We detected private TCR clonotypes in islet reactive CD4 T cells from all subjects. However, T1D subjects as a group expressed identical CDR3 junctions in multiple cells (expanded clonotypes) and at multiple visits. T1D cells with expanded clonotypes had a gene expression profile that was distinct from cells with non-expanded TCRs and HC cells, characterized by a unique subset of T cell activation/differentiation genes. The specificity of expanded TCRs was confirmed by TCR re-expression and T cell cloning. Our results illuminate TCR clonotype/phenotype relationships of islet-reactive CD4 T cells that may impact the immunopathology of subjects with T1D, and serve as biomarkers and therapeutic targets.

OR.16. Accumulation of CD8 T Cells Expressing EOMES-Associated Transcripts in Anti-CD3 Treated Recent Onset T1D Subjects

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Clinical studies with several biologic agents in newly diagnosed T1D subjects have shown transient stabilization of C-peptide levels in some individuals, followed by loss at the same rate as control groups. We are using cutting edge systems biology approaches to better understand immunologic mechanisms associated with C-peptide stabilization in new onset T1D subjects treated with the anti-CD3 monoclonal antibody, teplizumab. Using RNA sequencing (RNAseq) analysis, we have identified an unbiased whole blood gene expression signature in patients showing the greatest C-peptide stabilization (Responders). This signature comprises molecules associated with the transcription factor, EOMES, and includes both cytotoxic and inhibitory receptor transcripts. Importantly, kinetics of the EOMES signature paralleled beneficial clinical responses, and increased EOMES transcript levels correlated significantly with C-peptide levels, and with memory CD8 T cell frequencies. To further define the cellular basis of the EOMES signature, we performed flow cytometry analysis of PBMC from selected Responders and Non-responders. Frequencies of memory CD8 T cells expressing high levels of TIGIT, EOMES, and/or KLRG1 (termed MCD8TEK cells) were increased in Responders. Sorted MCD8TEK cells from selected Responders had expanded TCR clonotypes, expressed EOMES signature genes, and upon polyclonal stimulation, increased expression of multiple inhibitory receptor genes (TIGIT, KLRG1, CD160, LAG3) and decreased expression of cell cycle genes relative to TIGIT-KLRG1- memory CD8 T cells. Together, these data suggest that MCD8TEK have an exhaustion-like phenotype. Additional studies to functionally define MCD8TEK cells, and their role in transient immune tolerance induced by teplizumab, may facilitate development of more durable therapies for T1D.

OR.33. Circulating $CXCR5^+PD\text{-}1^+ICOS^+$ Follicular T Helper Cells are Increased near the Onset of Type 1 Diabetes in Subjects with Multiple Autoantibodies

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Type 1 diabetes (T1D) is thought to be a primarily T cell-driven autoimmune disease. However, autoantibodies produced by B cells are the best currently available biomarker for early islet autoimmunity and disease risk. These antibodies are produced by autoreactive B cells, the activation of which is largely dependent on the function of $CD4^+CXCR5^+$ follicular T helper cells (Tfh).

In the present study, we extensively characterized circulating Tfh cells in a clinical cohort of 55 children with recent-onset T1D, 68 at-risk children positive for multiple autoantibodies and 142 age-matched healthy controls. Markers associated with Tfh phenotype and function (PD-1, ICOS, CCR6, CXCR3, CCR7, IL-21) were analyzed by multi-color flow cytometry.

The frequency of activated $CD4^+CXCR5^+PD\text{-}1^+ICOS^+$ Tfh cells was markedly increased in the peripheral blood of patients with recent-onset T1D. A similar increase was observed only in the subset of autoantibody-positive at-risk subjects that displayed impaired glucose tolerance (IGT), demonstrating that Tfh activation is associated with advanced beta cell-autoimmunity and dysglycemia. Intriguingly, the increase of activated Tfh cells was evident only in subjects with T1D or IGT that were positive for ≥ 2 biochemical autoantibodies.

Together, our findings demonstrate that alterations in the circulating Tfh compartment are observable near the onset of T1D. Moreover, positivity for multiple autoantibodies appears to delineate a subgroup of T1D patients with pronounced peripheral blood Tfh activation. Our observations have important implications for both the potential use of Tfh cells as biomarkers of disease progression as well as for stratifying patients for future immunotherapy trials.

OR.57. Correction of Treg Activation Defect in Type 1 Diabetic Humans with *In Vitro* TNFR2 Agonism

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Tumor necrosis factor receptor 2 (TNFR2) is obligatory for induction, maintenance and expansion of activated regulatory T cells (aTregs), which are known to prevent or halt various forms of autoimmunity in animal models and humans. We show that although type 1 diabetics (T1D) have normal numbers of total Tregs, they have an increase in resting Tregs (rTregs) and a decrease in aTregs compared to controls (n= 55 T1D, n=45 controls, p=0.01), defined by CD45 protein. A large cross-sectional study of children and adult patients with T1D reveals that this Treg activation defect is lifelong (n=100 T1D, p<0.01). Lower numbers of aTregs were associated with having less residual C-peptide secretion from the pancreas (p=0.08) and poorer HbA1C control (p=0.03). Using two separate *in vitro* Treg expansion protocols, TNFR2 antibody agonism corrected the T1D activation defect by triggering conversion of rTregs into aTregs (n=54 T1D, p<0.001). TNFR2 antibody agonism was superior to standard protocols of Treg expansion and superior to tumor necrosis factor (TNF) in expanding the most potent subsets of Tregs. TNFR2 antibody expansion protocols exclusively expanded Treg cells but not CD4 T cells, thus creating homogenous populations of potent human Tregs in culture. In T1D, TNFR2 agonist-expanded Tregs were functionally potent by virtue of suppressing autologous cytotoxic T cells in a dose-dependent manner compared to controls. Targeting the TNFR2 receptor for Treg expansion *in vitro* and perhaps *in vivo* may be a way to correct the Treg activation defect in T1D.

F.06. A Stochastic Epigenetically-Triggered Mendelian Oligogenic (SEMO) Disease Model For Type 1 Diabetes

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The incidence of type 1 diabetes (T1D) is increasing 3-5%/year. The cause has been attributed to an undefined changing environment. However, there is much evidence against the environment (or any changing non-genetic mechanism) in causing the rising incidence: 1) If the environment causes both the failure of all identical twins of patients to have T1D and the rising incidence, the concordance rate among these twins should be rising, but it is not; 2) Migrants from high- to low-incidence countries continue to have high-incidence children; 3) T1D concordance among fraternal twins is the same as siblings of patients in general; 4) T1D incidence in the offspring of two T1D parents is identical to the identical twin rate. These observations argue strongly against the environmental hypothesis. Additionally, T1D genetic association studies show strong susceptibility in the major histocompatibility complex but many optional additive genes of small effect increasing T1D risk with little to no genetic linkage. We developed an alternative model to genetic "risk" and environmental influence involving three recessive interacting causal genes, all located on human chromosome 6, and a stable stochastic epigenetic trigger. The model yields testable predictions and explains many puzzling T1D features, including its rising incidence, the high risk of HLA-DR3/DR4 heterozygotes, the rarity of affected relatives of patients, and T1D incidence among first-degree relatives of patients. Since selection against any causal gene could prevent T1D, we postulate the rising incidence results from increasing mixing of parents from previously isolated populations that had selected against *different* causal genes.

F.07. Constitutively Active STAT5b Signaling in Dendritic Cells Confers Diabetes Protection and Halts Diabetes Progression in NOD Mice

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Defects in dendritic cells (DCs) development and function lead to autoimmune disorders. Autoimmune diabetes in humans and NOD mice results from breakdown of self-tolerance, ending in T cell-mediated beta-cell destruction. DC dysfunction in NOD mice results in part from a defect in the JAK-STAT5 signaling pathway associated with the *idd4* susceptibility locus. The involvement of STAT5b in DCs tolerogenic functions remains unknown. We generated transgenic mice (NOD.CD11c$^{Stat5b\text{-}CA}$) expressing a constitutively active form of the STAT5b gene (Stat5b-CA) under

control of the CD11c promoter. All NOD.CD11c$^{Stat5b\text{-}CA}$ mice were protected against diabetes. The protection from diabetes was associated with increased Treg pool and decreased percentages of CD8$^+$ T cells. Splenic DCs of NOD.CD11c$^{Stat5b\text{-}CA}$ mice acquired a mature phenotype, enhanced Treg number, Treg suppressive activity and induced CD4$^+$CD25$^-$ T cells to acquire suppressive function. In addition, DC$^{Stat5b\text{-}CA}$ promoted non-inflammatory Th2/Tc2 response and conversion of CD4$^+$CD25$^-$Foxp3$^-$ T cells into CD4$^+$CD25$^+$Foxp3$^+$ Tregs. Importantly, a single injection of DC$^{Stat5b\text{-}CA}$ to 10-week old NOD mice halted diabetes progression and educated their splenocytes to loose their diabetogenic potential when transferred to NOD.SCID mice. This is the first report that an active form of STAT5b restored DCs tolerogenic functions and re-educated T cells to acquire and sustain long-term protective immune response against diabetes in NOD mice.

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F.08. Efficient Targeting of Both CD4$^+$ and CD8$^+$ diabetogenic TC from Endogenously Delivered Epitopes

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Diabetogenic T cells are beta-cell antigen-reactive T cells that eluded tolerance mechanisms and mediate the destruction of beta-cells, causing Type 1 diabetes. Specifically targeting these T cells for deletion or reprograming is the main objective of antigen-specific immunotherapies. However, many recognized antigens turn out to be modified or hybrid epitopes that are best mimicked by mimotopes. Delivering mixed native epitopes and mimotopes that efficiently target both CD4$^+$ and CD8$^+$ diabetogenic T cells to tolerogenic antigen-presenting cells (APCs) would constitute an attractive therapeutic approach.

When epitopes are endogenously expressed in a single polypeptide, CD8 epitopes are effectively presented on MHC class I while CD4 epitopes are poorly presented. Conversely, the use of endosome-targeting signals improves presentation of CD4 epitopes, but is detrimental to stimulation of CD8 T cell clones. We show that that proper epitope arrangement combined with post-translational segregation of CD4 and CD8 epitopes allows each group of epitopes to be optimally presented to reactive T cells. We performed our analysis on different APCs, including dendritic cells, lymph node stromal cells and modified fibroblasts. We observe, as expected, that mimotopes are superior to the corresponding native epitopes. However, mutation improving recognition of a particular epitope may abrogate recognition of an overlapping epitope, indicating a need to avoid such overlapping. Among four endosome-targeting signals tested, the short invariant chain signal ((Ii1-80) was found to be the best in our constructs, which can be delivered to tolerogenic APCs *ex vivo* or *in vivo* to help reinstate tolerance.

F.09. Update on Clinical Trial Program Testing the BCG Vaccine in Established Type 1 Diabetes

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The bacillus Calmette-Guerin (BCG) vaccine has been the most continuously used vaccine in world history, and is also considered among the safest. Currently, 10 human clinical trials globally are testing repeat BCG vaccination in diverse forms of autoimmunity and allergies for both prevention and treatment (including patients with new onset and long standing conditions). Phase I study of the BCG vaccine in longstanding type 1 diabetics (T1D) reveals potential disease modulating effects after repeated BCG vaccination, including death of autoreactive cells, transient and modest restoration of insulin secretion and induction of beneficial regulatory T cells (Tregs). A Phase II clinical trial using multi-dose BCG in longstanding T1D was initiated in June 2015. This double-blinded, placebo controlled immuno-interventional trial protocol was approved by the FDA and is unique in testing the efficacy of the BCG vaccine in long-term diabetic subjects (average disease duration: 15-20 years) with small but detectable levels of C-peptide secretion from the pancreas. Based on published Phase II clinical trial data of BCG in multiple sclerosis subjects, the therapeutic effects of this vaccine appear to improve over the passage of time; therefore, potential clinical benefits in the diabetes trial will be followed for 5 years. The primary endpoint is decrease in HbA1c in treated

vs placebo subjects. The selection of BCG as an immuno-intervention in T1D is based on the protective host TNF response, including induction of Tregs, and potential long-term modulation of the immuno-inflammatory profile of vaccinated subjects.

F.10. Evolution of CD4 T Cell Phenotypes During Type 1 Diabetes Development

S. Alice Long[1], Peter Samuels[1], Michael Mason[1], Megan Tatum[1], Karen Cerosaletti[1], Carla Greenbaum[1], Jane Buckner[1] and Type 1 Diabetes TrialNet Study[2]. [1]Benaroya Research Institute, Seattle, WA; [2]TrialNet, Bethesda, MD

Islet inflammation and autoreactivity precede the development of clinical type 1 diabetes (T1D). CD4 T cells play a key role in this process and display altered function in T1D. However, the point at which they contribute to disease development is unknown. We measured IL-2 responsiveness and resistance of effector T cells (Teff) to regulation via regulatory T cells (Treg); phenotypes present in established T1D subjects. Using phospho flow and inhibition of proliferation assays, we performed an observational study with longitudinal samples from the TrialNet Pathway to Prevention study composed of 2 cohorts, 20 subjects each, of autoantibody (autoAb) positive subjects at risk for developing T1D. One cohort developed clinical diabetes by the second blood draw and the second did not. These autoAb$^+$ cohorts were compared to 25 autoAb$^-$ first degree relatives. IL-2 response was reduced and anti-CD3 induced proliferation was increased in Teff of both islet autoAb$^+$ cohorts as compared to first degree relatives. These early phenotypes were associated with fixed traits: age and DR4 respectively. In contrast, Teff resistance to Treg was associated with greater numbers of autoAb and decreased beta cell function near the time of T1D diagnosis, confirmed using multivariable logistic regression. Thus, alterations in T cell function precede clinical diagnosis of T1D, with IL-2R signaling defects preceding Teff resistance. Identifying the sequence of immune phenotypes has implications for better understanding disease progression and ultimately, improved selection and timing of therapies for prevention and treatment of T1D.

F.11. Genetics and Disease Contribute to T and B Cell Phenotypes of T1D Subjects as Revealed by Reproducible Flow Analysis of Large, Well-defined, Cross-sectional Cohorts

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Both genetic and environmental factors are known to influence type 1 diabetes (T1D) pathogenesis. Yet, linking these factors to cellular phenotypes has been challenging due to patient heterogeneity and cellular variability. Thus, we designed a large (n=100 subjects per group), age and gender matched, cross-sectional study of control and T1D cohorts genotyped for 18 selected T1D associated SNP's. We measured approximately 300 traits of B and T cells using 3 reproducible flow panels of >12 colors and 4 cytokine stimulations, optimized and standardized overtime. We validated previous findings of increased transitional B cells and memory CD4 T cells in T1D, while demonstrating a clear role for age and genotype for both of these immune features. In addition, we observed increased expression of HLA DR, CD95, and CXCR3 on lymphocyte subsets of T1D. Response to cytokines positively correlated with receptor expression. Yet, genotype and disease also impacted these traits. Consistent with previous results, some immune traits of controls were clearly associated with genotype. Interestingly though, some of these phenotypes were observed in T1D subjects even when genotype was held constant, suggesting common phenotypes influenced by known genetic alleles and disease. These findings offer a more comprehensive understanding of the phenotypes associated with T1D, help identify pathways associated with disease, and have implications for selection and stratification of subjects for treatment of T1D with immune-based therapies.

F.12. HLA-DQ Associated Signaling Differences Correlate with Reduced Treg Induction in Subjects with Type 1 Diabetes Susceptible Haplotypes

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Association studies indicate that the HLA-DQ locus is the most prominent genetic risk factor for type 1 diabetes (T1D). The functional mechanisms by which HLA-DQ molecules confer susceptibility in human T1D are not completely characterized, but clearly involve the biochemical influence of position 57 of the HLA-DQ beta chain. Susceptible alleles have an uncharged β57 residue that influences the charge and preferences of the peptide binding surface, thereby affecting epitope presentation and thymic selection of T cells. However, the residue at β57 also profoundly influences the stability of class II dimers because an uncharged residue at this position eliminates a crucial salt bridge with the DQ alpha chain. Our recent work indicates that differences in HLA-DQ stability may influence T cell signaling. In particular, through experiments with T cell clones, we demonstrate a hierarchy of decreased signaling in which DQ6 restricted clones upregulated the highest levels of pSTAT5, followed by DQ2 and then DQ8. Thus, observed levels of Stat-5 upregulation in response to activation exactly mirrored HLA-DQ stability. We further demonstrate that polyclonal T cells from subjects with DQ8 haplotypes have an impaired capacity to generate FoxP3 positive Tregs in comparison to subjects with protective DQ6 haplotypes. These results may help to explain prior observations that T cells from T1D patient exhibit defects in signaling and Treg fitness that are independent of minor risk alleles such as PTPN22, implicating a new mechanism that contributes to the loss of peripheral tolerance in T1D.

F.13. Neonatal Tolerance to Proinsulin is Sufficient to Prevent Autoimmune Diabetes

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All individuals are born with a certain level of self-tolerance to tissue specific antigens partly due to presentation of self-antigens in the thymus. Those with genetic predisposition, who may have less robust self-tolerance, may develop autoimmune disease. Augmenting the level of antigen-specific tolerance may prevent autoimmunity. We aimed to define whether augmentation of pre-existing tolerance for a limited period could be effective. For this, we expressed two different autoantigens, proinsulin and islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP) in the antigen presenting cells (APCs) of autoimmune diabetes-prone non-obese diabetic (NOD) mice during defined periods and tracked self-antigen specific T cells. Proinsulin expression from birth until weaning was sufficient to completely protect NOD mice from diabetes, insulitis and development of insulin autoantibodies. Insulin-specific T cells were significantly diminished (424±50 vs. 181±24), were naïve and did not express IFNg (14±1.7 vs. 4±0.9) when challenged. We tracked IGRP specific $CD8^+$ T cells in NOD mice expressing IGRP in APCs. When IGRP was expressed until weaning, IGRP specific $CD8^+$ T cells were not detected later in life (1797±786 vs. 50±4). Thus, islet specific auto-reactive T cells are uniquely produced in early life. Our finding that a brief exposure to proinsulin confined to neonatal life in NOD mice imparts long lasting protection from diabetes leads us to suggest that neonatal life is a vulnerable period for the escape of insulin-specific T cells. Therapies bolstering proinsulin antigen presentation during early life in high-risk human subjects may provide the best chance of prevention of diabetes development.

F.14. Hyaluronan Content Governs Tissue Stiffness in Pancreatic Islet Inflammation

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We have identified a novel role for hyaluronan (HA), an extracellular matrix polymer, in governing the mechanical properties of inflamed tissues. We recently reported that insulitis in type 1 diabetes (T1D) of mice and humans is preceded by intra-islet accumulation of HA, a highly hygroscopic (water-attracting) polymer. We asked whether

autoimmune insulitis was associated with changes in the stiffness of islets and whether HA was responsible. To measure islet stiffness, we used atomic force microscopy (AFM) and developed a novel "bed of nails"-like approach using quartz glass nanopillars to anchor islets, solving a long-standing problem of keeping tissue-scale objects from moving while performing AFM. We measured stiffness via AFM nanoindentation with a spherical indenter and found that insulitis made islets mechanically soft compared to controls. Conversely, treatment with 4-methylumbelliferone (4-MU), a small-molecular inhibitor of HA synthesis, reduced HA accumulation, diminished swelling, and restored basal tissue stiffness. Our results indicate that HA content governs the mechanical properties of islets by driving fluid shifts into sites of inflammation. Because tissue mechanotransduction has decisive effects on cellular responses, these findings open up an exciting new avenue for research in understanding the fundamental pathogenesis of tissue-specific autoimmunity.

F.15. IL-**7Rα Blockade During Islet**-specific Immunization of NOD Mice Promotes Treg Expansion and Activation

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Blocking antibodies for IL-7Rα prevent and reverse type 1 diabetes (T1D) in non-obese diabetic (NOD) mice by inhibiting autoreactive T cell responses and increasing Treg frequencies. Tregs play a pivotal role in the prevention of autoimmunity: increased numbers and enhanced suppressive activity of this population have shown therapeutic efficacy in diabetic mouse models. Therefore, targeted treatments increasing the suppressive potential of islet-specific Tregs represent an attractive therapeutic strategy for T1D, while avoiding the risk of general immunosuppression. To determine the impact of IL-7Rα blockade during activation of islet autoantigen-specific T cells *in vivo*, we immunized prediabetic NOD mice with islet antigens combined with LPS or alum in the absence or presence of anti-IL-7Rα antibodies and evaluated T cell responses using *in vitro* restimulation assays. Collectively, these experiments show that blocking IL-7Rα during an active autoreactive T cell response leads to preferential activation and expansion of Tregs, demonstrated by increased frequencies, proliferation and CD25 expression. This study suggests that the enhanced regulatory potential induced by IL-7Rα blockade during antigen-specific immunization could have significant implications in the development of novel immunotherapies for T1D. Moreover, the limited treatment time may reduce the risk of long-term immunosuppression and toxicity.

F.16. A Novel CD57-Negative Effector Memory Population is Enriched in Beta Cell-specific CD8 T Cells in HLA-A24$^+$ Type 1 Diabetes Patients

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Background: Autoreactive CD8 T cells play a key role in type 1 diabetes pathogenesis. The HLA-A24 class I gene confers significant risk of disease and early onset. We sought to characterize CD8 T cells specific for novel beta-cell epitopes in HLA-A24$^+$ T1D patients versus healthy controls.

Methods: PBMC from HLA-A24$^+$ T1D patients (n=10) and healthy controls (n=10) were labeled with surface antibodies and HLA-A24 tetramers specific for preproinsulin (PPI), insulin B (InsB) and CMV.

Results: Using bioinformatics tools for dimensionality reduction (viSNE) and unsupervised clustering analysis (FLOCK), 29 CD8 T cell subsets were identified in an unbiased manner based on expression of CCR7, CD45RA, CD27, CD57 and CD95. Assessment of the frequency of these subsets within the beta-cell specific CD8 T cell populations in patients and controls revealed that three effector memory populations were present in patients but absent in controls, and one effector memory population was significantly enriched in patients compared to controls.

Analysis of surface marker expression of these T1D-specific/enriched subsets showed they were CCR7-CD45RA-CD27-CD95$^+$ effector memory cells lacking CD57 expression. Traditional flow cytometry analysis confirmed the frequency of the CD57-negative effector memory cell population was significantly higher within InsB and PPI-specific CD8 T cell populations in patients versus controls (p=0.03 and p=0.04). There was no difference in the frequency of CCR7-CD45RA-CD27-CD95+ effector memory cells expressing CD57.

Conclusion: A novel autoreactive CD57-negative effector memory CD8 T cell subset is significantly enriched in beta-cell specific CD8 T cell populations of T1D patients and may serve as a disease biomarker.

F.17. Gene Expression Profiling to Identify Early Biomarkers of Disease Susceptibility and Progression 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 performed gene expression analysis in whole blood RNA samples to identify potential biomarkers of disease risk and progression. RNA samples from T1D patients, AA- first-degree relatives of T1D patients (FDRs), and AA$^+$ FDRs who do or do not progress to T1D (progressors and non-progressors) were obtained from the TrialNet Pathway to Prevention Study. Gene expression was measured by one-color microarray analysis and compared against that of non-T1D-related controls. Data were analyzed using Genespring and confirmed by qPCR. We identified a “diabetes-centric” gene expression signature that is shared among all T1D-related individuals, and a panel of genes within that signature that may serve as potential biomarkers of risk. In addition, we identified a gene signature can could distinguish AA$^+$ FDR progressors from non-progressors, and found that the most striking changes in gene expression observed in AA$^+$ progressors occurred >3 years before the onset of hyperglycemia. These genes could serve as potential biomarkers of disease progression, and if validated, would allow T1D to be diagnosed earlier in the course of disease, giving patients a chance for intervention therapy before substantial beta cell destruction.

F.18. JAK Inhibitor Ruxolitinib Inhibits Autoimmune Responses and Insulitis in NOD Mice

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Type 1 diabetes is an autoimmune disease, in which pancreatic islet β cells are destroyed by diabetogenic CD8 T cells. IFNγ and IL2 play important roles in pathogenesis of autoimmune diabetes. Ruxolitinib, a JAK1/2 inhibitor, inhibits receptor signaling of IFNγ and γ chain cytokines. The aim of this study was to investigate effects of ruxolitinib on immune responses and diabetes development in NOD mice. Female NOD mice were treated with ruxolitinib (50 mg / kg) or vehicle as control daily for 10 days through oral gavage, pancreatic LNs (PLN) and pancreas were detected for Th1 and Th17 cells, IFNγ and NKG2D expression, cytotoxic function and insulitis. It was noted that the numbers of DCs, CD4 and CD8 T cells were lower in ruxolitinib-treated NOD PLNs compared to vehicle-treated control. Strikingly, IFNγ$^+$CD4$^+$ and IFNγ$^+$CD8$^+$ T cells significantly decreased in NOD pancreas after ruxolitinib treatment. NKG2D expression was diminished on CD8 T cells within ruxolitinib-treated NOD pancreas. *In vivo* killing of islet peptide –pulsed CFSE-labeled splenocytes was significantly reduced in ruxolitinib-treated NOD PLNs. Adoptively transferred diabetogenic CD8 T cells did not proliferate in mice treated with ruxolitinib, unlike control mice. Further, insulitis scores were lower in ruxolitinib-treated NOD pancreas than in control NOD pancreas. Taken together, our study demonstrates that ruxolitinib treatment inhibits Th1 immune response, CD8 T cell activation, cytotoxicity and insulitis in a NOD mouse model. The effects of ruxolitinib on the diabtogenic immune response in NOD mice suggest a novel treatment of autoimmune diabetes.

F.20. Characterizing GAD65-specific CD4$^+$ T Cells in T1D Patients During Treatment with Ustekinumab

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Type 1 diabetes (T1D) is characterized by a loss of tolerance towards insulin-secreting beta cells in the pancreas, resulting in their destruction by T cells. Evidence suggests these self-reactive T cells produce IFN-γ and/or IL-17, contributing to inflammatory signalling pathways. In an attempt to block these cytokines and inhibit/alter the function of self-reactive T cells, we are conducting a phase I clinical trial of ustekinumab, a humanized mAb that inhibits the shared p40 subunit of IL-12 and IL-23, in adults with new-onset T1D. To measure changes in auto-reactivity we are tracking and characterizing GAD65-specific CD4$^+$ T cells using an assay in which antigen-specific CD4$^+$ T cells are detected by induced co-expression of CD25 and OX40 (CD134) after 44h incubation with antigen. Assays are also stained with antibodies to CD45RA, CD39, CCR7, CXCR3, CCR6 and CCR4 to characterize the proportion of Th lineages within the GAD65-specific population. For n=13 patients the average proportion of GAD65-specific CD4$^+$ T cells at baseline was 0.41% (range: 0 – 1.68%). The majority of these cells (85%) had a CCR7$^+$CD45RA$^-$ effector memory phenotype and were comprised of Th1, Th2 and Th17 cells at an approximate 2:1:3 ratio. At week 16 post-treatment, in n=5 patients the proportion of GAD65-specific CD4+ T cells was reduced by an average of 8 fold compared to baseline, whereas in n=3 patients the proportion increased by an average of 4 fold, and in n=5 there was no change. These data will help us identify how ustekinumab affects the proportions and phenotype of GAD65-specific CD4$^+$ T cells in T1D.

General Autoimmunity

OR.36. Phenotypic and Functional Heterogeneity of cTfh Cells in Systemic Lupus Erythematosus

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Follicular helper T (Tfh) cells play a key role in promoting autoreactive B cell maturation in systemic lupus erythematosus (SLE), with autoantibody production and promotion of tissue injury. Tfh cells via aberrant cytokine secretion initiate B cell activation in lupus, contributing to disease pathogenesis. A counterpart of Tfh cells circulates in the blood, so-called circulating Tfh (cTfh) cells, and may serve as a disease biomarker, assuming these cells can be carefully analyzed. It has been demonstrated that these cells are expanded in SLE, producing effector cytokines such as IL-21, critical for autoreactive B cell help. Yet, the distinguishing features of potentially pathogenic cTfh cells remain obscure, a hindrance to their utility in monitoring disease and understanding pathogenic mechanisms. We recently developed a single-cell highly multiplexed cytokine profiling microchip technology that, for the first time, enables the full-spectrum delineation of T cell effector function and polyfunctionality by co-measurement of up to 42 cytokines at the level of single T cells. This technology was used to delineate cytokine function heterogeneity and, in particular, determine the effector cytokine signature of pathogenic Tfh cells from a cohort of patients with SLE, compared to healthy and disease controls. Single-cell RNA-seq was then exploited to measure the transcriptional profile of cTfh cells at a single cell level, correlating this with their individual secretome patterns to determine the mechanistic basis of their polyfunctional heterogeneity and their potential role in SLE pathogenesis.

OR.43. The *IFIH1 A946T* Autoimmune Risk Variant Exhibits Enhanced Signaling and Promotes T1D Pathogenesis

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Genome wide association studies have linked SNPs in innate signaling programs to an increased risk for autoimmune disease. One SNP, rs1990760, within the IFIH1 viral sensor (leading to an A to T amino acid change at codon 946) is strongly associated with multiple autoimmune diseases including type 1 diabetes (T1D). The impact of this variant on viral sensing and autoimmune pathogenesis, however, has not yet been determined. Using an overexpression system, we demonstrate that IFIH1-A946T exhibits heightened basal and post-stimulation of innate immune signaling and increased viral control that corresponds to enhanced levels of type 1 interferon (IFN) production. Consistent with this finding, primary PBMCs from healthy subjects expressing the variant exhibit heightened innate immune signaling. To study IFIH1-A946T *in vivo*, we generated a novel knock-in murine model. Both heterozygous and homozygous variant mice display enhanced basal IFN mRNA expression and splenomegaly. Moreover, homozygous pups manifest an embryonic survival defect. Further, IFIH1-T946 mice exhibit increased frequency of STZ-induced T1D. Finally, intercross of IFIH1-T946 mice with animals expressing the T1D risk variant in PTPN22 lead to a synergistic increase in T1D development. Together, our data demonstrate that IFIH1-A946T alters innate immune antiviral signaling and strongly supports the model that increased IFN production driven by the variant leads to an inflammatory state that promotes autoimmunity, particularly in association with other autoimmune risk alleles.

F.22. Development of the Microarray Technology for Profiling Autoantibodies in Hepatocellular Carcinoma Patients. Afrakoma Afriyie-Asante, Ola Negm, John Robertson, Ian Todd, Paddy Tighe and Lucy Fairclough.

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Hepatocellular carcinoma (HCC) is characterized by mutations in the cellular machinery, which induce the liver cells to replicate at a higher rate. It is the third most common cause of cancer deaths worldwide. Most current diagnostic tools are sensitive only at the late stages, which compromise effective treatment. Autoantibody detection presents a robust means of diagnosing HCC since they are upregulated at the early stages and this has generated great interest in autoantibodies as an effective approach to detecting cancer. Therefore, studying their effect on HCC could help provide a comprehensive understanding of their role in the disease.

Antigen microarray, a new generation probing technique, has emerged as a promising tool for the examination of humoral immune responses such as profiling autoantibodies to tumour associated antigens (TAAs) in a multiplexed and high throughput manner. Part of this work aims to develop the in-house microarray to identify TAAs and profile autoantibodies in sera obtained from HCC patients. In this regard, the performance of the in-house microarray technology was validated by assessing the reactivity of serum samples with ten candidate TAAs. Additionally, parameters such as slide surface chemistries, edge effect, humidity, micro-titre volumes of samples and printing buffer were optimised to ensure a reproducible technology. Ongoing work is being carried out to produce a panel of recombinant autoantigens suitable for the entire study. This developmental work has explored multiple parameters in optimizing the antigen microarray to obtain reliable results, which could be applicable in screening for autoantibodies as a diagnostic platform.

F.23. A Role for Endogenous Retroviruses in Multiple Sclerosis and Systemic Lupus Erythematosus

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Human endogenous retroviruses (HERVs), which comprise approximately 8% of the genome, have been implicated in the pathogenesis of autoimmune diseases such as multiple sclerosis (MS) and systemic lupus erythematosus (SLE). Though $CD4^+$ T cells play a central role in both diseases, almost nothing is known about the expression level of HERVs in $CD4^+$ T cells from patients with MS or SLE. Real-time PCR was used to compare the mRNA expression

of HERVs in CD4+ T cells *ex vivo* and after activation from patients with MS or SLE to those from healthy controls. CD4+ T cells from MS patients and healthy controls displayed similar expression of numerous families of HERVs. Furthermore, after examining over 20 HERVs, we report that certain HERVs were significantly downregulated in CD4+ T cells from SLE patients as compared with those from healthy controls. Probing this observation, we sought to compare the protein-level expression of HERVs on different cell types. Using FACS, we report the intriguing finding that some HERVs were upregulated at the protein-level in SLE patients compared with healthy controls in certain cell types. Given that HERVs are expressed as ssRNA, which is a natural ligand for toll-like receptor 7 (TLR7) and a unique role for this receptor in CD4+ T cells has been described by this team, our ongoing work seeks to determine whether HERVs signal through TLR7 in CD4+ T cells on SLE patients and underlie the aberrant CD4+ T cell phenotype in these patients.

F.24. Study of Gluten-specific T Cell Repertoire in Blood and Gut During Gluten Challenge in Celiac Disease Patients

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Celiac disease is a chronic inflammatory disease of the small intestine caused by hypersensitivity to dietary gluten in genetically disposed individuals who express human leukocyte antigen (HLA)-DQ2.5 (95%) or HLA-DQ8. CD4+ T cells are the key players in causing intestinal inflammation in celiac disease by specific recognition of gluten epitopes.

In order to understand the T cell response to gluten exposure, we analyzed the T cell receptor (TCR) repertoire of gluten-specific T cells in gut and blood in treated celiac disease patients during 14 days oral gluten challenge. We isolated single gluten-reactive effector memory CD4+ T cells by FACS sorting of HLA-DQ2.5-gluten-tetramer-staining cells followed by cDNA synthesis and high-throughput sequencing. Preliminary results show clonal expansion, biased V-gene usage, pairing preferences and expansion of identical gluten-specific T cell clones in the blood and gut. We are in the process of sequencing the TCR repertoire of gluten-specific T cells of a total 10 celiac disease patients during the course of the gluten challenge. The analysis of this large data set of TCR sequences specific to gluten epitopes will allow us to dissect the disease causing immune response of celiac disease in great detail.

F.25. IL-6R Signaling Inhibits Generation of Th3 Cells and is a Promising Therapeutic Target for Enhancing Oral Tolerance Induction

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Oral antigen administration results in systemic hyporesponsiveness to a subsequent challenge with the fed antigen (oral tolerance) and has been shown to inhibit autoimmune disease in multiple animal models. However, transfer of this strategy to the clinics has not yet been achieved. Low dose oral tolerance depends on the presence of Th3 type Tregs, characterized by surface expression of latent TGF-beta (LAP and TGF-beta) and its adapter protein GARP (LRRC32). We established an *in vitro* system to induce Th3 like cells that express LAP and GARP on their surface. *In vitro* induced CD4+LAP+ T cells suppress proliferation of naïve T cells and production of IL-17A by Th17 cells. RT-PCR showed that membrane bound TGF-beta correlated with mRNA transcription levels of GARP but not TGF-beta, suggesting that the availability of GARP directly limits the expression of membrane bound TGF-beta. Screening for modulators of GARP expression identified IL-6 as a potent inhibitor. IL-6 inhibited transcription of GARP in a STAT3 dependent manner. To test the therapeutic potential of targeting IL-6R signaling during oral tolerance induction we assessed the DTH (delayed-type hypersensitivity) response in wild-type or $CD4^{Cre}IL6Ra^{fl/fl}$ mice after feeding with ovalbumin for 5 consecutive days. Importantly, oral tolerance was significantly improved in mice that lack IL-6R signaling in CD4+ T cells. In the same vein administration of neutralizing antibodies against IL-6 during antigen

feeding significantly improved oral tolerance induction. Our data demonstrates that blocking of IL-6R signaling is a promising approach to enhance oral tolerance induction.

F.26. Do CD107a-Expressing Human B Lymphocytes Release FasL$^+$ Cytotoxic Exosomes?

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Fas ligand is important for inducing immune cell death and maintaining peripheral tolerance. We have previously demonstrated constitutive FasL expression and antigen-specific T_H cell killing by mouse CD5$^+$ B cells. We also found that EBV-transformed human B-LCL possess an intracellular pool of FasL that can be released on cytotoxic exosomes. This study focused on further identification and characterization of human B cells that express FasL and their ability to release FasL$^+$ exosomes. We found that LAMP-1 (CD107a) was present on the surface of B-LCL, multiple myeloma and follicular B cell lymphoma cell lines that express FasL. A small population of circulating CD107a$^+$ B cells was found in the blood of healthy donors. CD107a$^+$ B cells were enriched among human CD5$^+$ B cells and displayed higher expression of CD43, CD27 and CD24 compared to total B cells. A patient who recently presented to the retina clinic with sudden-onset vision loss was found to have mild monoclonal B lymphocytosis consisting of a high proportion of CD43$^+$CD27$^+$ B cells that were CD107a negative. Treatment with rituximab led to marked depletion of circulating B cells, reduced T_H1 response toward recoverin, and transient improvement in vision. Early reconstituting B cells consisted exclusively of CD43$^+$CD27$^+$ B cells that were CD107a positive, but CD107a expression decreased as vision loss relapsed. These data suggest that CD107a expression correlates with FasL expression in transformed B cells, is more common among CD43$^+$CD27$^+$ B cells, and that CD107a$^+$ B cells may be important in ocular immune tolerance.

F.27. IRX4204, a Novel RXR Agonist, Alters Cytokine Secretion of T Cells and Monocytes, and Increases *In Vitro* Adaptive T Regulatory Cell Proliferation

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Retinoid receptors have been shown to be involved in several important immune pathways, in particular in the generation of anti-inflammatory adaptive T regulatory cells (aTreg). Retinoid X receptor is a nuclear receptor that is activated by 9-cis retinoic acid and dimerizes with other nuclear receptors. IRX4204 is a novel RXR agonist that has shown encouraging results in early trials for prostate cancer. Furthermore, IRX4204 has been demonstrated in preclinical mouse studies to have a potent effect on the *in vitro* differentiation of T cells and *in vitro* cytokine production by both T cells and monocytes. To gain a further understanding of mechanism of action of IRX4204, we examined the effect of the agonist in several *in vitro* analyses with a cohort of healthy control subjects. IRX4204 reduced IL-17A/F, TNFa, TNFb, IL-9 and CCL20 secretion from T cells incubated under Th17 skewing conditions. The presence of IRX4204 reduced the levels of IL-4, IL-5, IL-9, IL-13 and IL-21 secreted by naïve T cells stimulated with anti-CD3/CD28, a polyclonal activator. When naïve CD4$^+$ T cells were cultured under aTreg skewing conditions, addition of IRX4204 gave rise to increased numbers of aTreg. IRX4204 also altered the cytokine secretion profile of monocytes stimulated with LPS in a dose-dependent manner. Taken together, these results indicate that IRX4204 may be a promising anti-inflammatory therapeutic.

F.28. Autoantibodies, Altered Soluble Mediators, and Clinical Features Discriminate Incomplete Lupus Patients from Unaffected Relatives and Relatives with Classified SLE

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Blood relatives (Rel) of lupus patients have increased risk of developing SLE. Some have autoantibodies and SLE clinical features, but do not meet ≥ 4 ACR criteria needed to reach SLE classification (incomplete lupus, ILE). ILE relatives may transition to classified SLE, yet many will remain ILE patients without major organ involvement. We examined 77 individuals enrolled in the Lupus Family Registry and Repository (LFRR) who only met 3 ACR classification criteria and did not meet Systemic Lupus Collaborating Clinics (SLICC) SLE classification during medical record review, designated as ILE. ILE patients were matched by race, gender, and age (± 5 years) to unaffected Rel and unrelated controls (Ctls), with a subset of ILE patients (n=55) matched to medical record-confirmed SLE patients in the LFRR. ILE patients had significantly higher SLE-specific screening questionnaire (CSQ) scores than unaffected Rel and Ctls (*p<0.0001*). A number of soluble mediators positively correlated with CSQ scores and were highest in ILE patients, including SCF (*p=0.0001*), BLyS (*p=0.0018*), MCP-3 (*p=0.0167*), and TNFRI (*p=0.0196*), as well as ANA titer (*p<0.01*) and number of lupus-associated autoantibodies (*p<0.01*). Comparing ILE and matched SLE patients, SLE patients had higher rates of arthritis, serositis, and renal disease (*p<0.004*), number of autoantibody specificities (*p<0.004*), and levels of BLyS (*p=0.0138*), IL-2Rα (*p=0.0201*), IP-10 (*p=0.0269*), and TNFRII (*p=0.0309*). Yet, ILE patients had higher levels of the regulatory mediator TGF-β (*p=0.0454*). Identification of factors which distinguish relatives at increased risk of developing SLE may help curtail inflammatory damage and identify individuals for prevention trials.

F.29. Altered Treg Function and Increased Alloreactivity Following Aire-deficiency in Rats

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

To address the role of Aire, we first analyzed its expression in naïve rats. Interestingly, we observed the expression of Aire at mRNA and protein levels in the thymus and in the periphery of wild type rats particularly in a cellular subset in lymph nodes, suggesting a similar pattern of expression of Aire in rats and in human.

We then generated a model of Aire-deficient rats. First, compared with mouse models showing a mild auto-immune phenotype, we observed that Aire-deficient rats displayed an APECED-like disease with strong auto-immune symptoms such as alopecia and vitiligo occurring at 6 month, auto-immune histological injuries such as destruction of exocrine pancreas and numerous circulating auto-antibodies. Additionally, our preliminary results demonstrated an altered suppressive capacity of both $CD4^+CD25^+$ and $CD8^+CD45RC_{low}$Tregs in Aire-deficient rats. Finally, in a model of MHC-incompatible cardiac-allograft, we showed a strong and early development of acute allograft rejection lesions in the Aire-deficient rat compared with WT rat, suggesting a role of Aire on alloreactivity. Altogether, our results demonstrate that Aire-deficient rats expressed APECED-type auto-immune features and showed altered Treg function and increased alloreactivity.

F.30. PTEN Balances Glycolytic Metabolism in Naturally Occurring Regulatory T Cells

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Foxp3$^+$ regulatory T cells (T_{regs}) act at the interface between immunity and metabolism, processes that can dictate cellular fate and function. T_{regs} originate in the thymus (nT_{regs}) as well as extrathymically in the periphery (pT_{regs}). Although it is known that iTregs (induced *in vitro* from T_{conv} cells) rely predominantly upon lipid oxidation more than glycolysis or glutaminolysis, the metabolic properties of nT_{regs} are not fully elucidated. Here we show that in contrast to iTregs, activated nT_{regs}, like T_{conv} cells, are more dependent upon glycolysis and glutaminolysis. PTEN, a lipid phosphatase, that inhibits PI3K/Akt signaling and promotes T_{regs} stability and function, has been shown to negatively regulate glycolysis and glutaminolysis in cancer cells. Given this, we hypothesized that PTEN loss in T_{regs} (PTEN-DT_{regs}) that leads to their instability and autoimmunity occurs due to dysregulated T_{reg} metabolism. We found that PTEN-DT_{regs} exhibited enhanced glycolytic flux with no alterations in glutaminolysis. Concomitantly, PTEN-DT_{regs} showed increased expression of pyruvate dehydrogenase kinase isoform 4 (PDHK4), an enzyme that enhances glycolysis by blocking pyruvate dehydrogenase (PDH). Although, pan-blockade of PDHKs by dicholoroacetate decreased the glycolytic flux of PTENDT_{regs} and percentages of activated CD4$^+$ T cells, PTEN-DT_{reg} mice still succumbed to autoimmunity suggesting that blockade of glycolysis alone was not sufficient to rescue autoimmunity. Subsequently, we found that PTEN-DT_{regs} showed enhanced nucleotide and lipid synthesis that was associated with reduced AMPK signaling. Overall, these findings indicate that overactivation of PI3K/Akt (that occurs during inflammation) can lead to metabolic dysregulation and disruption of Treg homeostasis.

F.31. Despite Increased Type 1 IFN in Autoimmune NOD Mice, Downstream IFNAR Responses In Dendritic Cells and Nuclear Localization STAT1 are Reduced

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Innate immune signals help break self-tolerance to initiate autoimmune diseases such as type 1 diabetes. In this study, we address how chronic innate signals like type 1 IFN levels in prediabetic NOD mice contribute to the subsequent regulation of disease progression. To this end, we compared the basal and CpG-stimulated type 1 IFN levels in prediabetic NOD and control C57BL/6 (B6) mice. NOD mice had more type 1 IFN transcripts and/or serum IFNα/β both before and after CpG stimulation suggesting that prediabetic NOD mice already have a type 1 IFN mediated chronic-type environment. Interestingly, though NOD mice produce more type 1 IFN and express higher levels of CD40, and NOD monocyte DCs make more TNF, the overall CpG-induced transcriptional response is muted in NOD cDCs. Of relevance the costimulatory proteins CD80/CD86, signals needed for regulatory T cell homeostasis, are upregulated less on NOD cDCs. Similarly, NOD Rag1$^{-/-}$ mice also display a defect in CpG-induced CD86 upregulation compared with B6 Rag1$^{-/-}$, indicating this particular innate alteration precedes adaptive autoimmunity. The impaired response in NOD DCs is likely downstream of the IFN-α/β receptor because DCs from NOD and B6 mice show similar CpG-induced CD86 levels when anti–IFN-α/β receptor Ab is added. IFN-α–induced nuclear localization of activated STAT1 is markedly reduced in NOD CD11c$^+$ cells, consistent with lower type 1 IFN responsiveness. Therefore, NOD DCs display altered innate responses characterized by enhanced chronic-type 1 IFN and activation of monocyte-derived DCs but diminished cDC type 1 IFN response.

F.32. New Methods Enabling Molecular Characterization of Autoantigen Formation in an Autoimmune Disease, IgA Nephropathy

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IgA nephropathy (IgAN), a frequent cause of end-stage renal disease, is an autoimmune disease wherein immune complexes consisting of IgA1 with galactose-deficient *O*-glycans (Gd-IgA1; autoantigen) and anti-glycan autoantibodies deposit in the glomeruli and induce renal injury. Serum IgA1 has 3-6 clustered *O*-glycans, some of which are deficient in galactose and thus expose terminal *N*-acetylgalactosamine (GalNAc). Patients with IgAN usually have elevated serum levels of Gd-IgA1, but the mechanisms involved in Gd-IgA1 production are not fully understood. Our group has shown that abnormal expression/activity of *O*-glycan galactosyltransferase C1GalT1 contributes to Gd-IgA1 production. Moreover, we hypothesize that abnormalities in the initial step of *O*-glycosylation can also lead to production of Gd-IgA1. The initiation step in the synthesis of *O*-glycans is catalyzed by GalNAc-transferases (GalNAc-Ts), represented in humans by a family of 20 enzymes. As our pilot experiments indicated that some GalNAc-Ts contribute to production of Gd-IgA1, we sought to characterize the mechanism(s) at a molecular level. Toward this goal, we developed new methods using high-resolution mass spectrometry to analyze the site-specific preference of GalNAc-Ts and the degree of GalNAc incorporation. In proof-of-principle studies, we found that GalNAc-T2 depends on its lectin domain to efficiently add clustered glycans to IgA1. Furthermore, a disease candidate GalNAc-T(s) may exhibit differential site-specific preferences. Together, these data demonstrate new methods that enable characterization of specific GalNAc-Ts and their roles in the production of Gd-IgA1, the key autoantigen in IgAN, or other glycoproteins with abnormal *O*-glycosylation in other diseases, such as MUC1 in breast cancer.

F.33. Wnt Signaling Links High Salt and Proinflammatory Signature in Treg

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Aberrant cytokine expression by regulatory T cells (Tregs) is associated with loss of function and autoimmune disease. In this regard, high salt is one of the environmental factors that induce Treg IFNγ expression diminishing Treg function. Here, we examined the role of the Wnt/β-catenin signaling in maintaining Treg function in relationship to salt induced IFNγ production. The common Wnt pathway target gene TCF7 was more abundantly expressed in human circulating IFNγ^{+} Treg than IFNγ^{-} Tregs. Moreover, β-catenin was highly upregulated in Tregs cultured under Th1 conditions. To further define the role of Wnt/β-catenin signaling on Tregs *in vivo*, we generated Treg specific β-catenin stabilized mice and observed that these mice showed scurfy-like autoimmune phenotype. β-catenin stabilized Tregs lost their suppressive function and produced excessive IFNγ and GM-CSF. Moreover, high salt stimulation induced activation of Wnt/ β-catenin signaling in both human Treg and mice iTreg, and Treg specific depletion of β-catenin attenuated high salt induced SGK1-Foxo1/3a axis in mice iTreg. Taken together, these data demonstrate that Wnt/β-catenin signaling plays a critical role in maintaining Treg function and excessive IFNγ production. Furthermore, our data suggests that high salt conditions activate Wnt/β-catenin signaling acting upstream of SGK1-Foxo1/3a axis in Tregs.

F.34. Melanocortin Peptides – Endogenous Mediators of Inflammation

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Timely resolution of inflammatory exacerbations is a key principle in clinical management of autoimmune disorders. Over the last decades, several novel therapies for autoimmune disorders across multiple disease states have been developed to modulate a dysregulated immune response. In general, the underlying mechanism of action of these therapies is to target and downregulate a specific inflammatory mediator, such as a cytokine/chemokine, adhesion molecule, or certain signaling pathway. Rather than targeting a single inflammatory mediator or pathway, multimodal, endogenous resolution of inflammation could serve as a potential alternative mechanism to manage autoimmune disorders. Activation of melanocortin receptors (MCRs) could constitute such a potential alternative mechanism for multimodal, endogenous resolution of inflammation. MCRs are an evolutionarily conserved group of G-protein-coupled receptors. Five MCRs have been identified and shown to be expressed on many cell types and tissues

throughout the body, including immune cells, muscle, kidney, bone/cartilage, and CNS tissue. Melanocortin peptides are derived via enzymatic cleavage from a precursor protein pro-opiomelanocortin (POMC) in the pituitary gland and include ACTH, α-melanoctye-stimulating hormone (α-MSH), β-MSH, and γ-MSH. Melanocortin peptides bind to one or more MCRs and potentially modulate intracellular signaling by restoring homeostasis between pro- and anti-inflammatory mediators. Evidence from preclinical studies from autoimmune, inflammatory disorders in various therapeutic areas suggests that activation of MCRs may provide a potential alternative mechanism to resolve inflammation and restore homeostasis in an endogenous manner.

Genetics

OR.11. Pre-Adaptive Innate Transcriptional Signatures Associated With Immune Responses After Seasonal Influenza Vaccine

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The aim of this study was to identify pre-adaptive transcriptional signatures associated with immune responses (Day 28-Day 0) after influenza vaccination. We used pre-vaccination (Day 0) and early post-vaccination (Day 3) mRNA-Seq transcriptional profiling on samples from 159 subjects (50-74 yo) following the receipt of trivalent influenza vaccine containing the A/California/7/2009/H1N1 virus. Influenza-specific serum hemagglutination-inhibition (HAI) and virus-neutralization antibody (VNA) titers and mRNA-sequencing on PBMCs were performed using samples obtained at Days 0, 3 and 28 after vaccination. Permutation tests were used to identify 33 genesets that had a change from Day 0-to-Day 3 in mRNA expression in the genes (geneset p-value<0.005). The genes in each of the genesets were used in cross-validated penalized-regression models to identify associations with changes in HAI/VNA (Day 28-Day 0) after vaccination.

The median (IQR) age of the study subjects was 59.5 (55.3, 66.3) years (98.7% Caucasians). Day 0 influenza-specific median HAI/VNA titers (1/80; 1/40-1/320) showed the presence of pre-existing antibodies. There was no change in HAI or VNA titers from Day 0-to-Day 3; however, HAI/VNA titers increased by Day 28 (1/320; 1/160-1/640, p<0.001).

The pre-adaptive genes/genesets associated with HAI response are the dystrophin-associated glycoproteins (*SGCD*), lipid-linked alpha-1,2-glucosyltransferases (*ALG10*) and ssRNA-binding proteins (*PABPC4*). The pre-adaptive genes/genesets associated with VNA include genes playing a fundamental role in pathogen recognition/activation of innate immunity, including *TLR8*, *ADARB2*, protoplasmic signalling and cell-to-cell interaction, ssRNA-binding and mRNA *ZFP36*protein genes.

The identification of pre-adaptive/innate gene signatures associated with HAI/VNA responses may provide a better understanding of genetic markers of very early immune response.

OR.45. FOXP3 Controls Human Thymocytes Maturation and Peripheral T Cell Homeostasis

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FOXP3 is the master transcription factor of regulatory T cells (Tregs), which are key players for maintaining peripheral immune tolerance. While the fundamental role of FOXP3 in controlling the functionality of human Tregs is well established, its role in the differentiation of both thymic derived T regulatory (tTreg) and conventional (Tconv)

cells has only been postulated. To address this issue, we implemented the NSG-based humanized mouse model (huMice) to allow efficient T lymphopoiesis, and studied *in vivo* human T cell differentiation in presence of FOXP3 overexpression, knock-down (KD) or knocked-out (KO). Immune-phenotypic analysis of huMice showed decreased Tconv differentiation and dampened T cell response when FOXP3 was ectopically expressed, while in FOXP3 KD/KO huMice Tconvs were expanded, in particular in the $CD4^+$ memory cell fraction. Tregs arising in all experimental conditions expressed normal levels of FOXP3, denoting their advantage over FOXP3-modulated Tregs and indicating the need for FOXP3 expression in the development of human Treg. We found clonal expansion of Tconvs in FOXP3 KO/KD huMice coupled to altered expression of FOXP3-controlled genes in these cells. Moreover, FOXP3 KD/KO mice showed altered thymocyte differentiation with episodes of lethal lymphoproliferation, indicating a major role of FOXP3 in controlling the early differentiation of Tconv cells. Overall, our data confirmed *in vivo* the dependency of human Tregs differentiation on FOXP3 expression and provided for the first time evidence of the intrinsic role of FOXP3 in controlling homeostasis and differentiation of human conventional T cells.

OR.46. Engineering Human T Cell Circuitry

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Functional testing of human genome sequences in primary immune cells has been largely impossible until our advances in genome engineering methods that now permit direct DNA editing in human primary T cells. CRISPR/Cas9 has facilitated genome engineering in many cell types, but in human T cells Cas9 efficiency had been limited and Cas9 had not allowed targeted nucleotide replacements. We have now developed a CRISPR/Cas9-based platform that enables both knock-out and knock-in genome editing in primary human T cells by electroporation of Cas9:single-guide RNA ribonucleoproteins (Cas9 RNPs). Cas9 RNPs paired with homology-directed repair (HDR) template oligonucleotides can generate a high frequency of knock-in targeted genome modifications in primary T cells. This Cas9 RNP technology holds great potential for therapeutic genome engineering of human T cells for treatment of cancer, HIV, primary immune deficiencies, and autoimmune diseases. The technology also enables unprecedented explorations of genetic mechanisms that regulate T cell differentiation and function. Recently, we have applied a combination of *in vitro* human cell editing and mouse germline editing to functionally dissect the complex regulation of *IL2RA* (Interleukin 2 Receptor Alpha). These studies are beginning to reveal how specific non-coding genetic variants in the locus cause cell-type specific dysregulation and increased risk of human autoimmune diseases. We aim to understand how sequence variation throughout the human genome affects T cell circuits in health and disease.

T.36. Both Canonical and Noncanonical NF Kappa B Signalling are Disrupted by a Mutation of Noncanonical NFKB2 Causing a Primary Immunodeficiency Syndrome

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Common variable immunodeficiency is a heterogeneous primary immunodeficiency disorder. Single gene defects have been associated with it in approximately 20% of patients. Recently we identified a novel germline mutation within the c-terminal region of *NFκB2*gene, which leads to defective processing of the NFκB2 protein. Here, we investigated the effects of this mutation on the activity of all members of canonical and noncanonical NFκB2 signaling pathway.

Epstain-Barr-Virus-transformed lymphoblastoid cell lines (LCLs) from both patient and her healthy sibling were used to study the NFκB pathway. We demonstrated that the nuclear levels of p52 and Rel B from the patient's LCLs were markedly decreased compared to control, both before and after CD40L stimulation. The nuclear level of c-Rel from patient's LCLs was increased compared to control. However, there was no further nuclear c-Rel induction in patient's LCLs after CD40L stimulation. In addition, there were no significant difference in p50 and p65 nuclear translocation

between patient's LCLs and control. We further investigated the DNA-binding capacity of NFκB family members with the TransAM analysis, and confirmed that the basal level of DNA-binding capacity of p52 and Rel B from patient's LCLs was significantly reduced to 39±9% and 24±8%, respectively, compared to control. However, c-Rel DNA-binding capacity in patient's LCLs was significantly decreased compared to control. The IkBα level was significantly reduced in patient's LCLs, compared to control.

This disruption of the cross-talk between canonical and noncanonical NFκB pathways may partially account for the characteristic phenotype of patients carrying this mutation.

T.37. High Repertoire Divergence of Genetically-identical Human Thymocytes Generated in Identical Thymi

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VDJ recombination leads to random generation of diverse T cell receptors in the thymus. Most thymocytes undergo apoptosis during positive and negative selection. We used humanized mice to determine whether or not exposure to the same antigens for positive and negative selection in the same thymus would lead to the formation of similar TCR repertoires in different individuals. Three NSG mice were sublethally irradiated and transplanted with fetal liver $CD34^+$ cells and autologous fetal thymus. After fourteen weeks, we euthanized the mice and sorted thymic graft Single positive (SP) CD8 cells, SP Treg-depleted CD4 cells and Tregs (SP $CD4^+$ $CD25^+$ $CD127^-$ cells). Genomic DNA was isolated and underwent high-throughput TCRB CDR3 sequencing (Adaptive Biotechnologies). The Jenson-Shannon Divergence (JSD) comparing the CDR3 repertoires (amino acid level) of any two mice was close to 1 for each cell type [CD4: 0.95, 0.96, 0.97; CD8: 0,97, 0.98, 0.96; Treg: 0.98, 0.99, 0.99], meaning that the repertoires were highly divergent. However, there were 306, 164 and 11 clones overlapping among CD4 cells, CD8 cells and Tregs of these three mice, respectively. There was more overlap among the most abundant clones (top 500 in frequency). In conclusion, our data indicate that formation of TCR repertoire is largely stochastic and can be almost totally divergent in mice with identical hematopoietic stem cells, thymus, genetic background and environments. This finding may help to explain the incomplete penetrance of genetically-controlled autoimmune diseases in identical twins.

T.38. The Genetic Basis for the HLA-DR3/4 Excess in type 1 Diabetes Patients

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We developed a stochastic epigenetically-triggered Mendelian oligogenic (SEMO) model for type 1 diabetes (T1D). The model's genetic component includes recessive inheritance for the major histocompatibility complex (MHC) susceptibility gene(s) based on MHC haplotype sharing by affected sib pairs and the fit in some populations of MHC alleles/haplotypes to the Hardy-Weinberg equilibrium (HWE). However, many patient populations exhibit an HLA-DR3/4 heterozygote excess. Many investigators believe MHC susceptibility recessive inheritance is unlikely due to this lack of MHC HWE. We previously provided evidence for greater parental subpopulation mixing in T1D families (54%) compared to control families (27%), thus explaining their higher offspring T1D incidence and rising population incidence. We postulate specific *HLA-DRB1*03* and *DRB1*04* haplotypes mark previously isolated populations that had selected against different causal T1D non-HLA loci. There are two HLA-DR3 and at least five HLA-DR4 T1D susceptibility conserved extended haplotypes (CEHs) that presumably arose in different European subpopulations. Our model suggests the HLA-DR3/DR4 excess involves only some susceptibility CEHs. We therefore analyzed Type 1 Diabetes Genetics Consortium data for patients from several thousand T1D families organized into nine geographic cohorts. The distribution of *HLA-DRB1/-DQB1* haplotype combinations varied significantly between cohorts. Some HLA haplotypes fit the HWE among many patient cohorts while others did not. Specific haplotypes appear to explain most of the lack of DR3/DR4 HWE in many patient cohorts. Thus, it was not HLA-DR3 or DR4 specificities that

increased T1D "risk" but specific MHC haplotype combinations that distorted (but did not negate) the HWE expected from Mendelian recessive inheritance.

T.39. Understanding Microglial Gene Expression in Alzheimer's Disease

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Alzheimer's disease is pathologically characterized by the accumulation of neuritic β-amyloid plaques and neurofibrillary tangles in the brain and clinically associated with a loss of cognitive function. The dysfunction of microglia cells has been proposed as one of the many cellular mechanisms that can lead to an increase in Alzheimer's disease pathology. Investigating the molecular underpinnings of microglia function could help isolate the causes of dysfunction while also providing context for broader gene expression changes already observed in mRNA profiles of the human cortex.

We have used mRNA sequencing to construct gene expression profiles of microglia purified from the cortex of 11 subjects from a longitudinal cohort of aging, Rush Memory Aging Project (MAP). By studying these microglia gene expression profiles in the context of tissue-level profiles of the cortex of 542 subjects from the MAP and Religious Orders Study (ROS) we are able to address dual problems. By using information from the large ROSMAP cohort, we are able to identify and separate genes associated with processes such as metabolism and translation in the microglia signature, allowing us to isolate the genes which are strongly associated with immune response. Conversely, we illustrate that the microglia signature can be used to highlight predefined sets of coexpressed genes in ROSMAP that are highly enriched for microglia genes ($p<10^{-100}$). Addressing these two questions allows us to identify sets of microglia specific genes which are associated with various Alzheimer's disease traits further emphasizing the molecular consequences of microglia dysfunction in this disease.

T.40. Transcriptomic Fingerprints Reveal an Immune Phenotype Shared by Melanoma Patients and a Subset of Healthy People

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Several components of precision medicine, like differential immune phenotypes and responses to immune perturbations, owe to vast immune variability among humans. We approach precision medicine by capitalizing on this variability and using RNA-Seq to derive individual transcriptomic immune "fingerprints" containing genes whose expression in peripheral blood $CD4^+$ cells is temporally stable yet differential among healthy people. Interestingly, the same genes are also uniquely expressed among stage IV melanoma patients, and in each cohort individuals segregate into groups via hierarchical cluster based on relative expression of the genes. Hierarchical clustering of the healthy individuals and melanoma patients together reveals a healthy group with which no melanoma patients cluster and another healthy group with which the patients do cluster. Fingerprint genes that are differentially expressed between the healthy individual groups, which we term the cancer phenotype derived gene set ($PDGS^{CA}$), cluster a second cohort of independent melanoma patients with the same healthy donor group as the first patient cohort ($p=0.002$). Conversely, the $PDGS^{CA}$ segregates a second cohort of independent healthy donors relatively evenly among the first healthy cohort, verifying that the $PDGS^{CA}$ identifies a melanoma-like phenotype in healthy individuals. The fingerprint and $PDGS^{CA}$ genes were re-derived using both cohorts of healthy individuals after adjusting batch effects between them, and the original observations were maintained. Pathway analysis implicates upregulated T cell activation in the healthy group that clusters with melanoma patients. Future directions include clarifying the biological

meaning behind the PDGS^{CA} and predicting melanoma patient PD-1 blockade outcomes using our transcriptomic fingerprint method.

T.41. Dissecting the Mechanisms of Disease for Autoimmunity Variants in the IL2RA Super-enhancer
Dimitre Simeonov, Alice Chan, Barbara Celona, Frederic Van Gool, Brian Black, Mark Anderson and Alexander Marson. University of California, San Francisco, CA

Hundreds of autoimmunity loci have been identified in the human genome, yet our understanding of the nucleotide changes within these stretches of DNA and how they predispose to disease remains limited. By combining genetic and epigenetic data with a CRISPR/Cas9 genome engineering approach we have begun to interrogate autoimmunity associations at the *IL2RA* locus. *IL2RA* encodes an interleukin-2 receptor that plays an intricate role in maintaining normal immune function through constitutive expression on regulatory T cells and transient induction in activated effector T cells. The *IL2RA* locus exemplifies the complex landscape of *cis*-regulation. A cluster of putative enhancers occupied by master transcriptional regulators form a "super-enhancer" at this locus. Fine-mapped variants for eight autoimmune disorders fall within distinct segments of the super-enhancer. Our initial genetic perturbation studies have identified an intronic enhancer that is required for *IL2RA* induction in human T cells. This element harbors a common autoimmunity variant that lands in a MEF2 motif. By ablating the MEF2 factors we have shown they are critical for *IL2RA* induction in T cells and the autoimmunity variant disrupts MEF2 binding to the intronic enhancer. To better understand the biological mechanisms by which dysregulation of this genetic circuit predisposes to autoimmunity we have generated "knock-out" mice in which the MEF2 binding site is deleted or "knock-in" mice that have the autoimmunity variant. These studies promise to reveal new insights into mechanisms of autoimmunity.

Immune Monitoring

OR.44. Identification of Plasma Protein Biomarkers of Acute Renal Allograft Rejection
Maura Rossetti, Connor Fitzpatrick, Nicholas Harre, Ying Zheng, Neil Todd Mercer, Gemalene Sunga, David Gjertson and Elaine Reed. University of California, Los Angeles, CA

Upon presentation of clinical symptoms, renal transplant recipients routinely undergo needle core biopsy to diagnose acute graft rejection. This procedure is invasive and associated with patient morbidity. Our aim was to validate the use of plasma proteins as biomarkers of rejection, and to develop a less invasive and cost-effective diagnostic panel for renal allograft. We measured 10 proteins, previously identified by mass spectrometry as differentially present at the time of rejection, in the plasma of 96 adult renal transplant recipients by ELISA. The cohort included 45 non-rejectors and 56 rejectors, the latter sampled at the time of rejection (-7/+3 days of a positive biopsy) or after the event (>30 days after the positive biopsy). We were able to confirm statistically significant differences both cross-sectionally (rejectors vs non-rejectors) and longitudinally (rejection vs post-rejection) in Apolipoprotein A1, as previously published. In addition, we found statistically significant differences in alpha-2-Macroglobulin cross-sectionally, and in Alpha-1-Antichymotrypsin and Apolipoprotein E longitudinally. Finally, complement Component 4 and the Inter-alpha-trypsin Inhibitor Heavy Chain 4 showed trends cross-sectionally (the former) and both cross-sectionally and longitudinally (the latter). Next, we sought to determine whether combinations of these markers could be used to classify patients as rejecting or non-rejecting. Multivariate logistic regression and CART analyses demonstrated that different combinations of these proteins might be used to this aim, warranting their further validation in a larger independent renal cohort. In conclusion, these plasma proteins could potentially be used in a panel as a novel method to diagnose acute cellular renal allograft rejection.

T.18. Predicting Disease Flare in African-American Lupus Patients: Elevated Pre-flare Levels of Innate,

Adaptive, and TNF-superfamily Soluble Inflammatory Mediators Mark Impending SLE Disease Flare, While Regulatory Mediators Distinguish Periods of Non-flare

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SLE is a complex autoimmune disease characterized by immune dysregulation and varied disease activity. Identifying mechanistic mediators of altered disease activity could help prevent damage and improve disease management. We evaluated changes in 52 plasma soluble mediators 6-12 weeks preceding clinically-defined disease flare in 13 African American (AA) SLE patients compared to matched SLE patients without impending flare (NF) and healthy controls. In addition, mediators within samples preceding SLE disease flare and during a clinically stable period (SNF) from the same individual were compared in 18 AA SLE patients. Correcting for multiple comparison, SLE patients with impending flare had significant alterations in 32 soluble mediators at baseline, with significantly higher levels of pro-inflammatory innate ($q \leq 0.008$) and adaptive cytokines ($q < 0.05$), chemokines ($q < 0.002$), and TNF superfamily members ($q < 0.04$). Baseline levels of regulatory cytokines, including IL-10 ($q = 0.005$) and TGF-β ($q < 0.001$), were higher in non-flare SLE patients. A normalized and weighted combined soluble mediator score was significantly higher in pre-flare SLE patients versus those with stable disease ($p < 0.0001$) and clearly differentiated pre-flare and non-flare periods of disease within the same patient ($p < 0.0001$). No differences in the number or type of autoantibody specificities, nor differences in medication use, between pre-flare and NF or SNF SLE patients were noted. Pro-inflammatory soluble mediators are elevated prior to disease flare, while regulatory mediators are elevated during periods of stable disease. Alterations in the balance between inflammatory and regulatory mediators may help identify AA patients at risk of disease flare and help decipher SLE pathogenic mechanisms.

T.19. Towards Selecting Biomarkers to Evaluate the Effectiveness of T Cell Therapy Products

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Background: Virus-specific T cells (VST) have been used effectively to treat or prevent viral infections following hematopoietic stem cell transplantation (HSCT), and cell surface profiles may be valuable biomarkers to evaluate the performance of VST *in vivo*. We hypothesized that surface memory markers on VSTs are a reliable biological measure to predict the likelihood of antiviral responses in patients following HSCT.

Methods: We prospectively studied T naïve (Tn), central memory (Tcm), effector memory (Tem) and TEMRA expression by multicolor flow cytometry in 10 VST products targeting adenovirus, CMV, and EBV that were infused to patients following HSCT. VST were monitored *in vivo* using IFNg-ELISpot assays and clinical responses evaluated by virus PCR assays 4 weeks after infusion.

Results: Our preliminary results show that VSTs possess a distinct T cell phenotype compared to control PBMCs with no significant difference in $CD4^+$ or $CD8^+$ Tem cells (p=0.83 and 0.17; respectively) but with a lower frequency of TEMRA cells (median TEMRA VSTs 4.7% v.s. TEMRA controls 39.5%; p<0.0001, Mann-Whitney test). Patients who achieved a complete clinical response (CR) based on resolution of viremia received VST products that contained a predominant Tcm population. Tcm cells were significantly lower in VST products administered to patients with no response (NR) or partial response (PR) as compared to patients who achieved a CR (median Tcm CR 2.7% v.s. Tcm NR/PR 0.015%; p=0.0045). We propose that further study will enable us to validate memory surface markers as reliable predictors of VST performance *in vivo.*

T.20. RImmPort: An R Package that Enables Ready-for-analysis Immunology Research Data

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Public access to raw clinical and immunological research data has created a tremendous opportunity for data-driven science. It has allowed the researchers to evaluate new research hypotheses that were not originally formulated in the studies; by promoting data reuse, reproducibility and reanalysis of multiple datasets from published studies. In order to perform such analysis of disparate data presupposes a) uniform representation of research data using data standards and b) easy access to such standard representations of clinical research data in analytical environments.To streamline the accessibility and interoperability of publicly accessible NIAID-funded research study data sets in ImmPort (immport.org), we have developed RImmPort package that prepares the data for analysis in R. RImmPort comprises of three main components: 1) a specification of R classes that encapsulate study data, 2) foundational methods to load data for a specific study, and 3) generic methods to slice and dice data across different dimensions in one or more studies. Furthermore, RImmPort supports open formalisms such as CDISC standards on open source bioinformatics platforms such as Bioconductor to ensure that ImmPort curated study datasets are seamlessly accessible and ready for analysis, thus enabling innovative bioinformatics research in immunology.

T.21. CD146 Is Preferentially Expressed on Recently Activated Switched Memory B Cells in Peripheral Blood of Healthy Volunteers

Pradeep Kumar Dagur, Angelique Biancotto, J. Philip McCoy Jr. National Heart Lung and Blood Institute, Bethesda, MD

MCAM (CD146) is primarily expressed on endothelial cells. In past, we have shown that it can also be expressed on effector memory T lymphocytes. Here, we report that approximately 1.264 ± 0.845 % (Mean±SD, n=99) of total B cells in healthy individuals express CD146. Characterization of $CD146^+$ B cells revealed that CD27, CD38, CD39 CD49D, CD29, CD80, CD86, CD58, CD99 are widely co-expressed on these cells whereas CCR7, CD69 are not coexpressed by $CD146^+$B cells. This antigen expression is indicative of effector memory phenotype, similar to what is observed on T cells. Furthermore, coexpression of CD49d, CD29, CD39 and CD99 is indicative of the recently activated B cells. When compared to total B cells, $CD146^+$ B cells have higher expression of IgA, IgE, or IgG compared to IgM or IgD. Additionally, up to 40% of IgA expressing plasmablasts express CD146. The finding of co-expression on $CD146^+$ B cells of a second adhesion molecule, CD58, is an interesting, but not yet understood observation. Similar to its role on T cells, the presence of CD146 on B cells may endow these cells with the ability to readily adhere and possibly migrate through endothelium and transit to sites of inflammation.

T.22. TEMRA Subsets Fighting Exhaustion- What You See is not What You Get

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Terminally differentiated memory RA^+ T cells (TEMRA) play an important role in a broad range of pathologies. We and other groups found an association between high TEMRA cells level and different pathologies (e.g. disturbed bone fracture healing or aortic valve stenosis). These pathologies are discussed to be associated with the aging of the immune system, resulting in increased levels of TEMRA cells.

Commonly, TEMRA cells are defined by the combination of distinct surface markers ($CD45RA^+/CCR7^-$ vs. $CD57^+/CD28^-$). However, these differences in definition also result in diverse TEMRA populations. We performed detailed multicolor analysis to characterize phenotypic different subsets among conventionally defined TEMRA cells.

The goal of this study is, to analyze different TEMRA cell subsets in terms of their proinflammatory potential and the expression of additional surface markers, which are associated with impaired or activated cell function. We analyzed

PBMCs from healthy donors as well as from patients suffering from severe aortic valve stenosis to understand TEMRA subset function in more detail.

For the first time we could show, that TEMRA cells can be divided into functionally diverse subsets. Most surprisingly, subsets expressing high levels of the exhaustion marker PD-1, also showed a high proinflammatory activity, rather than signs of exhaustion.

We hypothesize that conflicting data in literature are related to the heterogeneity of “TEMRA”, therefore a detailed understanding of these potential subsets is closely linked to a better understanding of several pathologies, especially of those that are related to an “aged” immune system.

T.23. Lyophilized Antibody Cocktails Offer Long Term Shelf Life and Excellent Reproducibility for Longitudinal Studies

Anagha Divekar, Matthew Rogers, Michael Lee, Haewon "Josh" Chong, Xifeng Yang and Craig Monell. BioLegend, Inc., San Diego, CA

Multi-color flow cytometric assays require laboratory personnel to mix from four up to 15 antibodies on a daily basis, which is time and labor intensive and can lead to manual errors due to missing antibodies, pipetting incorrect amount of the antibody or cross contamination or spillage during the workflow. In addition, liquid cocktails with tandems dyes such as PE/Cy7 and APC/Cy7 suffer from stability issues, leading users to prepare a fresh cocktail for every use. One solution to prevent these issues is to lyophilize antibody cocktails allowing increased shelf-life and providing researchers with the ability to easily conduct long-term, multi-site studies to obtain reliable data. Using our unique lyophilization technique, we have successfully lyophilized a variety of multi-color immuno-phenotyping cocktails. Here we show that a lyophilized CD3 APC/Cy7, CD4 PE/Cy7, CD8a Alexa Fluor™ 700, CD14 APC, CD16 PE, CD19 Pacific Blue™, CD45 PerCP, CD56 PE, HLA-DR Alexa Fluor® 488, CD11c PE/Dazzle™ 594, and CD123 Brilliant Violet 510™ cocktail performs similar to (i.e., % positive, MFI and compensation parameters) a freshly mixed cocktail. Stability studies have shown that these lyophilized cocktails are stable for up to 3 months under heat (37°C) stress storage conditions. These results suggest that the lyophilized cocktail will be stable for several years. Real-time stability studies are ongoing.

T.24. Veri-**Cells™ Leukocytes and PBMC (CD4$_{low}$)**, Lyophilized Human Blood Cells are Reliable Controls for Flow Cytometric Assays

Anagha Divekar, Myra Gordon, Matthew Rogers, Xifeng Yang, Donna Williams and Craig Monell. BioLegend, Inc., San Diego, CA

Control cell populations offer the ability to monitor assay performance and variability for longitudinal studies. Veri-Cells™ Leukocytes, a lyophilized cell preparation, is an excellent human immunophenotyping control as it includes all leukocyte subsets (lymphocytes, monocytes and granulocytes). Our Veri-Cells™ PBMC and PBMC (CD4$_{low}$) are available as clinically useful controls allowing monitoring of normal and low levels of $CD4^+$ cells. These cell preparations can be used to assay most CD markers and chemokine receptors such as CXCR5, CCR6, CCR4 and CCR7. Our Custom Solutions Team offers the option to tailor control cells to their specific requirements ranging from pre-lyophilization staining with live/dead dyes, cell activation and selective depletion/enrichment. Lyophilized cell lines provide the convenience of on demand testing without the need for expensive tissue culture equipment or incubation/contamination delays. Combinations of cell lines or sorted isolates can be added to leukocytes as analogs for abnormal or rare event staining controls. These lyophilized cell preparations exhibit a scatter and staining profile similar to that of freshly prepared cells. Real time stability data demonstrates excellent stability for two years; longer term stability studies are under way. Single or multi-test custom lots can be manufactured at almost any size for use in long term or multisite clinical trials.

T.25. Establishing the Natural Variability of Circulating Immune Cells by Flow Cytometry: Impact of Age, Gender and Environmental Factors in Healthy Donors

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Immunophenotyping of blood cells by flow cytometry is an important procedure in both clinical and research settings. The interpretation of results across different studies remains challenging due to technical variance caused by different analytical procedures and the inherent natural variance in human populations. It is therefore critical to define reference ranges of immune blood cells in large cohorts of healthy subjects of different age, gender and infection status.

With this in mind, we took advantage of the Milieu Intérieur cohort of 1,000 healthy donors stratified by gender and age and extensively annotated for demographic, clinical, and genetic characteristics. Fresh whole blood was analysed by semi-automated flow cytometry procedures that allowed the characterization and quantification of 164 different immune traits. The impact of age, gender and different environmental factors on these traits was evaluated.

We observed a significantly higher number of eosinophils, NK cells and monocytes in men and an increase of MAIT cells in women. We detected an age-related decline of innate immune cells, including NKT and MAIT cells, CD56bright NK cells, ILCs, pDC and cDC1. Naïve T cells also showed a decline with age, whereas activated, memory T cells and activated regulatory T cells increased. CMV seropositivity was strongly associated with the presence of $CD4^+$ and $CD8^+$ TEM and TEMRA compartments, but did not impact the frequency of naïve T and TCM cells.

These results will help set healthy reference value ranges for future immunophenotyping studies and have important implications for guiding vaccination strategies and establishment of personalized therapies.

T.26. Can Immune Parameters, $CD16^+/CD56^+/CD3^+$ NK Cells and T Regulatory Cell ($CD4^{+high}/CD25^{+high}/Foxp3^+/CD127^{dim}$) be Used as Predictors for Long Surviving of Larynx Cancer Patients?

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Aim: In this study, we aimed to focus on the relationship between T-regulator cell and NK on effection of long-term survival in laryngeal squamous cancer patients.

Patients and Methods: In this study, we evaluated laryngeal squamous cancer patients in Research Clinic were diagnosed and previously untreated was conducted on 19 patients. We measured NK% ($CD16^+/CD56^+/CD3^+$) and Treg% ($CD4^{+hi}/CD25^{+hi}/CD127^{dim}/Foxp3^+$) using by flow cytometry A view of the charts of 19 cancer patients (2 females, 17 males; mean age 55, 2 years; range 42 and 74 years) surgically treated and followed up January 2008 - and January 2016 and 15 healty control persons was evaluated. Mann-Whitney U and Wilcoxon Signed Ranks Test have been utilized for statistical analysis.

Results: NK value of 9 patients living for 7 years after the operation was measured as significantly higher (p <0.001) compared to healthy control and T-regulator cell level was low value according to the healty control group (Treg of healthy group(n=15) value: 8.27+/-2:31 Treg of Survival patients(n=9) value:7.71+/-4.29). NK and Treg value of patients living for 7 years after the operation were found significantly differences compared to healty control group (NK, p <0,007, Treg, p <0,008).

Conclusion: These results suggest that immun-survillance at the side of NK and T-regulator cells are very important in anti-tumor immun-treatment in patients with head and neck squamous cell cancers. Consequently, consideration of this immunological variables in Laryngeal cancer diagnosis protocols, may provide new perspectives on the strategy of the prognosis and treatment.

T.27. High-dimensional Cytometric Time-of-flight (CyTOF) Analysis of Human Peripheral Blood $CD4^+$ T Regulatory Cells Provides New Insights Into Their Frequency and Function

Matthew Kunicki, Kara Davis, Rosa Bacchetta and Maria-Grazia Roncarolo. Stanford University, Stanford, CA

Human $FOXP3^+$ $CD25^+$ $CD127^{lo/-}$ T regulatory (FOXP3 Treg) and $CD49b^+$ $LAG3^+$ type 1 T regulatory (Tr1) cells are essential for maintenance of peripheral immune tolerance. The mechanisms responsible for the induction and activation of these Treg cell subsets in health and disease have not been fully elucidated. In addition, understanding the relation between Treg cells and $CD4^+$ T helper (Th) cell subsets is important for defining the interplay between Treg and Th cells and their respective role in immune homeostasis and response.

To improve detection of FOXP3 Treg and Tr1 cells, and at the same time define their unique response to cytokine and TCR stimulation, we constructed a panel of 32 intracellular and surface markers to be used in CyTOF analysis. First, we performed a baseline comparison of transcription factors and surface markers expressed by FOXP3 Treg, Tr1, Th1, Th2, Th17, and T follicular helper (Tfh) cells isolated from peripheral blood of healthy subjects. We next tested the phosphorylation of STATs and other signaling molecules after activation.

Our preliminary data shows that we can detect by CyTOF a discrete population of FOXP3 Treg and Tr1 cells, and suggests additional specific biomarkers. Using high-dimensional single-cell analysis (viSNE), we see differential expression of CXCR5, CXCR3, CCR4, or CCR6 among our T cell populations, such as the $CCR4^+$ FOXP3 Treg and $CXCR3^+$ Tfh subsets. In addition, more heterogeneity in surface marker expression and signaling pathways can be observed within $CD4^+$ cell populations that would indicate the presence of cell subsets currently unreported.

T.28. Associations of $CD8^+$ T Cell Activation Markers and Alcoholic Liver Disease in Patients with Alcohol Use Disorder

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Only a third of patients with alcohol use disorder (AUD) will develop alcoholic liver disease (ALD) and the role of $CD8^+$ T cells in the pathogenesis of ALD is controversial. We aimed to analyze $CD8^+$ T cell activation using a combination of membrane markers including the nonspecific $CD38^+$ and the antigen-dependent $HLADR^+$.

Patients and Methods: AUD patients admitted for detoxification between 2013 and 2015. Laboratory parameters, including liver function tests and peripheral blood for immunophenotyping (FACSCalibur, BD Biosciences) were obtained the day of admission. Four profiles of activation were defined: 1.- $HLADR^-/CD38^-$, 2.- $HLADR^-/CD38^+$, 3.- $HLADR^+/CD38^-$ and, 4.- $HLADR^+/CD38^+$. ALD was defined by 2 out of: AST >74< 300U/L, AST/ALT>1.5 and/or total bilirubin >1.2mg/dL. Patients with HCV, HIV, liver cirrhosis, cancer, autoimmune disease and immunosuppressive treatment were excluded.

Results: 53 patients eligible (83% men); age was 49-years-old [IQR:44-54 yrs] and alcohol consumption was 145 g/day [IQR:90-205g/day]. Patients with ALD (23%) presented with low absolute numbers of $CD3^+$ (1,117 c/μL ±469 vs. 1,449 c/μL ±650, p=0.05), $CD4^+$ (720 c/μL ±376 vs. 919 c/μL ±437, p=0.08) and $CD8^+$ T cells (339 c/μL ±177 vs. 460 c/μL ±241, p=0.05), with respect to patients without ALD. $CD8^+$ T cells not expressing activation markers (CD38-/HLADR-) were more frequent in patients without ALD (327 c/μL ±178 vs. 218 c/μL ±116, p=0.05). Although not statistically significant, $CD8^+$ T cells expressing $HLADR^+$ were more frequent in those patients with ALD (73 c/μL ±92 vs. 60 c/μL ±53, p=0.53).

Conclusion: heavy drinkers without ALD have a reduced expression of $CD8^+$ T cell activation markers.

T.29. Differences in Immune Cell Signaling Between CAV and Propensity-matched Non-CAV Patients

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Cardiac allograft vasculopathy (CAV) is the major cause of mortality in heart transplant patients. Gene expression data implicates that interferon gamma (IFNγ) inducible pathways play an important role in the pathophysiology of CAV. At this time, it is unclear whether cytokine signaling pathways in stimulated PBMC are altered in patients with CAV. In this study, we analyzed 6 lymphocyte subsets and monocytes for pSTAT-1, pSTAT-3, pSTAT-5 and Phospho-p44/42 MAPK (Erk1/2) signaling. PBMCs were stimulated with cytokines including IFNα, IFNγ, IL-2, IL-6, IL-7, IL-10, IL-21, GM-CSF, MIG (monokine induced by IFNγ), and IP-10 (IFN-inducible protein 10), then stained using 9-color flow cytometry. Using a cross-sectional study design, 23 patients with CAV and 21 age- and sex-matched patients with non-CAV were recruited from the Stanford heart transplant clinic. A greater proportion of patients with CAV have more chronic kidney disease, diabetes or heart failure. All transplant patients were on immunosuppressant treatment, either on Sirolimus or MMF (mycophenolate mofetil) or both. The results suggest a greater pSTAT-5 signaling response in monocytes (p=0.002), stimulated with MIG and IP-10 for patients with CAV compared to the non-CAV patients. If further validated, this could prove a potential biomarker to monitor risk of CAV or response to immunosuppression therapy.

T.30. Implementing CyTOF and Barcoding to Immune Monitoring and Clinical Studies

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CyTOF (Cytometry by Time of Flight) is a novel technology that presents a significant advance in multiparametric single cell analysis (over 50 parameters/cell). CyTOF is already being used in medical fields including immunology, hematology, and oncology. Combining CyTOF with mass-tag cell barcoding (MCB) opens new possibilities for single cell interrogation and consistency. MCB eliminates variability between samples, shortens processing time and reduces reagent consumption and instrument use. We present here results we obtained using CyTOF2 instrument with important methodology considerations regarding sample preparation, storage and barcoding. We compared CyTOF to flow cytometry, evaluated the stability and storage of samples and we developed a protocol for sample preparation that combined both MCB and use of robotics to allow the preparation several samples simultaneously, increasing throughput and consistency. We found that CyTOF was tightly comparable to flow cytometry, when analyzing some of the most commonly used immune markers. Using CyTOF, we were able to combine 10 flow cytometry biomarker panels into one single CyTOF panel (45 markers) reducing not only antibody consumption but also the number of cells to needed. We used MCB in a clinical study and were able to collect data from different subjects and different time points simultaneously, eliminating the variability between samples if they were run separately. CyTOF and MCB allow consistency and economy, in highly dimensional analysis at the single cell level. However more tuning is still needed for personalized projects as the MCB procedure interfered with some of the surface markers due to fixation/permeabilization process.

T.31. A Proficiency Testing Comparison of Site Choice (SC) vs. Standardized Intra-cellular Staining Assays (ICS) Conducted by the External Quality Assurance Program Oversight Laboratory (EQAPOL)

Janet Staats, Twan Weaver, Jennifer Enzor, Wes Rountree, Christopher Todd, Ambrosia Garcia, Linda Walker, Marcella Sarzotti-Kelsoe, Jessica Frazier, Ana Sanchez, Kent Weinhold and Thomas Denny. Duke University, Durham, NC

The goal of proficiency testing is to assess a site's ability to conduct test methods, comparing their data against a known standard and/or data from all participating laboratories. For assays without a known standard, EQAPOL employs a highly standardized assay with consensus grading to measure overall site performance. Centralized analysis is used to delineate assay and analysis performance. Unfortunately, the EQAPOL standardized assay does not enable evaluation of a site's in-house assay. Therefore, EQAPOL piloted an optional SC component, whereby sites could utilize EQAPOL-provided PBMCs and peptides with their own assay. In a followup survey regarding the SC pilot, sites reported a strong preference to include both SC and EQAPOL standard components (73%), an ability to provide their own SC reagents (87%), and a willingness to harmonize SC endpoints to aid data comparisons (67%). There was mixed feedback as to whether SC data should be compared to other sites' assay data or the EQAPOL assay data. In conclusion, SC results were highly variable since the assays used are mostly non-standardized, require harmonizing reportables to express values in common measures for statistical analysis, and preclude the ability to perform centralized analysis. There was strong consensus that EQAPOL provide feedback in the two main areas where sites struggle: instrument setup and data analysis. As a result, EQAPOL developed a two-tiered program including both the EQAPOL standardized assay, to provide feedback on site performance, specifically instrument standardization and data analysis, paired with a SC component to enable comparison of site's own assay.

T.32. Comparison of Biological Control Cells to Use with Standardized HIPC Panels for Immune Monitoring

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Biological controls are used in flow cytometry to develop reference ranges that aid in interpreting clinical data and to assess inter-assay variability that facilitate comparisons of disease stages, conditions, therapies, and vaccines in the context of immune monitoring for clinical trials. The most common type of control used is peripheral blood obtained from normal, healthy donors (NHD). This type of control is confounded by biological variation due to disparities in age, gender, and race. The use of biological controls is further muddled by technical variations in the assay owing to differences in specimen collection and storage, preparation methods, reagents, instruments, statistical analysis, and even reporting units (%, cells/uL, ABC). To identify the most effective biological control for use with standardized HIPC panels, we compared cryopreserved peripheral blood mononuclear cells (PBMC) obtained from thirty NHD, collected at two time points each, to thirty staining's of custom Veri-Cells™ PBMC, a lyophilized cell preparation pre-stained with Zombie Green viability dye. We evaluated markers for subsetting T, Treg, T-helper, B, NK, monocytes, and dendritic cells using standardized HIPC panels. The T-helper panel, modeled after OMIP 017, includes chemokine receptors CD196, CD183, CCR10, CD161 and CD194 for identification of Th1, Th17, Th2, Th9, and Th22 subsets. Of the 43 subsets measured, similar frequencies were obtained for NHD PBMC and Veri-Cells™ across 30 populations (70%). Veri-Cells™ staining patterns are similar to PBMC; however, greater reproducibility ensures a more reliable biological control for immune monitoring to evaluate overall assay variability and reagent performance.

T.33. BLISS: Development of a Non-radiation Based Assay to Determine Glucocorticoid Sensitivity in Healthy Volunteers

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More than half a century after their discovery, glucocorticoids remain the first-line treatment for almost all non-infectious inflammatory diseases. However, patient response, in terms of both efficacy and side-effects, is highly variable. Key to personalised immunotherapy is biomarker development that determines glucocorticoid responsiveness, aiming to avoid the iatrogenic harm currently resulting from repeated empirical cycles of treatment. Preliminary data in a range of conditions indicates that *in vitro* suppression of patients' lymphocyte proliferation using the synthetic glucocorticoid dexamethasone, correlates with their *in vivo* clinical glucocorticoid sensitivity. However, these studies quantify cell division using tritiated thymidine, and this reliance on radiation is impractical for translation to a clinical laboratory setting. We therefore sought to evaluate an alternative non-radioactive colorimetric method detecting the incorporation of bromodeoxyuridine (BrdU), a synthetic nucleoside analogue, into proliferating lymphocytes. This BrdU lymphocyte incorporation of steroid sensitivity (BLISS) assay was compared with the radioisotope based gold standard (DILPA) in 101 healthy individuals. The quantification of lymphocyte suppression with dexamethasone using both techniques correlated well (Pearson's correlation coefficient of 0.66; $p<0.0001$). Categorical data was analysed in a contingency table, calculating the sensitivity, specificity, and positive and negative predictive values of BLISS against DILPA for the diagnosis of steroid resistance. Fisher's exact test showed a close association ($p<0.0001$) with a negative predictive value of 84%. BLISS intraexperimental and repeated measures also showed minimal variation. These data demonstrate that the BLISS assay is a non-radioactive alternative for quantifying steroid sensitivity. Its utility now needs to be validated in prospective clinical studies.

Immunity and Infection

OR.05. Generation of Pro-inflammatory B Cell Subsets in Aging Mice and Humans can be Assisted by Visceral Adipose Tissue

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B cell function is decreased with age and associated with increased chronic low-grade systemic and metabolic inflammation and with an increase in visceral adipose tissue (VAT). We have previously shown that an optimal influenza vaccine response is associated with higher percentage of switched memory B cells and decreased late exhausted B cells which produce inflammatory cytokines. Chemokines from adipocytes are involved in the chemotaxis of immune cells which infiltrate the VAT and contribute to the inflammatory process. To identify contributors to the phenotypic and functional changes observed in aged B cells, we studied B cells in the VAT of obese mice and humans (epididymal and abdominal VAT, respectively). We found macrophages, B cells and T cells in VAT in a ratio of 1:3:5 and higher percentages of pro-inflammatory and lower percentages of anti-inflammatory B cell subsets in VAT as compared to peripheral B cells (from mouse spleen and human blood). B cells isolated from VAT express higher levels of inflammatory immune activation markers (TNF-α/IL-6/IL-8), significantly higher NF-kB activation and phospho-STAT3, and secrete higher amounts of Ig antibodies specific for fat (self) antigens as compared to peripheral B cells. When adipocytes from the VAT were co-cultured with peripheral B cells for 72 hours, a higher percentage of pro-inflammatory B cell subsets, similar to what we have seen in the fat, were observed. Moreover, we found that adipocytes produce several pro-inflammatory chemokines. These results are the first to show a direct effect of adipocytes on pro-inflammatory B cells.

OR.12. Determining Optimal Adjuvanticity of a Q Fever Vaccine Using A Tri-Agonist Compound Library

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Vaccines are powerful tools that both prevent disease outbreak and provide lasting immunity to the vaccinated individual. However, the development of more effective vaccines is still necessary for diseases, such as malaria, HIV, and Q fever. *Coxiella burnetti* is an infectious agent that is the cause of Q fever, a potential bioterrorism agent. Currently, a vaccine for Q fever exists, but the vaccine is only licensed in Australia and concerns about safety and efficacy necessitate the development of a safer and more effective vaccine. Recently researchers demonstrated that administration of a target antigen with multiple immune agonists, especially Toll-like receptor (TLR) agonists, successfully activate the immune system and enhance immune responses to the target antigen. Our lab has developed a novel vaccine adjuvant system by conjugating three TLR agonists together (tri-agonist adjuvant). Using this novel tri-agonist adjuvant construct we probed immune system responses both *in vitro* and *in vivo*. In both settings, we elicited a more balanced Th1 and Th2 innate immune response and successfully increased antibody scope and diversity, suggesting downstream changes in immune signaling and adaptive immune activation. Here, we examined how different covalently linked TLR agonist combinations affect NF-kB activity and cytokine production. Finally, our novel tri-agonist adjuvant construct was used in a Q fever vaccination model to examine changes in immune activation including T cell, antibody, and cytokine responses. Our studies suggest that our tri-agonist adjuvant aids significantly in the design of more effective vaccines.

OR.18. How Does the Addition of Surface Protein Antigens to a Potential Vaccine for *Staphylococcus aureus* Affect the Immune Response it Induces?

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Staphylococcus aureus is a bacterium capable of causing severe human disease for which there is currently no vaccine. This pathogen can evade host immune responses by expressing a polysaccharide capsule which coats the organism, thereby impeding innate recognition of the bacterium. For other bacterial pathogens, capsular polysaccharides (CPs) conjugated to carrier proteins have been highly effective as vaccines for preventing diseases. The mechanism is proposed to be through induction of humoral responses, including memory B cells, which generate antibodies that facilitate the killing of the pathogen via opsonophagocytocis or complement mediated bacteriolysis. A novel *S. aureus* four antigen vaccine (SA4Ag) is in development which, in addition to CP-conjugates, also includes two *S. aureus* surface proteins. The aim of these studies is to evaluate the impact that these protein antigens have on the humoral and cellular immune responses induced by vaccination with CP-conjugates alone. We have employed micro-engraving, a single-cell analysis technique developed by our laboratory, in which antibodies secreted from individual B cells can be screened for antigen-specificity, thereby enabling the quantification, identification, and isolation of antigen-specific memory B cells. Using this approach, together with Luminex technology, opsonophagocytic assays, and flow cytometry, we have measured how the inclusion of these additional protein antigens affects functional properties of circulating antibodies, memory B cell responses, T cell responses, and levels of somatic hypermutation following vaccination of non-human primates with SA4Ag compared to CP conjugates alone.

OR.39. Eros is a Novel Transmembrane Protein Essential for the Phagocyte Reactive Oxygen Burst and Host Defense

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The generation of superoxide and hydrogen peroxide via the phagocyte reactive oxygen burst is crucial for effective innate immunity. The transfer of electrons to molecular oxygen is mediated by a membrane bound heterodimer,

cytochrome b558, comprising gp91 and p22 subunits. Deficiency of either of these subunits in mouse or man leads to severe immunodeficiency that is characterised by susceptibility to bacterial and fungal infection, including *Salmonella* and *Listeria* species. Abnormalities in the reactive oxygen burst have also been linked to autoimmunity and cancer. We show that a novel protein, *Eros* (Essential for Reactive Oxygen Species), encoded by a previously undesrcibed mouse gene is essential for host defense to infection via the phagocyte NAPDH oxidase. *Eros* contributes to the generation of ROS because it is required for the normal expression of the NADPH oxidase components, gp91 and p22. Consequently, *Eros*-deficient mice die rapidly after infection with *Salmonella* or *Listeria*. *Eros* is also necessary for the formation of ROS-dependent neutrophil extracellular traps (NETS) and impacts on the immune response to melanoma metastases. *Eros* is an orthologue of the plant protein Ycf4, necessary for expression of proteins of the photosynthetic photosystem 1 complex, itself also an NADPH oxioreductase. We thus describe the key role of the previously uncharacterized protein *Eros* in host defense.

F.35. Production of Interleukin 10 by T Cells Promotes Systemic *Salmonella* Infection in Mice

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Salmonella enterica serovar Typhimurium (*S.* Typhimurium) produces systemic disease in numerous hosts. In mammals, oral inoculation is followed by intestinal colonization and subsequent dissemination into deeper tissues, which may lead to severe pathogenesis. In this study, we evaluated how the anti-inflammatory cytokine interleukin-10 (IL-10) contributes to *S.* Typhimurium pathogenesis. We assessed the role of IL-10 in *S.* Typhimurium infection *in vivo* using IL-10 knockout mice (IL-$10^{-/-}$) and found that these animals displayed a notorious resistance to the systemic disease, as compared to wild-type mice. IL-$10^{-/-}$ mice had significantly reduced bacterial loads both, in the spleens and livers. Although *S.* Typhimurium survival was reduced within bone marrow-derived macrophages from IL-$10^{-/-}$ mice, restoring IL-10 production by these cells did not result in significant changes in the resistant of IL-$10^{-/-}$ mice to *S.* Typhimurium infection. However, IL-10 production by T cells fully restored the susceptibility of IL-$10^{-/-}$ mice to systemic *S.* Typhimurium infection. These findings support the notion that IL-10 production by T cells is a key process in the infective cycle of *S.* Typhimurium in mice, due to generation of a tolerogenic immune response that favor bacterial dissemination.

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F.36. Effect of Sustained and Intermittent Stimulation for Sleep Deprivation **in IL1, IL6 and TNF α** Cytokines and Mortality in Rats with Endotoxic Shock

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Interrupted sleep, fragmented sleep or restricted sleep is a corollary of many psychiatric, neurological and respiratory disorders and also results from disruptive environments such as that of the intensive care unit (ICU). Animal models have demonstrated that sleep deprivation alone is associated with increased mortality. Septic insult with sleep deprivation results in enhanced mortality in a murine model. Recent rodent studies have revealed that sleep interruption (SI) can have more significant consequences for cognitive and neurophysiological variables than were expected and may even be equivalent to those of total sleep deprivation. Here we describe in detail a method for intermittent but sustained stimulation using light, vibratory and electric stimulation for interrupting sleep in

a rat model and the effect that produce a septic insult in inflammatory cytokines such as IL1, IL6 and TNF α and mortality in rats with and without sleep deprivation using sustained, intermittent stimulation.

Methods: We included four groups of 25 rats each one, distributed as follow; 1 y 2; exposed to endotoxin shock by *Escherichia Coli*; with and without sustained and intermittent stimulation for sleep deprivation, 3 y 4 rats; with and without sustained, intermittent stimulation for sleep deprivation as a controls.

Results: Sustained, intermittent stimulation for sleep deprivation alone increased TNFα e IL-6 similar to rats with endotoxic shock, and synergized the effect of the septic insult. The mortality also increased dramatically.

Conclusions: The sustained and intermittent stimulation for sleep deprivation provokes a synergy deleterious effect in TNF α, IL-6 and mortality in rats with endotoxic shock.

F.37. Association Between STR -794 $CATT_{5-8}$ and the SNP -173 G/C Polymorphisms of the Macrophage Migration Inhibitory Factor (MIF) and Development of Lepromatous Leprosy in Patients from Western Mexico

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Leprosy is caused by *Mycobacterium leprae* that invades Schwann cells in peripheral nerves, leading to incapacities. Lepromatous leprosy (LL) is the most aggressive presentation and the most common in Mexico. LL patients are unable to activate an inflammatory response against *M. leprae* due to host-related reasons. The macrophage migration inhibitory factor (MIF) is one of the first cytokines to trigger inflammatory reactions and it has been shown to be effective against *M. tuberculosis*. Two polymorphisms have been reported for human *MIF:* STR -794 $CATT_{5-8}$ and the SNP -173 G/C. Specifically, 7 to 8 CATT repeats at -794 and the C allele at -173 are associated to higher serum levels of MIF. Therefore, the aim of this work is to find whether the -794 $CATT_{7-8}$ and -173 C polymorphisms in *MIF* gene may provide a protective effect against LL. We carried a case and controls study with 100 LL patients and 100 healthy subjects (HS). PCR was used for genotyping of STR -794 $CATT_{5-8}$ polymorphism and PCR-RFLP for -173 G/C. We measured serum levels of MIF by ELISA. We found that patients carry high -794 CATT repeats (47.1%) significantly more often than HS (32.7%), but we found no differences in -173 SNP genotypes distribution. However, we found a tendency for patients with -173 C/C genotype to show reduced MIF serum levels (37.93 ng/mL) compared to HS with the same genotype (84.17 ng/mL). These results provide insight into novel MIF genetic regulation in leprosy infections.

F.38. The Presence Of Microbiota Protects Against *Pseudomonas aeruginosa* Induced Keratitis

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The impact of microbiota on ocular immunity is unclear. Here, we report that in health, the presence of microbiota strengthens the ocular innate immune barrier function and promotes resistance to pathogenic insult. Consistent with this view, Germ-Free (GF) mice on a SW background that are typically resistant to *P. aeruginosa*-induced keratitis were rendered susceptible by the lack of microbiota. This was exemplified by increased corneal bacterial burden and elevated pathology of the GF mice when compared to the conventionally maintained specific pathogen free (SPF) SW mice. The elevated susceptibility to the infectious challenge correlated with significant decrease in the antimicrobial capacity of neutrophils derived from the GF SW mice against *P. aeruginosa*. Oral antibiotic treatment of SPF SW mice reduced the ability of neutrophils to kill *P. aeruginosa in vitro* and *in vivo*, demonstrating that microbiota-derived cues maintain neutrophil priming in the bone marrow against this specific pathogen. In contrast, topical antibiotic treatment reduced ocular commensal presence at the conjunctival surface, but did not alter the resistance to infection. Reconstitutions of GF mice with either mouse or human-derived gut microbiota restored the resistance to the infectious ocular challenge. To determine the mechanisms of commensal priming, RNAseq

experiments comparing the phenotype of GF-derived neutrophils and SPF-derived neutrophils were carried out and showed significant alterations in type I interferon-dependent gene expression profiles, implicating IFN-dependent pathways in regulating the microbiota-dependent neutrophil maturation. Indeed, type I interferon priming of GF mice reconstituted neutrophil bactericidal activities against *P. aeruginosa*.

F.39. The CRISPR/Cas9 System as an Anti-Viral Treatment to Prevent Primary Infection by Human Cytomegalovirus Positive Hematopoietic Stem Cells

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HCMV, a beta-herpes virus, induces a lifelong latency in hematopoietic stem cells (HSC) after an asymptomatic primary infection. After a HSC transplantation HCMV-positive donor cells can infect the HCMV-negative recipient, which might lead to end-organ diseases. The current available anti-viral treatments cause side effects and are not able to target HCMV in latent state.

We used the CRIPSR/Cas9 system to directly alter the HCMV genome in donor cells. Therefore we knock-out the immediate early gene (IE) encoding essential viral proteins for lytic replication and end of latency.

We transduced low HCMV-permissive U373-MG cells with a lentiviral vector encoding the Cas9-GFP and either one (singleplex) or three (multiplex) gRNA targeting the IE gene. We FACS-sorted those cells based on their Cas9-GFP expression and infected them with laboratory or clinical HCMV strains.

After infection with TB40 or Toledo, IE-positive cells show a reduction of 50% with the singleplex and of 75% with the multiplex strategy. Infection with the clinical strain VR1814 is even more affected by singleplex and multiplex strategies, reaching 70% and 80% reduction respectively. Viral genome analysis in singleplex U373-MG show mutations at the target site with a frequency of up to 70%. Furthermore we observe a decrease of new virion release especially with the multiplex strategy of about 99% in comparison to untreated controls. Finally, those observations were not made with cells expressing an unspecific gRNA/Cas9.

We are now planning to test this strategy on HCMV latently infected $CD34^+$ hematopoietic stem cells to prevent viral reactivation during HSCT.

F.40. Promotion of Filamentous Growth in *Candida albicans* by *Pseudomonas aeruginosa* Secretions: Identification of Novel Players in Cross Kingdom Communication

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Pseudomonas aeruginosa (PA) and *Candida albicans* (CA) are commonly found together *in vivo* with the antagonistic or synergistic nature of their interaction still under debate. We have developed an assay to study the effect of PA secretions on CA growth. Our data suggest that wild type (wt) PA secretions induce filamentation of CA, a key virulence attribute of this fungus. Using appropriate PA mutants, we have shown that this observation is not due to the production of rhamnolipids, exotoxin A, or dependent on the virulence regulator *ToxR*. Interestingly, promotion of filamentous growth does not take place when using supernatants from a PAO1 mutant having a spontaneous 58-Kb chromosomal deletion (PAO1-LΔ58Kb), indicating that the gene(s) responsible for promoting hyphal growth in CA is/are encoded within this region.

Both biochemical and genetic approaches are being exploited to determine the nature of the active components and mechanism of action. Activity is eliminated by heat (95°C, 10 min), showing the possibility of the hyphal-inducing

agent being a protein. Using Amicon® Ultra-15 centrifugal filters to fractionate PA supernatants; the CA hyphal induction activity was retained in ultrafiltrates of molecular weights above 10 kDa, which is in agreement with preliminary SDS-PAGE analysis showing the presence of two 8– 12 kDa bands in the wt supernatants. Considering the influence of cross-kingdom communication on microbial pathogenesis and the difficulty in treating polymicrobial infections, this work presents new insights into the interactions that occur between PA and CA during co-infections in Cystic Fibrosis patients and burn/trauma victims.

F.41. Gestational Hypothyroxinemia Causes Poor Viral Immunity In The Progeny

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Hypothyroxinemia is a clinical condition characterized by low levels of T_4 and T_3, and normal levels of TSH. During pregnancy, only T4 can cross the placenta and reach the fetus. Previous studies show that hypothyroidism and gestational hypothyroxinemia early can cause an irreversible damage to the central nervous system (CNS) of the fetus. Furthermore, maternal hypothyroxinemia can significantly increase the severity of autoimmune encephalomyelitis experimental in the progeny. Since gestational hypothyroidism alters immune population of the progeny, here we evaluated whether maternal hypothyroxinemia (Hpx) can influence the immune response against viral infections in the progeny. With this aim, we induced gestational hypothyroxinemia (Hpx) to pregnant female mice by administrating 0.02% methimazole in the drinking water during days 10-15 of pregnancy. After 6 to 8 weeks, the progeny gestated by control or hypothyrocinemic mothers were infected intranasally with 10^5 PFU of respiratory syncytial virus (RSV). We observed that the progeny gestated hypothyroxinemic mothers showed greater weight loss as compared to control mice. With respect to the viral load, the mice the animals were sick by RSV, but did not differ in their viral loads between the progeny gestated in hypothyroxinemic mothers and in control mothers. Furthermore, as compared to control mice, animals that were gestated by hypothyroxinemic mothers showed increased infiltration of neutrophils, which correlated with higher clinical scores in the lungs after hystopathology analyses. Furthemore, the progeny gestated under hypothyroxinaemia showed a significant reduction in the lung infiltration of $CD8^+$T cells, as well as in the production of gamma interferon.

F.42. Heme Oxygenase-1 Reduces The Viral Replication And Lung Diseases After Human Respiratory Syncytial Virus infection

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Human respiratory syncytial virus (hRSV) is the principal cause of severe lower respiratory tract infection in childhood. HRSV infection alters the production of a proper adaptive T cell response by blocking the immunogenicity of dendritic cells (DCs). However, the pathological mechanisms by which hRSV impairs DC activity still remain unknown. Our group evaluated the role of Hemeoxygenase-1 (HO-1), a potent cytoprotective and anti-inflammatory host enzyme, during hRSV infection and their effect in the development of viral-associated immunopathology. Our data suggest that hRSV induces HO-1 expression in DCs, a condition that favor hRSV replication in these cells, since treatment of DCs with Cobalt protoporphyrin IX (CoPP), an inducer of HO-1, increases hRSV-N replication. However, *in vivo* CoPP-mediated HO-1 induction aided mice in recovering body weight and reduced viral replication in their lungs after hRSV infection. Likewise, CoPP-mediated HO-1 induction in the A549 carcinomic human alveolar basal epithelial cell line reduced hRSV replication. These results indicate that HO-1 induction affects the replication of hRSV positive or negatively, depending on the cell type, which is infecting. In addition, these data suggest that CoPP could be used as a therapeutic treatment to ameliorate hRSV infection in lungs and disease.

F.43. A Single, Low Dose of a Current Good Manufacturing Practice (cGMP) Recombinant BCG Vaccine Confers Protection Against Human Respiratory Syncytial Virus (hRSV) infection and Lung Pathology in Mice
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Human respiratory syncytial virus (hRSV) is a major health burden worldwide, causing most of the hospitalizations due to bronchiolitis and pneumonia in children below the age of two years. HRSV causes year-to-year outbreaks of disease, which also affects the elderly and immunocompromised individuals. Furthermore, both hRSV morbidity and epidemics are explained by a consistently high rate of re-infections that could be established throughout the host life. Importantly, currently there are no licensed vaccines for the prophylaxis of this important human pathogen. Here, we describe a novel, recombinant Bacillus Calmette-Guerin (BCG) vaccine expressing the nucleoprotein (N) of hRSV (herein rBCG-N) and its protective capacity in the BALB/cJ model of infection. A single dose of 3×10^5 cfu of the rBCG-N vaccine protected mice against infection with 1×10^7 pfu of the 13018-8 hRSV A2 strain. Compared to infected controls, vaccinated mice displayed reduced weight loss and infiltration of neutrophils within the airways, as well as reduced viral loads in bronchoalveolar lavages. Interestingly, vaccinated mice displayed increased activation of T cells within the airways and no significant antiviral antibodies at the time of sacrifice, which suggested that the rBCG-N vaccine induced a strong antiviral T cell immunity. Indeed, *ex vivo* re-stimulation of splenic T cells at 28 days post-vaccination activated a repertoire of T cells secreting IFN-gamma and IL-17, which further suggest that the rBCG-N vaccine induced a mixed, $CD8^+$ and $CD4^+$ T cell response capable of both restrain viral spread in the lungs and prevent the pulmonary pathology.

F.44. IL-2 Production by Activated Naïve and Effector CD8 T Cells Predicts Memory Formation
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IL-2 acts as a differentiation factor that drives effector CD8 T cell formation and also programs robust memory recall responses. However, the roles of intrinsic IL-2 production in shaping effector and memory CD8 T cell differentiation remain uncertain. To address this, we utilized novel IL-2 and IFN-γ double cytokine reporter systems to trace the cytokine production profiles and fate decisions of newly activated naïve, effector, and memory virus-specific CD8 T cells. We discovered that, within hours of activation, recently activated naïve CD8 T cells segregate into populations that produce only IL-2, only IFN-γ, co-produce IL-2 and IFN-γ, or do not produce either cytokine. Later, as effector and memory CD8 T cells develop, a shift towards IFN-γ production occurs with a smaller subset capable of co-producing IL-2 and IFN-γ. Newly activated naïve CD8 T cells that fail to produce IL-2 and IFN-γ are biased to adopt effector attributes. Conversely, activated naïve and effector CD8 T cells that produce IL-2 preferentially develop memory traits including the ability to mount robust recall responses. Notably, recently activated naïve CD8 T cells that produce IL-2 appear refractory to signaling through their STAT5 pathways, which potentially directs their developmental choices. Although the properties of IL-2-producing and non-producing effector cells are distinct, their established memory counterparts are more similar and elicit equivalent secondary responses. Together, these data illustrate that the intrinsic ability to produce IL-2 can dictate and predict the formation and protective efficacy of CD8 T cell subsets.

F.46. Novel Neutrophil Models for the Study of Host-pathogen Interactions
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Neutrophils are a critical first line defense against bacterial and fungal infections. The study of neutrophil function has been a challenge owing to their short life-span following isolation and the inability to genetically manipulate neutrophil

function *in vivo* or *ex vivo*. In this abstract, we describe the use of conditionally immortalized granulocyte-macrophage progenitor (GMP) cell lines that are capable of unlimited expansion and which are genetically tractable.

These GMP cell lines were derived from mice through the transduction of a MSCVneo ER-HoxB8 retrovirus such that HoxB8 factor-dependent GMP cell lines emerge in the presence of stem cell factor (SCF) and beta-estradiol (E2). Upon removal of E2, these GMP cells differentiate to fully functional neutrophils over 4-5 days.

To confirm mature terminal neutrophil function Mac-1-high and kit-low expression was determined by flow cytometry. Using confocal microscopy, HoxB8-derived neutrophils were capable of generating neutrophil extracellular traps (NETs). In addition, mature HoxB8-derived neutrophils produce equivalent superoxide, and are capable of chemotaxis similarly to primary bone-marrow derived neutrophils. More importantly, these HoxB8-derived neutrophils can phagocytose and eliminate the human fungal pathogens, Candida glabrata and Candida albicans, as evidenced by *in vitro* fungal colony assays and viability indicators. Of particular note, transplantation of these cells into neutropenic mice challenged with lethal Candida inoculum showed prolongation of survival.

Overall, HoxB8-derived neutrophils represent a biological model of neutrophil biology, an inexhaustible and genetically tractable supply of cells that should provide the field of immunology with a much-needed tool for the study of neutrophil pathogenesis.

F.47. Chemokines and Their Function in Neonatal Immune Responses

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Background: Neonates and young infants are at a higher risk of infections. The human neonatal immune response is functionally distinct from that of adults. Newborns rely heavily on innate and mucosal immunity to defend against infections. Previous work has revealed developmental-specific innate-like functional features of naïve $CD4^+$ T cells. However, it is unclear whether other adaptive immune mechanisms may compensate for attenuated innate immune functioning in early life.

Methods: To identify neonatal naïve T cell-specific cell surface markers and cytokine signatures, we analyzed published and our genome wide datasets of differential gene expression comparing cord blood and adult peripheral blood naïve $CD4^+$ T cells ($CD3^+CD4^+CCR7^+CD25^-CD45RO^-$) at baseline and following *in vitro* stimulation. Candidate cytokine genes were validated at the protein level by flow cytometry and ELISA.

Results: We found that CXCL8 is strongly expressed by neonatal T cells. By contrast, we found that CXCL16 is abundantly expressed in both neonatal and adult T cells. In addition, CCL20 RNA expression was elevated in neonatal T cells although CCL20 protein could not be detected by ELISA or flow cytometry. Several potential surface markers specific to neonatal (naïve) $CD4^+$ T cells were identified using this approach.

Conclusions: Further experiments are required to demonstrate the functional roles of the chemokines produced by neonatal T cells, and to address the possibility that distinct CCL20 protein isoforms may be expressed by neonatal T cells. Our combinatorial approach will facilitate a comprehensive functional characterization of neonatal $CD4^+$ T cells.

F.48. A New Saponin Based Adjuvant Candidate (Astragaloside VII) for Viral Vaccines

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The majority of the vaccines require association with adjuvants capable of increasing the potency or stimulating the appropriate immune response. In the present study, adjuvant potential of an immunomodulator saponin, namely Astragaloside VII (Ast VII) from *Astragalus trojanus* Stev., on the cellular and humoral immune responses of Swiss albino mice against model viral antigen Newcastle Disease Virus (NDV) was investigated. Swiss albino mice were immunized subcutaneously with live 6 log10 EID50 LaSota strain alone or with Ast VII (120 μg) on Day 1. A boosting injection with inactivated 6 EID50 LaSota containing Ast VII (120 μg) formulated with Astragalus polysaccharide, squalene or monophosphoryl lipid A (MPL) were given 2 weeks later. Splenocytes and sera were collected 6 weeks after the second immunization for splenocyte proliferation assay and measurement of antibody and serum IL-2, IFN-¥ and IL-4 cytokines level by ELISA. NDV-specific IgG, IgG1 and IgG2b antibody titers and IFN-¥, IL-2 in serum levels were notably enhanced by Ast VII (120 μg) alone and Ast VII (120 μg) formulation with polysaccharide, squalene or MPL as an adjuvant compared to the commercial vaccine. Ast VII also enhanced the Concovalin A and NDV induced splenocyte proliferation in all combinations. Ast VII significantly stimulates specific antibody and cellular responses via Th1-dependent pathway, verifying its potential to be used in NDV based viral vaccines as an adjuvant. Based on the promising results, we have decided to design further studies to see Ast VII's adjuvant potential in human seasonal influenza viral vaccines.

F.49 Ccdc88b is a Novel Regulator of Maturation and Effector Functions of T Cells During Pathological Inflammation

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Inflammation is a normal protective physiological response to infection, tissue injury and organ malfunction. Dysregulated inflammation causes debilitating pathologies such as cerebral malaria (CM), sepsis, inflammatory bowel diseases (IBD), arthritis and asthma. Inflammatory diseases remain a significant burden on human health worldwide, due to a lack of understanding of the inflammatory mechanisms involved. Several anti-inflammatory drugs exist (steroids, and NSAID), but they are not completely effective and have serious side effects. Cerebral malaria (CM) is a significant threat to global health with ~1.5 million deaths per year, mostly in children under the age of 5 and pregnant women. CM is caused by an inflammatory response triggered by the trapping of *Plasmodium*-infected red blood cells at the blood brain barrier. In susceptible mice, CM can be induced by infection with *Plasmodium berghei* ANKA (PbA), where infected mice succumb from severe cerebral symptoms such as shivers, paralysis, coma, and ultimately death. Our lab and others have shown that mutations in pro-inflammatory genes such as TNFα and IFNγ, as well as transcription factors that regulate their expression protect mice from CM.

We have set-up a genome-wide mutagenesis screen in mice to identify genes and pathways, that when mutated, protect animals from lethal neuroinflammatory diseases such as CM. We found a mutation in the gene, Coiled-Coil Domain Containing protein 88b (*Ccdc88b*), has been identified as conferring resistance to CM in mice. Although the function of *Ccdc88b* is unknown, it is expressed primarily in T cells of wildtype animals but not in mice that have the mutation giving insight to a protective effect in mice normally susceptible to CM. Further more, analysis has shown that mice with this mutation have impaired T cell maturation, significantly reduced T cell activation and impaired cytokine production. Currently, these findings show that *CCDC88B* is a novel regulator of maturation and effector function of T cells during inflammation. The human *CCDC88B* gene maps to a portion of chromosome 11 that is associated with susceptibility to several inflammatory and auto-immune disorders. Our findings strongly suggest that CCDC88B is the morbid gene underlying the effect of the susceptibility locus on inflammation. Discovering genes and proteins that play a key role in the host pathological inflammatory response will provide targets for intervention in CM, and shed light for anti-neuroinflammatory drug discovery.

F.50 The Effect Of Plasmodium Berghei Infection on Bcg-induced Memory Responses Against

Mycobacterium Tuberculosis in Wild Type C57BL/6 Mouse

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Tuberculosis (TB) remains a major global health threat. BCG vaccination against TB is a widely used strategy to control TB; however, it is known to show variable efficacies in pulmonary TB. A possible reason could be coinfections with parasites including Plasmodium. While previous studies in mouse pulmonary TB on the less virulent strain, *Plasmodium yoelli* have indicated that malaria infections do not abrogate BCG-induced immune responses against *Mycobacterium tuberculosis*, it is not certain what the effect of the more virulent strain *Plasmodium berghei* would be.

The aim of the study was to investigate the effect of *P. berghei* infection on BCG vaccination in wild type C57BL/6 mice and to elucidate the immune mechanisms involved.

C57BL/6 mice were infected with *P. berghei* six weeks after BCG vaccination. They were killed at various time points and the spleens collected and stained for analysis by flow cytometry.

It was observed that *Plasmodium berghei* infection induces a time-dependent destruction of the central memory T cells ($CD44^{+}CD62L^{hi}$) and the marginal zone B cells ($B220^{+}AA4.1\text{-}CD1d^{lo}$). There was a strong effector T cell ($CD44^{+}CD62L^{lo}$) response following *P. berghei* infection which was enhanced by BCG vaccination. It remains to be confirmed whether loss of central memory T cell results in loss of protection against *Mycobacterium tuberculosis.*

Therefore, malaria may hamper the immunity induced by BCG and the effect is virulence driven. Further investigation on the mechanisms involved would suggest new strategies to the search for more effective vaccines that could withstand the pressures from coinfections.

F.51. Comparative Study of Histopathological Characteristics in Experimental Periodontitis Previous and During Pregnancy in a Murine Model

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Periodontitis is a chronic inflammatory disease caused by bacteria (oral biofilm) that affects dental support tissues (periodontium), producing bleeding and gradual bone loss. This affects over 23% women in reproductive age, and increases up to 56% in pregnant women. Pregnancy modifies clinical, cytological and microbiological aspects due to sexual hormones which are able to alter tissue responses to oral biofilm. The relationship of cytokine expression in pregnant women and periodontitis development has not been stablished punctually. Elevated levels of Th2 cytokines as IL-6 and IL-10 found in pregnant women could favor disease progression. In this study, using 7 weeks old BALB/c female mice, we studied 4 experimental groups to stablish the pregnancy-periodontitis relationship; 1) Experimental Periodontitis (EP) on pregnant mice, 2) EP on non-pregnant mice, 3) pregnant mice (control 1) and 4) non pregnant, non-EP (control 2). We register clinical and histological changes in all groups and measured serum levels of Th1 and Th2 cytokines. We associated periodontitis development and severity with cytokine profile expression. Severe histological alterations in junction epithelium integrity in EP-pregnant mice were observed compared with EP non-pregnant mice. The EP groups (pregnant or not) had higher levels of TNF-α, IL-6 and IFN-γ compared to controls (pregnant or not without EP). Non differences in IL-10 expression were observed between groups. In conclusion, the cytokine profile developed due pregnancy condition favors the severity of experimental periodontitis, in a murine model.

F.52. Cortisol Enhances the Gamma Interferon Stimulated Pro-inflammatory Response of Human Monocytes

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Background: Recent studies show that glucocorticoids (GCs) can augment innate immune inflammatory responses (1). especially those of monocyte/macrophages. We previously reported that high concentrations of GCs enhance the gamma interferon (IFNg) augmentation of human monocyte immunoglobulin G Fc receptor expression (2). Recent reports show that physiologic concentrations of GCs also augment the innate immune response (3).

Methods: With IRB approval, human monocytes were cultured for 16 hours with or without GMCSF (100pg/ml), IFN-g (20ng/ml) and a physiologic (50nM) or pharmacologic (1uM) concentration of cortisol. Lipopolysaccharide (LPS, 1ng/ml) was then added and supernatant collected after 4hr for IL-6 ELISA assay.

Results: Eight experiments from 4 donors (2m:2f) showed: Cells cultured with IFNg or GMCSF increased IL-6 release compared to controls ($p<0.005$). IFNg and GMCSF together did not augment IL-6 beyond either alone. Cells co-cultured with 50nM or 1uM cortisol significantly increased IL-6 release in the presence of GMCSF alone (1366pg/ml 50nM cortisol and 1498pg/ml 1uM cortisol vs. 542pg/ml for GMCSF alone; $p<0.05$ and $P<0.005$, respectively). In the presence of IFNg and GMCSF, cells co-cultured with cortisol significantly increased IL-6 release (1892pg/ml 50nM cortisol and 3155pg/ml 1uM cortisol vs. 764pg/ml for IFNg/GMCSF; $p<0.05$ and $P<0.001$, respectively). GC alone did not significantly affect IL-6 release.

Discussion: GCs are not uniformly anti-inflammatory. GCs can enhance IFN-g induced augmentation of LPS-stimulated inflammation in GMCSF-cultured monocytes. The mechanisms for these observations warrant investigation.

References: 1) Brain Behav Immun; 24:17-18, 2010. 2) J Immunol; 138:3235-41, 1987. 3) Crit Care Med; 37:2727-32, 2009.

F.53. Preoperative Glucocorticoid Does Not Alter *In Vivo* Clearance of Bacteria in a Rodent Model of Surgical Site Infection

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Background: Glucocorticoids (GCs) are often administered before and during surgery for their anti-inflammatory, analgesic and/or anti-emetic effects. Concerns remain that GCs may increase infection risks due to immunosuppression[1]. We examined the effect of a single preoperative GC injection on *in vivo* clearance of S. *aureus* bacteria from a surgical incision.

Methods: Following IACUC approval, male C57/Bl6 mice were given corticosterone, 5mg/kg, or saline subcutaneously (n=10/group). They were anesthetized and the nape of the neck shaved, sterilized and a 1cm full thickness skin incision was made. The wound was inoculated with three $5x10^6$ bioluminescent S. *aureus*(strain SH1000) in 5ul. Injections were made into dermis on each side of the wound and at the wound base. The incision was closed with a nylon mattress suture. Wounds were imaged serially for photon emission (Xenogen, Caliper Life Sciences) as a quantitative measure of viable bacteria[2].

Results: Average radiance ($p/cm^2/sec$) was the same in control and treatment groups at 8 (5.5+/-4.3 vs. 6.0+/-5.7), 24 (9.1+/-6.4 vs. 8.6+/-7.4), 48 (8.5+/-8.8 vs. 6.9+/-5.6) and 72 (4.4+/-3.9 vs. 5.4+/-4.6) hours. Total radiance (p/sec) was also no different at these time points. No wound showed evidence of abscess formation or wound breakdown.

Discussion: GCs are widely administered to patients who undergo high-risk surgery such as colorectal and cardiac surgery. Concerns remain regarding the potential immunosuppressive consequences of GCs. This study did not show any effect of a single GC injection on clearance of bacteria from an experimentally infected wound.

References: 1) *Acta Anaesth Scand*:57;823-34, 2013. 2) *J Invest Derm*:131;907-15, 2011.

F.54. Dll4/Notch regulation of RSV-induced immunopathology through T_{reg} Cell Development

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Respiratory Syncytial Virus (RSV) infection is the most common and severe infection in pediatric clinic. Regulatory T cells (T_{reg}) prevent RSV-induced immunopathology. Recent studies have shown that one Notch ligand— Delta-like ligand 4 (Dll4) was up-regulated on antigen presenting cells after RSV infection, but the role of Dll4 in T_{reg} development during RSV infection is still unknown. Here we found that Dll4 was expressed on $CD11b^+$ myeloid DC in the lung and draining lymph nodes after RSV infection. Dll4 neutralization exacerbated RSV-induced mucus production and mononuclear cells infiltration in lung accompanied by a decreased of $CD62L^{hi}CD44^{lo}Foxp3^+$ central T_{reg} cells in draining lymph node. In addition, examination of $Foxp3^+$ T_{reg} cells showed a decrease in inhibitory molecules OX40 and ICOS after Dll4 blockade during RSV infection. Finally, Dll4-exposed iT_{reg} cells maintained more $CD62L^{hi}CD44^{lo}$ central phenotype, increased Foxp3 expression, and became more suppressive *in vitro*. These results suggest that Dll4 may function to maintain a central T_{reg} cell phenotype and control RSV infection.

Immunodefiency- Primary or Acquired

OR.07. Clinical Laboratory Standard Capillary Protein Electrophoresis Alerted of a Low C3 State and Lead to the Identification of a Factor I Deficiency Due to a Novel Homozygous Mutation

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Introduction: Complement factor I (CFI) deficiency is associated to recurrent infections by encapsulated microorganisms, and, less commonly to autoimmunity. This study describes a novel mutation in CFI causing complete factor I deficiency leading to low C3 that was detected because the capillary protein electrophoresis (CPE) in the serum of a patient that was being investigated for a unrelated condition, lead to CFI suspicion.

Materials, methods and results: A serum sample from of a 53-year-old male patient being investigated for benign non-alcoholic fatty liver disease (NAFLD) was found to lack of beta-2 peak. C3 was markedly reduced and the functional activity of the classical pathway was very low. Common causes of low C3 were excluded and complement regulatory factor I was undetectable. The patient had and infections record that included an episode of invasive meningococcemia at the age of 20 years and that there was consanguinity. Sequencing of the CFI gene revealed a novel mutation consisting in the homozygous deletion of 5 nucleotides in exon 12 causing a frameshift that lead to a truncated protein. Both his mother and his son were heterozygous carriers.

Conclusion: Serum protein profiling by CPE as used now in clinical laboratories can alert of possible states of low C3, which, once confirmed and common causes ruled out, can lead to CFI and other complement deficiency diagnosis. This is important since they constitute a still underestimated risk of invasive meningococcemia that can be greatly reduced by vaccination.

OR.08. Rapamycin Empowers the Suppressive Activity of FOXP3-mutated T Regulatory Cells In Atypical Immune Dysregulation Polyendocrinopathy Enteropathy X-Linked (IPEX) Syndrome

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Immune-dysregulation, Polyendocrinopathy, Enteropathy, X-linked (IPEX) syndrome is a lethal polyautoimmune disease caused by mutations in FOXP3, resulting in the dysfunction FOXP3$^+$ regulatory T cells (Tregs). Along with increasing awareness of the disease, late/atypical presentations have been reported. Due to severe gastritis with mucosal inflammatory infiltrates and high serum levels of anti-harmonin-autoantibodies (HAA), IPEX syndrome was suspected in a 10-year-old boy presenting with decreased growth and recurrent vomiting. Detection of the c.210+1G>C FOXP3 gene mutation confirmed diagnosis. The percentage of CD4$^+$FOXP3$^+$ T cells was low and *in vitro* Treg suppressive ability was almost absent. Under rapamycin treatment the patient had a rapid clinical improvement and FOXP3 expression in peripheral and gut-infiltrating Tregs increased, although it never reached normal levels. Notably, the frequency of thymic-derived Tregs, as determined by Treg-cell-specific-demethylated-region (TSDR) analysis, did not increase, suggesting that Rapamycin induced an increase of peripheral Tregs *in vivo*. Patient's Tregs induced under Rapamycin treatment showed restored *in vitro* function and their suppressive ability was partially dependent on TGF-β1. With the exception of low CD39, normal expression of Treg-associated molecules (including CTLA4, TIGIT, LAG3 mRNA, Helios and CD62L) was detected. The presence of several aberrant FOXP3 mRNA isoforms with N-terminal truncation was detected in PBMC therapy. The patient is now clinically stable with no additional autoimmune manifestations, although the presence of elevated HAA persists. In conclusion, we report a case of IPEX in which clinical response to Rapamycin is associated with improved Treg function, not previously reported in the presence of FOXP3 mutations.

OR.19. Intestinal Microbiota Sustains Inflammation and Autoimmunity Induced by Hypomorphic RAG Defects

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Hypomorphic Rag mutations cause Omenn Syndrome (OS) characterized by a profound immunodeficiency associated with autoimmune-like manifestations. Both in humans and mice, OS is mediated by oligoclonal activated T and B cells. The role of microbial signals in disease pathogenesis is debated. We found that Rag2R229Q knock-in mice developed an IBD-like disease affecting both the small bowel and colon. Lymphocytes were sufficient for disease induction, as intestinal CD4 T cells with a Th1/Th17 phenotype reproduced the pathological picture when transplanted into immuno-compromised hosts. Moreover, oral tolerance was impaired in Rag2R229Q mice and transfer of wild-type Tregs ameliorated bowel inflammation. Mucosal IgA deficiency in the gut resulted in enhanced absorption of microbial products and altered composition of commensal communities. The Rag2R229Q microbiota further contributed to the immunopathology since its transplant into wild-type recipients promoted Th1/Th17 immune response. Consistently, long-term dosing of broad-spectrum antibiotics in Rag2R229Q mice ameliorated intestinal and systemic autoimmunity by diminishing the frequency of mucosal and circulating gut-tropic CCR9$^+$ Th1 and Th17 T cells. Serum hyper-IgE, a hallmark of the disease, was also normalized by antibiotic treatment, indicating that intestinal microbes may play a critical role in the distinctive immune dysregulation of OS. Interestingly, skin inflammation was not affected by the antibiotic treatment. Further studies will clarify whether an independent pathogenetic mechanism sustains tissue inflammation at this site and/or whether other resident communities of indigenous microorganisms shape local immune cell differentiation and similarly influence the host health.

OR.37. X-linked Neutropenia Caused by Overactive Mutations in the Wiskott-Aldrich Syndrome Protein Renders Neutrophils Hyperactive

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The gene encoding the Wiskott-Aldrich syndrome protein (*WASp*) is highly expressed and upregulated during neutrophil maturation. Gain-of-function mutations in *WASp* that destroys the auto-inhibited conformation cause a rare form of X-linked neutropenia (XLN). Although neutrophil granulocytes are clearly affected in XLN patients, it remains largely unknown what role WASp plays in neutrophil development and function. Here, we generated two new mouse models that express the XLN patient mutations WASp-L272P and WASp-I296T and compared neutrophils from these mice to WASp-deficient neutrophils. We found that several neutrophil functions, such as chemotaxis, phagocytosis, adhesion, degranulation, and reactive oxygen species (ROS) production were regulated by WASp. Surprisingly, while WASP-deficient neutrophils exhibited defective actin polymerization, intracellular ROS production, phagocytosis, and chemotaxis, XLN neutrophils showed increased polymerized actin, intracellular ROS, phagocytosis rate, and chemotaxis. In the competitive setting of bone marrow chimeric mice, WASp-deficient neutrophils had a selective disadvantage when competing with wildtype cells. In contrast, XLN neutrophils had a clear advantage over wildtype neutrophils in entering peripheral tissues in naïve mice and under sterile inflammation. These data indicate that there are unique requirements for the presence and activation status of WASp in neutrophils and that activating mutations in WASp render neutrophils hyperactive.

OR.49. Clinical and Structural Impact of Mutations Affecting the Residue Phe367 of FOXP3 in Patients with IPEX Syndrome

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Immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome is a monogenic autoimmune disease characterized by early-onset life-threatening multisystemic autoimmunity. This rare hereditary disorder is caused by loss-of-function mutations in the gene encoding the forkhead box P3 (FOXP3) transcription factor, which plays a key role in the differentiation and function of $CD4^+CD25^+$ natural regulatory T cells (Tregs), essential for the establishment and maintenance of natural tolerance. We identified a novel mutation in the *FOXP3* gene affecting the Phe367 residue of the protein (F367V) in a family with three male siblings affected by IPEX. Two other mutations affecting the FOXP3 Phe367 residue (F367L and F367C) have been described previously. This unique situation of three mutations affecting the same residue in FOXP3 led us to study the molecular impact of these mutations on the structure of FOXP3 protein. Structure analysis showed that Phe367 is involved in a rich interaction network related to both monomer and dimer structure stabilization, and is crucial for FOXP3 regulatory activity. In addition to these findings, we provide an explanation for discrepancies between PolyPhen-2 and SIFT computational pathogenicity predictions for the F367V mutation. In summary, as assessment of the pathogenicity of a novel mutation is crucial to achieve a proper molecular diagnosis, we analysed the impact of mutations affecting the Phe367 residue using a combined approach that provides a mechanistic view of their pathogenic effect.

T.43. Case Series Highlighting the Variability in Initial Presenting Symptoms of Chronic Granulomatous Diseas

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Chronic granulomatous disease is a rare hereditary disorder of the immune system characterized by a deficiency of the oxidative burst function in phagocytes. The disease is inherited in an X-linked (XL) or autosomal recessive (AR) fashion. In 2000, Winkelstein J, *et al*. found a prevalence in the US of 1:200,000-1:250,000 with X-linked inheritance far more common than autosomal recessive. In this review we present two different cases of XL-CGD in male patients who were admitted to the same medical center within months of each other. While both patients have the same immunodeficiency, their presentations and subsequent workups were very different. Patient one, admitted at 19 days of life for fever and leukocytosis was found to have multifocal osteomyelitis on MRI. After weeks of IV antibiotics, patient had persistently elevated inflammatory markers but a negative oncology workup. Finally immunodeficiency workup revealed CGD. Patient 2 was admitted for IV steroids for persistent bloody diarrhea and weight loss despite outpatient immunosuppressive therapy. Working diagnosis was medically refractory IBD, but persistence of symptoms led to further workup and eventual diagnosis of CGD. We wish to highlight the variation in initial presentation of CGD and emphasize the importance of having CGD on the differential diagnosis in practice.

T.44. Primary Immunodeficiency Diseases in Iran: Update Report from the National Registry

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Primary immunodeficiency diseases (PIDs) are a heterogeneous group of inherited disorders which are characterized by variety of clinical manifestations, including infections, autoimmunity, lymphoproliferation, and malignancies. According to the recent classification of PIDs, about 300 different diseases have been described so far.

Iranian Primary Immunodeficiency Registry (IPIDR) was established in 1999 after conducting a students' research group for immunodeficiencies with supervision of clinical immunologists.

One thousand, six hundred sixty-one patients have been registered during last 17 years. Among new patients who have been registered during last 5 years, predominantly antibody deficiencies are the most common PIDs, where one third of cases are included in this category (32%), followed by combined immunodeficiencies (22%), congenital defects of phagocytes (17%), other well-defined syndromes (17%), autoinflammatory disorders (5%), diseases of immune dysregulation (3%), defects in innate immunity (2%), and complement deficiencies (1%). The patients with PIDs in now can benefit from standard and research-based testing and multidisciplinary comprehensive care. Conducting several multidisciplinary and international collaborative projects, holding meetings on immunodeficiencies, improvement in diagnosis and treatment, and establishment of the Iranian primary immunodeficiency association were some of the activities with focus on PIDs during these years. The overall activities in the field of PIDs led to an increased trend in recognition of more patients in the recent years and decreasing diagnosis delay.

It is to be hoped that continuing progress in education, infrastructural facilities and real translational research could lead to higher quality of life of patients with PIDs in the country.

T.45. How to Diagnose Primary Immunodeficiency Diseases?

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Primary Immunodeficiency Diseases (PIDs) are usually presented with one of the following eight characteristic clinical presentations: 1. Recurrent upper or lower respiratory tract infections; 2. Failure to thrive (FTT) from early infancy; 3. Recurrent pyogenic infections; 4. Unusual severe infections; 5. Recurrent infections with the same type of

pathogen; 6. Autoimmune or chronic inflammatory disease and/or lymphoproliferation; 7. Characteristic combinations of clinical features in eponymous syndromes; and 8. a number of characteristic presentations such as angioedema.

Suspicious to a number of certain PIDs could be made according to their clinical phenotypes. Meanwhile the first step in the diagnostic process starts from a limited set of tests, which is available in most hospital, including complete blood count/differential. Specific laboratory tests for each category of defects in the immune system is needed, considering the characteristic clinical presentations; e.g., 1. immunoglobulin assays for antibody deficiency or 2. CH50 and AP(AH)50 assays for complement deficiency in those with recurrent sinopulmonary infections with encapsulated organisms; 3. B- and T- lymphocytes enumeration for combined immunodeficiency in those with FTT or early onset severe infections; 4. chemotaxis, nitroblue tetrazolium (NBT) dye reduction test, dihydrorhodamine (DHR) oxidation test for phagocyte defects in those with recurrent pyogenic infections.

It should be noted that more sophisticated tests, including specific antibody responses to protein or polysaccharide antigens, lymphocyte proliferation tests, advanced immunophenotyping, random migration, phagocytosis, and intracellular microbial killing by phagocytes, and a chemiluminescence assay can be performed in immunological laboratories. Meanwhile the definite diagnosis of PIDs relies on genetic tests.

T.46. Human MDA5 Protects Against Respiratory Infections

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Most acute respiratory infections, which include the common cold, bronchiolitis, and pneumonia, are caused by viruses. By performing genomic, virological, and immunological analyses in a child with an autosomal recessive *IFIH1* mutation who has recurrent respiratory infections, we have identified a key physiological role for MDA5 in innate immunity against human rhinoviruses and respiratory syncytial virus. Our results also suggest that MDA5 participates in innate immunity against human coronaviruses and in the production of natural antibodies against T cell-independent type 2 antigens in viral capsids. Hence, RNA-sensing by MDA5 is important for local antiviral immunity in the human respiratory tract.

Immunodermatology

T.47. Induction of Human $CLA^+CD25^+Foxp3^+$ Regulatory T Cells by Nitric Oxide

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Atopic dermatitis is a prevalent skin inflammatory condition commonly treated by phototherapy despite its deleterious side effects including short-term risk of burning, and long-term premature skin aging and potential carcinogenesis. The molecular pathways responsible for ultraviolet (UV)-induced immunosuppression are not fully understood. UV induces the systemic release of nitric oxide (NO) from the skin, which may participate in phototherapy-induced immunosuppression. Indeed, we show that UVB-irradiation of human volunteers induces the release of NO. *In vitro* studies show NO induces *de novo* regulatory $CD25^+Foxp3^+CD127^{lo}$ T cells from human $CD4^+CD25^-$ T cells.

Moreover, NO promotes expression of the skin homing marker CLA on Tregs, suggesting their preferential migration to the skin. NO uses the sGC-cGMP pathway to promote these NO-induced Tregs. These Tregs are functional as able to suppress proliferation of autologous T cells in a contact dependent manner, and depend on TGFb and PD1-PDL1 interaction, but not on CTLA-4. Overall, our data show that NO is a potent immuno-regulator that likely contributes to the beneficial effect of UV phototherapy, and targeting of the NO pathway may therefore provide an alternative treatment to avoid the deleterious side effects of phototherapy.

T.48. Pathogenicity of Anti-ADAMTS13 Auto-antibodies: Endothelial Damage and Cardiac Injury in a New Mouse Model of Acquired Thrombotic Thrombocytopenic Purpura

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Background and objective: Acquired thrombotic thrombocytopenic purpura (TTP) results from severe deficiency of the von Willebrand factor-cleaving protease ADAMTS13, related to anti-ADAMTS13 auto-antibodies. Endothelial activation is thought to play an additional role in TTP induction, suggesting that endothelial activation may constitute a “second hit” in disease pathogenesis, mainly by exocytosis of multimers of von Willebrand factor (VWF) and formation of thrombi in microvascular territories such as heart. The objective of this work was to develop a new model of TTP to assess the pathogenicity of anti-ADAMTS13 auto-antibodies.

Patients/methods: Anti-ADAMTS13 IgG obtained from patients with acquired TTP were adoptively transferred to C57BL/6 mice, then triggered by injection of 2,000 units/kg of recombinant human VWF (rhVWF).

Results: Administration of human anti-ADAMTS13 IgG to mice resulted in a decrease in ADAMTS13 activity. After challenge with rhVWF, mice developed TTP-like symptoms (*i.e.* hematuria, pleural effusion and motor deficit) associated with severe thrombocytopenia, schistocytosis and anemia. On day 1, necropsy revealed moderate myocardial necrosis accompanied by lymphocytic infiltration and interstitial edema. Magnetic resonance imaging demonstrated a decrease in left ventricular perfusion associated with alteration of left ventricular ejection fraction and cardiac output, suggesting an early systolic dysfunction. This was associated with a decreased endothelium-mediated relaxing responses to acetylcholine in mesenteric arteries, demonstrating severe early endothelial dysfunction.

Conclusion: This new mouse model revealed that anti-ADAMTS13 auto-antibodies from patients are pathogenic and that endothelial dysfunction may represent an important trigger of the systemic organs failure occurring in TTP.

T.49. Differential Migration of Different Skin-resident Dendritic Cell Subsets in a Model of Autoimmune Dermatitis

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Dendritic cells (DC) that primarily reside in tissues carry antigens to local lymphoid organs to induce immunity or tolerance. We have previously reported that autoimmune skin dermatitis-prone MRL-Fas$^{lpr/lpr}$ (MRL-lpr) and MRL-Fas$^{+/+}$ (MRL$^{+/+}$) mice have a markedly impaired migration of langerin-expressing DCs from skin to skin-draining lymph nodes. Such defect in the migration of tissue-resident DCs may predispose to autoimmunity. Skin contains two subsets of DCs that express langerin: Langerhans cells (LC; Lang^{+}CD11b^{+}) and langerin^{+} dermal DCs (LangdDC; Lang^{+}CD11b$^{–}$). Here, we tracked the *in vivo* migration of skin-resident DCs at the steady state as well as by applying the fluorophore TRITC to the skin of MRL and wild-type mice. At the steady state, LCs were reduced, whereas

Lang^{+}dDCs were increased or unchanged in the skin-draining lymph nodes of MRL-lpr and MRL$^{+/+}$ mice as compared to control animals. Furthermore, the presence TRITC in the skin-draining lymph nodes of MRL-lpr and MRL$^{+/+}$ mice as compared to wildtype mice was 4-times lower after the application of the fluorophore, with reduced LCs in MRL mice. Such reduction in migration was corrected by treatment of mice with glycosphingolipid αGalCer that ameliorates dermatitis in these mice and leads to increased LCs but reduced LangdDCs in skin-draining lymph nodes. These data suggest a possible protective role of LC migration from skin to skin-draining lymph nodes in the pathogenesis of autoimmune dermatitis. Ongoing studies will investigate mechanisms underlying the differential control of the two subsets of langerin-expressing DCs and its consequences for the development of autoimmunity to skin.

T.50. The Versatile Behavior of Skin and Blood Derived Sézary Cells

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Background: We defined the CD158k/KIR3DL2 molecule as a first positive cell surface marker for Sézary cells (SCs). We recently found an unexpected heterogeneity, with elevated numbers of “naïve-like” or terminally differentiated CD158k^{+}T cells in some patients.

Materials and methods: To further characterize the phenotypic heterogeneity of SCs, in blood and skin, with a specific focus on T stem cell memory (TSCM) and skin resident memory T cells (TRM), 48 blood samples from Sézary patients were analyzed for their phenotypic characteristics including naïve and memory markers, chemokine and interleukin receptors and markers of activation. The skin of 12 patients with a heterogeneous blood pattern was simultaneously investigated.

Results: 46% of the blood samples displayed a phenotypic heterogeneity of naïve/memory subsets. 8 patients had fully TSCM SCs. When compared to the skin, we found a marked heterogeneity of SCs, with an advanced maturation pattern, including effector, transitional memory and terminal effector phenotypes, whereas few central memory SCs were present. Differences were also reported between skin and blood, regarding interleukin, chemokine receptors, activation markers and TRM subsets.

Conclusion: We demonstrate that SCs are unexpectedly highly heterogeneous both in skin and blood, some of which having a TSCM phenotype. The heterogeneity found in the skin is characterized by more mature phenotypes. We are currently analyzing the repertoire using NGS. We are also investigating the differentiation potential of these subsets *in vitro* to find mechanistic clues to their lineage relationships, potentially related to disease aggressiveness or response to treatment.

T.51. Autoantigen Microarrays Reveal Myelin Basic Protein Autoantibodies In Morphea

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The pathogenic mechanisms of morphea are poorly described and biomarkers associated with morphea activity or clinical features remain undefined. This study leverages resources of the Morphea in Adults and Children Cohort, which contains nearly 500 morphea patients with corresponding biospecimens, to better define the prevalence and clinical significance of morphea autoantibodies. We used protein microarrays to profile serum autoantibodies from morphea cases, healthy and scleroderma disease controls. We identified a unique autoantibody profile specific to morphea. Specifically, we found significantly elevated autoantibody reactivity to myelin basic protein (MBP) by protein

array and verified by ELISA in morphea compared to healthy and scleroderma controls ($p<0.01$ morphea vs controls). By protein array, 27.1% morphea exhibited MBP autoantibodies compared to 5.7% healthy and 0% scleroderma controls ($p<0.01$ morphea vs controls). Equivalent frequencies were identified by ELISA in which 30.5% morphea patients harbored MBP autoantibodies compared to 6.7% healthy controls ($p<0.01$ morphea vs controls). The specificity of serum MBP autoantibodies as a biomarker for morphea compared to healthy and scleroderma controls was 94.3% and 100%, respectively. There was significantly increased frequency of skin lesion pain, overall skin pain, and bodily pain in MBP-positive morphea versus MBP-negative morphea ($p<0.05$ MBP^+ vs MBP^- morphea for these parameters). Intriguingly, we identified that morphea MBP autoantibodies targeted distinct epitopes within the full-length MBP protein compared to multiple sclerosis MBP autoantibodies. The presented findings identify a molecular classification of morphea based on distinct autoantibody biosignatures, and in particular anti-MBP antibodies, and may represent a promising new approach to differentially classify morphea.

Immunology of the Eye

OR.28. Microbiome-dependent Modulation of Mucosal Immunity at the Ocular Surface

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Mucosal sites that interface with the environment and provide barrier function include the intestine, nasopharynx, lung, female reproductive tract and the ocular surface. Disruption of immune homeostasis at the ocular surface is associated with discomfort, inflammation and potential loss of vision. Immune cells are present within the conjunctiva and can be affected by environmental factors, potentially including microorganisms. However, proof that a resident ocular microbiome exists and influences local immunity has been elusive. We used a mouse model of ocular surface disease to study whether commensal microbes are present in ocular mucosa and modulate immunity. We found that IL-17 is constitutively produced within the conjunctiva-associated lymphoid tissue (CALT) and is necessary to recruit neutrophils to the ocular surface in the steady state and after a bacterial challenge. IL-17 sources in CALT include γδ T cells, αβ T cells and innate lymphoid cells (ILCs), in that order. Notably, a strain of Corynebacterium isolated from ocular tissue of mice, and known to also colonize the ocular surface of humans, induced the conjunctival γδ T cells to secrete IL-17, which modified the local inflammatory signature. This interaction appears necessary to regulate local immunity at the ocular surface, since elimination of these bacteria by antibiotic treatment, or their introduction into non-colonized mice, correlated inversely with severity of an experimental *Candida albicans* infection. Our results thus indicate that a relationship exists between commensals and immune cells at the ocular surface, which is critical for maintenance of homeostasis and host defense within the ocular mucosa.

T.52. Substance P Suppresses Foxp3 Expression in Regulatory T Cells in Dry Eye Disease

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Substance P (SP) is a neuropeptide released by peripheral nerves that mediate pain perception. Recent studies have supported that SP and its receptor NK1R are expressed on immune cells. Here, we aimed to investigate whether murine regulatory T (Treg) cells express the SP receptor (NK1R) and whether they respond to SP in healthy and dry eye mice. Dry eye disease (DED) was induced in wild-type (WT) C57BL/6 mice using the controlled environment chamber for 14 days.The draining lymph nodes (DLN) of WT (n=5) and DED (n=10) mice were harvested to assess NK1R and Foxp3 expression in Tregs ($CD4^+$ $CD25^+$) using flow cytometry analysis at days 7 and 14. The DLNs and spleens of WT mice mice (n=5) were harvested and Tregs ($CD4^+$ $CD25^+$) were sorted using magnetic-activated cell sorting. Sorted Tregs were co-cultured with interleukin (IL)-2 and SP, and Foxp3 expression of $NK1R^+$ Tregs was analyzed after 18 hours using flow cytometry. Using flow cytometry analysis, we found that 15 ± 4 % of Tregs were

NK1R$^+$ and that NK1R$^+$ Tregs express lower Foxp3 levels than NK1R- Tregs in WT mice. *In vitro* treatment of sorted naïve Tregs (CD4$^+$ CD25$^+$) with SP led to statistically significant reduction in Foxp3 levels in NK1R$^+$ + Tregs. Mice with DED showed increased NK1R epxression, but decreased Foxp3 expression in NK1R$^+$ Tregs on day 14. Our data demonstrate that SP directly suppresses Foxp3 expression in NK1R$^+$ Treg cells. Increased SP receptor expression and decreased Foxp3 expression in DED mice suggest that SP amplifies T cell mediated immunity in dry eye disease.

T.53. Vitreous Cytokines in Intermediate, Posterior and Panuveitis

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Purpose: To assess the levels of serum and vitreous cytokines from eyes undergoing fluocinolone acetonide (Retisert®) implantation for the treatment of intermediate, posterior or panuveitis in the Multicenter Uveitis Steroid Treatment (MUST) trial.

Methods: Vitreous specimens were obtained from patients with active intermediate or posterior/panuveitis who were randomized to the implant arm of the MUST trial (NCT00132691) and agreed to participate in the ancillary study (NCT00331331) done at the National Eye Institute, NIH. Multiplex bead array cytokine analysis was performed on both the vitreous and simultaneously obtained plasma

Results: There were 37 participants (15 intermediate uveitis, 22 posterior/panuveitis) with a mean age of 45.5 years, 78.4% were female. Serum PDGF, IL1ra, IL-17, eotaxin, G-CSF, IFN-gamma, IP-10, RANTES and TNF-alpha were elevated (*>100pg/ml*) in the entire cohort. Serum IL-6 , MCP-1 and VEGF were significantly elevated in intermediate uveitis compared to posterior or panuveitis. Vitreous IL-6, IL-13, G-CSF, GM-CSF, IP-10 (CXCL10), MCP-1, and RANTES were elevated in the vitreous of all patients. IL-6, IL-13, FGF-basic, GM-CSF, IP-10, MCP-1 showed a >2-fold increase *in vitreous* compared to serum in the majority of the specimens. Spearman rank correlation test showed IL-2, INF-gamma in the vitreous and INF-gamma in the serum may have significant correlation with age. We failed to detect any association of cytokine levels with anatomical location of uveitis, adjusting for age using logistic regression.

Conclusion: Th1, Th2 and Th17 related pro-inflammatory cytokines were found to be elevated in the vitreous and serum of patients with active posterior segment uveitis.

T.54. CD19$^+$CD24^{hi}CD38^{hi} B Regulatory Cells are Deficient in Human Non-infectious Uveitis

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In humans, IL-10-producing regulatory B cells (Bregs) are enriched within the transitional B cell population (CD19$^+$CD24^{hi}CD38^{hi}) in peripheral blood. Loss of Breg-mediated suppression, either through numerical deficiency or loss of function, has been implicated in the pathogenesis of several autoimmune and non-infectious inflammatory diseases. Recently, Bregs have been shown to suppress intraocular inflammation in a murine model of experimental autoimmune uveoretinitis (Wang et al, 2014, Nat Med, 20(6): 633-41), but little is known about the role of Bregs in human uveitis. In order to determine the role of Bregs in this patient group, flow cytometry was firstly used to quantify the frequency of CD19$^+$CD24^{hi}CD38^{hi} B cells in patients' peripheral blood (n=7) and compared with healthy donors

(n=8). Uveitis patients had a lower proportion of $CD19^{+}CD24^{hi}CD38^{hi}$ B cells compared to healthy donors (8.2% v 2.5%, p=0.0006) and this was particularly evident in patients with Birdshot chorioretinopathy (n=5)(8.2% v 1.5%, P<0.0001). Secondly, to assess the functionality of these Bregs, $CD19^{+}$ B cells and $CD4^{+}$ T cells were obtained from healthy donors and uveitis patients using fluorescence activated cell sorting. Following T cell co-culture, IL-10 production by B cells from uveitis patients was comparable to healthy controls when stimulated with anti-CD3 (2% v 3%, p=0.33) or CpG and anti-IgM (5% v 6%, p=0.58). Hence, $CD19^{+}CD24^{hi}CD38^{hi}$ Bregs are depleted in the peripheral blood of uveitis patients, but their functional capacity to produce IL-10 following activation is retained. These observations demonstrate that a deficiency in peripheral $CD19^{+}CD24^{hi}CD38^{hi}$ Bregs is a characteristic of human uveitis.

T.55. Regulation of Phagocytosis Within the Immune Privileged Eye

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Soluble molecules released by retinal pigment epithelial cells (RPE) suppress the phagocytic process within macrophages. This is mediated in part by the neuropeptides alpha-melanocyte stimulating hormone (α-MSH) and neuropeptide Y (NPY). In this work, we examined whether retinal microglial cells have suppressed phagolysosome activity, and whether this changes in response to the immunization used to induce experimental autoimmune uveitis (EAU). Also, we studied the effects of the neuropeptides on phagolysosome formation.

Microglial cells were isolated from healthy mouse retinas, and from retinas 3 days and 30 days after immunization for EAU. The isolated microglial cells were fed opsonized pHrodo-bioparticles for 24hr. Fluorescent intensity measurements for phagolysosome activity showed that microglia cells from healthy retinas have no phagolysosome activity, and possibly have no phagocytic activity. In contrast, microglial cells from mice immunized for EAU show phagolysosome activity at both 3 and 30 days even if the immunization was adjuvant only. Macrophages (RAW 264.7) treated with α-MSH and NPY were fed opsonized ovalbumin coated magnetic beads for 24 hours, lysed, and a magnet was use to isolate the magnetic-bead containing vesicles. Immunoblotting of the vesicle content showed that there was reduced OVA degradation, with reduced RAB 7 and LAMP-1 expression.

These results indicate that RPE suppress phagolysosome activation in antigen presenting cells (APC), and that to induce EAU there is a loss of this suppression. This suggests that a mechanism of ocular immune privilege is to alter APC processing of antigens to not present cognizant peptides, a form of antigen sequestration.

Immunoncology

OR.03. PD-1 Marks Dysfunctional Regulatory T Cells in Malignant Gliomas

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Immunotherapies targeting the immune checkpoint receptor programmed cell death protein 1 (PD-1) have shown remarkable efficacy in treating cancer. $CD4^{+}CD25^{high}FoxP3^{+}$ regulatory T cells (Tregs) are critical regulators of immune responses in autoimmunity and malignancies, but the functional status of human Tregs expressing PD-1 remains unclear. We examined functional and molecular features of PD-1^{high} Tregs in healthy subjects and patients with glioblastoma multiforme (GBM) combining functional assays, RNA-sequencing and cytometry by time of flight (CyTOF). In both GBM and healthy subjects, circulating PD-1^{high} Tregs displayed reduced suppression of $CD4^{+}$ effector T cells, production of IFNγ and molecular signatures of exhaustion. Transcriptional profiling of tumor-resident

Tregs revealed several genes co-expressed with PD-1 and associated with IFNγ production and exhaustion, as well as enrichment in exhaustion signatures compared to circulating PD-1^{high}Tregs. CyTOF analysis on circulating and tumor-infiltrating Tregs from patients with GBM treated with PD-1-blocking antibodies revealed that treatment shifts the profile of circulating Tregs towards a more exhausted phenotype reminiscent of the one of tumor-infiltrating Tregs, further increasing IFNγ production. Thus, high PD-1 expression on human Tregs identifies dysfunctional, exhausted Tregs secreting IFNγ that exist in healthy individuals and are enriched in tumor infiltrates, possibly losing function as they attempt to modulate the anti-tumoral immune responses.

OR.04. A Multidimensional Analysis of Hepatocellular Carcinoma Reveals an Immunosuppressive Microenvironment Shaped Along a Gradient Of Chemokines and PD-1 Expression

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Despite recent success in cancer immunotherapies, the dynamic relations between tumor-microenvironment and the systemic immumone remain elusive. We aim to address this by interrogating the “cancer immune-gradient” which encompasses the tumor (T) microenvironment, its adjacent non-tumor liver tissues (NT) and peripheral blood (PB).

In the current study, we integrated multiplexed technologies: Time of Flight of Mass Cytometry (CyTOF), Next Generation Sequencing (NGS) and NanoString technologies to explore this cancer immune-gradient in hepatocellular carcinoma (HCC). From CyTOF analysis, we found that tumor was infiltrated by inherently suppressive immune subsets: regulatory T, tumor-associated macrophages (TAM) and resident natural killer (NK_R), tissue resident memory (T_{RM}) and effector memory (T_{EM}) $CD8^+$T cells. NGS analyses on these T-enriched immune subsets revealed immunosuppressive phenotypes. Despite that, T_{RM} and T_{EM} cells remained functionally competent when activated *ex-vivo* and were significantly reduced with tumor progression. Compared to NT, T-infiltrating T_{RM} and T_{EM} cells expressed more PD-1 and less TNFα and IFNγ, indicative of a more immunosuppressive tumor-microenvironment. Further analyses evidenced a chemotaxis gradient for the recruitment of TAM via CCR6/CCL20, NK_Rvia CXCR3/CXCL10 pathways; and TBX21 as potential modulator of PD-1 expression in tumor.

The current approach provides a holistic view of mechanistically relevant dynamic interface across cancer immune-gradient and identified key immune subsets and pathways responsible for protective immunity in HCC.

OR.20. Exploiting Effector Functions of Tumorantigen-specific CD40-activated B Cells for Cancer Immunotherapy

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Adoptive transfer of *ex vivo* expanded or manipulated immune cells for treatment of malignant diseases is increasingly gaining attention. T cells have demonstrated significant potential and chimeric combinations of T cells with single chain variable fragments of B cells show remarkable success in the treatment of hematological malignancies. However, for technical and regulatory reasons their application in the clinic is currently limited. Here, we report of an alternative approach that directly exploits the advantages of B cell antigen specificity without the need of genetic engineering. Murine or human B cells specific for tumor antigens were isolated by use of antigen-biotin tetramers. Stimulation via CD40 ligand resulted in the development of an antigen-presenting phenotype and the induction of enhanced antigen-specific T cell responses *in vitro* and *in vivo* compared to polyclonal B cells. Furthermore, these cells showed a tumor-specific homing pattern in tumor-bearing mice. Differentiation of OVA-specific B cells into antibody-secreting plasma cells was achieved by stimulation with interleukin-21 and CD40 ligand.

Prophylactic and therapeutic treatment of tumor-bearing mice with OVA-specific CD40B-activated B cells and antibody-secreting plasma cells led to an anti-tumor immune response resulting in regression of tumors and a prolonged survival. Taken together, these results provide new insights into the role of activated antigen-specific B cells as APCs and their use for cancer immunotherapy.

W.84. A Case Report of Thyroid Cancer with Lung Metastasis

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Background: Follicular thyroid cancer (FTC) contributes to 15% of thyroid cancers. The incidence of distant metastasis is 11-25%. The majority of published cases of FTC metastases were found long after the primarily diagnosis. We present FTC with lung metastatic cancer presented on the time of diagnosis.

Case: A 64 year old man with a past medical history of GERD, hemorrhoids, hypertension, FTC with pulmonary metastasis status post RIA-131and total thyroidectomy, stage 4-T3N1M1(to the lung and L-sided neck-lymphadenopathy) presented to allergy clinic with urticarial on his thighs bilaterally. He applied aloe vera ointment to the affected area of the thigh with relief of symptoms. Physical examination was within normal limits. Chest radiography didn't visualize any masses. PET scan showed mildly hypermetabolic right level-2-cervical and bilateral hilar lymph nodes, which are not significantly changed compared to a prior study. No specific allergen was identified. The rash resolved and didn't reoccur.

Conclusion: FTC metastases to the lymph nodes as in our case is only 10% and 80% of FTC present initially with a solitary thyroid nodule. A series of 1,038 patients with FTC by Shaha reported 4% presented with distant metastasis and the distant metastasis total incidence was 11%. Emerick described only 2 cases or 3.6% with distant metastasis at the time of the primary diagnosis. The peak of FTC is 50-60 years old and our patient is 64. The prognosis of FTC varies with metastatic disease having a worse prognosis. This case report emphasizes distal metastases as possible initial presentation of FTC.

T.56. High Dose Vitamin C Inhibits Tumor Progression in the Mouse Mammary Gland PyMT Model

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Vitamin C, a water soluble vitamin, is essential for life because it serves as a cofactor for many enzymes. However, animal studies and clinical trials of vitamin C have yielded mixed results. The PyMT mouse is a representative model for human breast cancer. These mice express the polyoma virus middle T oncoprotein under the direction of the mouse mammary tumor virus gene promoter/enhancer, and develop mammary gland tumors starting from 5-6 weeks of age. In an epithelial cell line derived from mammary gland tumors of PyMT mice, we observed that low dose vitamin C enhanced growth of PyMT cells, while high doses of vitamin C, >0.5 mM, retarded their growth. High dose vitamin C induces cell cycle arrest and apoptosis of PyMT cells. Similarly, high dose of vitamin C (4 g/kg) retarded growth of mammary tumors in PyMT mice as well as tumors derived from PyMT cell implants. Biochemical and molecular analyses showed that vitamin C could have multiple anti-tumor effects. First, vitamin C lowers the lactate levels in blood as well in tumor tissues, which could suppress HIF-1a driven angiogenesis via VEGF expression. Second, vitamin C activates AKT which increases reactive oxidative stress and kill cancer cells which have a higher sensitivity to ROS-induced apoptosis. Together, the data suggest that high dose vitamin C could specifically target cancer cells by inhibiting aerobic glycolysis (the Warburg Effect) and ROS-induced programmed cell death.

T.58. Modulation of the Neuroblastoma Microenvironment by Polyamine Blockade

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High-risk neuroblastoma (NB), which accounts for a considerable portion of pediatric cancer-related mortalities, results from *MYCN*-amplification and alterations in Myc-regulated pathways. Despite improvements in therapy, long-term survival rates remain poor. High-risk NBs have elevated polyamine (PA) levels due to Myc's targeting of ornithine decarboxylase, the rate-limiting enzyme for PA synthesis. It has been shown that *in vivo* PA blockade using the drug DFMO lead to a greater reduction in NB growth than that seen *in vitro*, suggesting a tumor-cell extrinsic effect. Elevated PAs can drive the differentiation of immune suppressive cells, while blockade can reverse this by increasing tumor-infiltrating leukocytes (TILs). However, previous studies investigating immune effects of DFMO on the tumor microenvironment (TME) are incomplete. Therefore, we sought to characterize the NB TME in a spontaneous *MYCN*-driven NB mouse model with and without DFMO. Terminal disease tumors were dissociated, and the frequencies of various TILs were assessed. We observe that DFMO reproducibly alters the NB TME by increasing frequencies of DCs and NK cells while maintaining CD4-negative invariant NKT cells and granulocytic-MDSCs. These data support our hypothesis that PA blockade induces distinct immune changes in the TME that could allow for a more efficient anti-tumor response to NB. We hope that these studies will complement the data being accrued from phase I/II clinical studies using DFMO in various therapeutic strategies for NB and will allow for an increased understanding of how to employ PA blockade in the immunotherapy of this disease.

T.59. Different Activation Status of NK Cells Infiltrating Metastatic Lymph Nodes From Breast Cancer And Melanoma Patients

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There is growing evidence that Natural Killer (NK) cells, infiltrate human tumors and are implicated in the immune response against tumors. In the present studies, we investigated the phenotype and function of NK cell subsets in tumor draining lymph nodes (LN), first metastatic localization during breast cancer (BC) and melanoma progression. Thirty LN-NK cells from BC were characterized by an altered expression of activating NK receptors (NKp46, NKp30) and high expression of NKG2D. In melanoma, NK from 25 M-LN expression of NKp30 and NKp46 was high. LN cell suspensions contain high percentages of NK expressing CD62L and CXCR3. The proportions of invading tumor cells in M-LN from BC patients were modest (<20%) and did not correlated with the alteration of LN-NK cell phenotype. The NKp30 alteration reported in blood and tumor-derived NK cells of BC patients was also present in LN-NK cells indicating a recirculation of NK cells from the tumor site. In contrast, most M-LNs from melanoma patients were massively invaded by metastatic tumor cells (>70%) that might interfere with NK cell phenotype. In NK infiltrating M-LN from BC and melanoma patients, we have identified a new subset of cytotoxic $CD56^{bright}CD16^{+}$ NK cells that exhibit higher expression of activating receptors (NCRs, NKG2D) and perforin than $CD56^{bright}CD16^{-}$ NK counterparts. These data show particular regulation of NK cells in the same metastatic site of two types of solid tumors. The presence of mature and activated $CD16^{+}$ NK cells in M-LN may be target for efficient combined treatment using mAbs or cytokines.

T.60. Automated Discovery of Rare Cell Subsets: Identification of Leukemic Cells as Minimal Residual Disease (MRD)

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Flow cytometry is an excellent tool in the study of cancers of the blood and related tissues. Clinical analysis methods exist for use in diagnosis, efficacy monitoring during treatment, and remission/relapse monitoring. These methods have acceptance but also have shortcomings, one of which is a reliance on expert manual analysis, which can have researcher-specific bias resulting in misidentified or unidentified cells. In addition, manual analysis is difficult to scale

and standardize for clinical tests. Even subtle shortcomings in analysis can affect a clinical outcome, especially in the case of monitoring for minimal residual disease (MRD), where very small numbers of cells can be significant (e.g., < 1%). For these reasons, computational methods to assist or automate the detection and classification of aberrant cells in the blood and other tissues will be beneficial to clinical applications of cytometry. In this study, we use viSNE followed by CITRUS analysis to computationally assist in the identification of leukemic MRD in a sample created from healthy bone marrow mixed with a small amount of bone marrow from an individual with acute lymphoblastic leukemia (ALL) and assayed on the Fluidigm CyTOF mass cytometer. The small population of aberrant cells (16 / 10,000) is correctly identified automatically from among the majority background of healthy cells, indicating promise for the application of finding MRD in post-therapy disease monitoring for cancer. With these tools, an expert can focus on biological significance with less bias, improved efficiency, and with insight into the complete structure of the data.

T.61. Novel Conjugate CD1d-Ab Directs iNKT Cells to Tumor-specific Targets

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Invariant natural killer T (iNKT) cells are innate-like lymphocytes that have phenotypic and functional characteristics of both T and NK cells but are restricted by CD1d, non-classical MHC class I-like molecules that present glycolipid antigens (GAg). Previously, iNKT cells have been shown to participate in anti-tumor responses via direct cytotoxicity, production of pro-inflammatory cytokines, and alteration of the tumor microenvironment. We recently developed a strategy to focus and augment the activity of iNKT cells against $CD1d^+$ and $CD1d^-$ tumors. In this strategy, we generate conjugate antibodies against tumor-specific antigens (TSAs) to CD1d molecules displaying iNKT cell-stimulating glycolipid antigens (CD1d-GAg). We hypothesize that these CD1d-GAg:anti-TSAmAbs ("CAbs") will concentrate within tumors and activate direct and indirect iNKT cell-mediated anti-tumor responses *in situ*. To support this hypothesis, we demonstrate that tumor-targeted CAbs increase directed lysis of human and murine tumors by effector cell populations enriched in iNKT and NK cells *in vitro*. Additionally, we observe rapid intracellular production of IFN-γ and upregulation of granzyme B in iNKT and NK cells within hours of administration of CAb *in vivo*. Finally, our preliminary data suggest that the use of TSA-specific (but not control) CAbs retard the growth of exogenously-administered tumors in lymphocyte-deficient mice repleted with small numbers of iNKT and NK cells. Our ongoing and future studies will help clarify whether CAb and iNKT-mediated anti-tumor responses could be used as a platform for cellular immunotherapy.

T.62. Therapeutic Effects of Anti-LAP in Different Cancer Models

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Regulatory T cells (Tregs) play a key role in tumor-induced immunosuppression but there are few antibodies available that specifically target Tregs. We found that latency-associated peptide (LAP) serves as a biomarker of Tregs and plays an important role in anti-tumor immunity. Tumor-associated LAP^+CD4^+ T cells possess suppressive abilities by reducing the proliferation of naïve T cells. To study the regulatory role of LAP^+CD4^+ T cells in mouse models of cancer we developed a murine monoclonal anti-LAP antibody. We found that treatment with anti-LAP reduces tumor growth and increases survival in various cancer models, including colon carcinoma, glioblastoma and melanoma. Anti-LAP antibody is able to block TGF-b release from cells expressing LAP and also reduces both the number and suppressive abilities of tumor-associated LAP^+CD4^+ regulatory T cells. Anti-LAP treatment triggered a profound peripheral immune response by acting on both innate and adaptive parts of the immune system. We observed increased numbers of mature antigen-presenting cells, with higher MHCII and CD86 expression, and down-regulated tolerogenic dendritic cells expressing increased CD103 and PD-L1 in spleen after treatment with anti-LAP.

These changes were associated with increased infiltration of cytotoxic $CD8^+$ T lymphocytes with higher frequencies of $Ki67^+$ cells into tumors. Based on the TCGA dataset, the expression of LAP-associated genes correlates inversely with patient survival in a number of cancers that include, among others, glioblastoma, colon carcinoma and melanoma. In conclusion, anti-LAP antibody as monotherapy or combined with conventional anti-tumor modalities represents a novel immunotherapeutic approach for the treatment of cancer by targeting Tregs.

T.63. SCS Macrophages Suppress Melanoma by Restricting Tumor-dDerived Vesicle–B Cell Interactions

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Tumor-derived extracellular vesicles (tEVs) are important signals in tumor-host cell communication. Yet, how endogenously produced tEVs impact the host in different areas of the body remains unclear. Here we combine imaging and genetic analysis to track melanoma-derived vesicles at organismal, cellular and molecular scales to show that endogenous tEVs efficiently disseminate via lymphatics and preferentially bind subcapsular sinus (SCS) $CD169^+$ macrophages in tumor-draining lymph nodes (tdLNs) in mice and humans. The $CD169^+$macrophage layer physically blocks tEV dissemination but is undermined during tumor progression and by therapeutic agents. A disrupted SCS macrophage barrier enables tEVs to enter the LN cortex, interact with B cells and foster tumor-promoting humoral immunity. Thus, $CD169^+$macrophages may act as tumor suppressors by containing tEV spread and ensuing cancer-enhancing immunity.

T.64. Transcriptional Regulation of IL-9-Secreting CD4 T Cells (TH9) by the Transcription Factor Interferon Regulatory Factor 8 (IRF8)

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Th9 cells were defined as a new subset of CD4 T cells in 2008. These cells secrete high levels of IL-9 and are pro-inflammatory *in vivo*. While Th9 cells were initially described to contribute to the development of autoimmune and allergic diseases, we and others have recently shown that these cells also have anticancer functions. However, the molecular mechanisms responsible for Th9 cell development remain incompletely understood.

Our current data indicate that IRF8 affects the differentiation of Th9 cells. Accordingly, downregulation of IRF8 expression reduces IL-9 secretion from Th9 cells. The absence of IRF8 failed to affect the expression of the transcription factors PU.1 and IRF4, which are also involved in Th9 development. Instead, we found that IRF8 binds to PU.1 in Th9 cells and the PU.1-IRF8 complex subsequently binds to the promoters of il9, il10 and il21 and favors Il9, Il10 and Il21 expression. We further demonstrated that the expression of IRF8 in Th9 cells is dictated by TGFβ induced activation of transcription factor Smad3. Our findings collectively show that IRF8 participates in Th9 development in combination with PU.1. These results suggest that modulation of IRF8 functions in Th9 cells could enhance their anticancer activity.

T.65. Synthetic Vaccine Particles for Long-term CTL Responses and Anti-tumor Therapy

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We have reported that synthetic vaccine particle (SVP) technology enables nanoparticle encapsulation of antigens and TLR agonists resulting in augmentation of immune responses with minimal systemic production of inflammatory cytokines. Now we evaluated several antigen formulations for their ability to induce CTL activity in combination with

SVP-entrapped adjuvants. One of these formulations led to fast and efficient antigen processing, induced rapid and prolonged CTL activity and provided for expansion of $CD8^+$ T cell effector memory cells locally and centrally after a single immunization. This resulted in superior survival following therapeutic dosing in several mouse cancer models. Immunological memory in SVP-immunized animals persisted for 1-2 years. Treatment of tumors induced by cervical cancer model cell line TC-1 using a SVP-entrapped peptide led to suppression of tumor growth and a considerable delay in mortality. Similarly, treatment with SVP-encapsulated peptide derived from Trp2 protein generated cytotoxicity *in vivo* leading to prolonged survival in mice inoculated with B16 melanoma cells. SVP-encapsulated CpG provided superior therapeutic benefit when compared to equal or higher amounts of free CpG. Identical levels of immune memory, therapeutic activity and CTL induction were observed whether SVP-entrapped CpG contained a natural phosphodiester or modified phosphorothioate backbone. Mutated HPV-16 oncogenic proteins were then encapsulated into SVP and used for therapeutic treatment of mice inoculated with TC-1. This led to induction of a broad CTL activity and strong tumor suppression with majority of mice surviving the challenge even if treatment was started at a late time-point when nearly all animals bore sizeable tumors.

T.66. Abscopal Effect May Require the Presence of IL-17 in a Mouse Model of Pancreatic Tumor

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The abscopal effect is a phenomenon in cancer radiation therapy where localized treatment of a tumor affects the treated tumor and the other tumors. Accumulating evidence suggests that radiation can directly kill tumor cells as well as modulate immunity and attain the therapeutic effects. The up-regulation of anti-tumor immunity may be attributed to the abscopal effect. It becomes accepted that IFN-γ^+ and IL-17^+IFN-γ^+ cells both harbored strong anti-tumor activity. Therefore, we attempt to explore the role of IL-17 in the radiation indueced ascopal effect here. First, the radiation significantly reduced cell growth of the murine pancreatic tumor cells both *in vitro* and *in vivo*. Second, no difference of engrafted tumor growth was found between wild-type and the IL-17 deficient (IL-17KO) mice. However, radiation successfully inhibited the tumor growth in wild-type mice, while 20-30% less of the inhibition in the IL-17KO mice. Third, radiation might synergize with IL-17 and elicit inflammatory responses by examining the gene profile of the treated cells. Most importantly, the significantly increased levels of $CD8^+$ cytotoxic T cells as well as $CD4^+$IFN-γ^+ cells occurred only in the wild-type mice receiving radiation. In fact, a comparatively enhanced frequency of $CD4^+$IFN-γ^+IL-17^+ cells and $CD8^+$IFN-γ^+IL-17^+ cells was also only found in those wild-type mice, suggesting a cytotoxic phenotype of IL-17^+ cells after radiation treatment. Finally, the radiation treatment significantly induced abscopal effect in wild-type but not IL-17KO mice. These results suggest that IL-17 facilitates the control of tumor growth through the regulation of anti-tumor immunity in radiotherapy.

T.67. Differential Expression of the T Cell Inhibitor TIGIT in Glioblastoma and Multiple Sclerosis

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Multiple sclerosis and glioblastoma are CNS diseases that appear to represent opposite ends of an immune regulatory spectrum. Loss of immune regulation with activation of myelin-specific T cells leads to autoimmunity in multiple sclerosis, while impaired T cell responses in glioblastoma prevent tumor clearance. TIGIT is a newly identified receptor that inhibits T cell function by preventing assembly of the co-stimulatory receptor CD226 and by competing for their common ligand, CD155. Overexpression of TIGIT and CD155 has been reported in multiple cancers pathologies, while genetic studies and animal models link CD226 signaling to risk of developing multiple sclerosis. In order to establish whether the TIGIT/CD226 axis contributes to the T cell responses in multiple sclerosis and glioblastoma, we assessed expression of these receptors in lymphocytes in multiple sclerosis and glioblastoma

infiltrates, and in peripheral blood. We found that TIGIT was highly expressed on glioblastoma-infiltrating CD8 T cells, but was near-absent from multiple sclerosis lesions. Similarly, TIGIT was significantly upregulated in circulating lymphocytes of patients with glioblastoma as compared to multiple sclerosis and healthy controls. These data suggest that TIGIT may be a critical checkpoint receptor in regulating CNS T cell immune responses that may be amenable for therapeutic manipulation.

T.68. Do Inflammatory and Cancer Biomarkers Differentiate Between Oral Premalign and Malign Lesions?
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Clinical predictors of malignant transformation in oral leukoplakia have been recently reviewed. Few prevention studies have shown efficacy in preventing malignant transformation of leukoplakias. One of the major difficulties in Oral Preneoplastic Lesions prevention trials is identification of patients with higher cancer risk. The primary objective of this study is to determine, in a population from whom Erosive Oral Lichen Planus, Oral Leukoplakia and Squamous Cell Carcinoma samples are collecting whether considering local and systemic levels of anti-CCP, IL-1 beta, and TNF-alpha. A total of 27 subjects were included in the study. There were 10 premalign lesion, 7 malign lesion and 10 systemically healthy subjects. Saliva and serum samples were collected in each subject.Serum and saliva levels of anti-CCP, IL-1 beta, and TNF-alpha were determined by ELISA. Data were analyzed by Kruskal-Wallis and Mann-Whitney U tests and Spearman correlation analysis. The inflammatory response seems to be more pronounced in malign lesions. The observed increase in both local and systemic levels of inflammatory cytokines may suggest an interaction between malign lesions and inflammation. Upon confirmation of eligibility for enrollment in the study, complete medical and dental histories are obtained from each individual. All patients returned to the clinic for clinical periodontal measurements including probing pocket depth (PD), plaque index (PI), and the presence of bleeding on probing (BOP); Clinical periodontal measurements were performed by a single calibrated examiner

T.69. Her2/neu Protein Based Immunotherapy Against HER2-positive Breast Cancer
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Over-expression of HER2 protein provides cells a strong anti-apoptotic and more potent proliferative signals which lead to aggressive malignant cancer. HER2 over-expression can be seen in several cancers including 25% of breast cancers. Recent strategies point to the development of personalized immunotherapy and here in this study, the efficacy of a new personalized vaccine combination will be tested in a mice breast cancer model.

Balb/c mice will be receiving two weekly intraperitoneal immunizations of the immunotherapeutic vaccine combination containing HER2/neu protein, QS-21 adjuvant followed by a tumor challenge with syngeneic HER2 positive breast cancer cells, and tumor development will be monitored. To test the efficacy of the immunotherapy, mice splenocytes will be isolated and IFNg, IgG subtypes will be measured. To test for the HER2/neu specificity of CTL response, LDH release assay will be applied.

In this study, we are expecting to boost the immune response against HER2/neu expressing tumor cells and also prevent the immunosuppression mediated by tumor cells.

T.70. GoInVivo™ Antibodies, Validated Biofunctional Antibodies for Immune Checkpoint Research
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Immune checkpoints are molecules that have been described to control the immune response. Some important immune checkpoint molecules are gaining more and more attention as they regulate the balance between tumor

elimination and tumor escape. Tumor cells communicate with these receptors to trick the immune response into suppression, allowing the tumor cell to grow without immune cell intervention. To interfere with this process, some important checkpoint interactions can be manipulated with the use of bioactive antibodies. Well- studied combinations include PD-1/PD-L1, CTLA-4/CD80 and CD86, LAG-3/MHC II, and Tim-3/Galectin 9. Our GoInVivo™ antibodies, against some of these immune checkpoint molecules, offer several advantages. They have been tested by flow cytometry and *in vitro* bioassays, are pathogen-free as tested by qPCR, and have excellent price for large sizes, among others. Here we present our portfolio, validation methods and data, as well as *in vivo* applications. We characterize the specificity by flow cytometry staining and blocking capacity by target-ligand inhibition bioassays. We also study the effect of *in vivo* injection in the activation phenotype and percentage of lymphocytes, as well as the cytokine profile in serum.

Inflammatory Bowel Disease

OR.01. Lachnospiraceae Protect From Colitis By Regulating Colonic Group 3 Innate Lymphoid Cells

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In previous work, we demonstrated that host-specific bacteria are required for proper maturation of the small-intestinal immune system. In sharp contrast to gnotobiotic mice colonized with normal mouse microbiota (MMb), which have a well-developed small-intestinal immune system, we demonstrated that gnotobiotic mice colonized with normal human microbiota (HMb) have an immature small-intestinal immune system indistinguishable from that of germ-free (GF) mice. Using the dextran sodium sulfate (DSS)-induced colitis model that relies heavily on the innate immune system, we have now found that—similar to GF mice—MMb mice are exquisitely sensitive to DSS colitis with a 100% mortality rate; in contrast, HMb and specific pathogen-free (SPF) mice are protected. Based on flow cytometric analysis of the colonic immune systems, we identified group 3 innate lymphoid cells (ILC3s) as the immunologic correlate of disease. Co-housing MMb and HMb mice facilitated a bi-directional transmission of microbes that led to an intermediate disease phenotype in both groups. Bioinformatic analysis of the fecal microbiota of SPF, MMb, HMb, and co-housed mice identified the bacterial family *Lachnospiraceae* as a microbiological correlate for protection from disease. Using semi-selective media, we cultured a *Lachnospiraceae*-enriched pool of bacteria from HMb mice that—when gavaged to MMb mice—led to decreased numbers of colonic ILC3s and protection from DSS-induced mortality. This work provides an immunologic explanation for why multiple cohorts of patients with inflammatory bowel disease have demonstrated that *Lachnospiraceae* is associated with reduced risk of disease and provides a mechanistic foundation for the potential therapeutic use of these organisms.

OR.09. Circulating T Cells Specific for an Intestinal Bacterial Antigen Peptide Show a Distinctive Phenotype and Potentially **Proinflammatory Gene Expression Pattern in Crohn's Disease**

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

Methods: Using MHC-II tetramer-guided epitope-mapping (TGEM), we identified a specific peptide of OmpC recognized by peripheral T cells from HLA-DR1501$^+$ individuals. HLA-DR1501 tetramers loaded with this OmpC peptide or a known antigenic peptide from influenza virus (Flu) were then used to compare OmpC versus Flu-specific T cells from HLA-DR1501$^+$ healthy and CD donors via flow cytometry and multiplex qPCR.

Results: OmpC-specific T cells more frequently expressed the Th17 marker CD161 (49% v 26%; p=1.1e-18) and the gut-homing integrin α4β7 (43% v 10%; p=1.8e-13) than autologous Flu-specific T cells. Conversely, the Th1 marker CXCR3 was more commonly seen on Flu than OmpC-specific T cells (72% v 61%; p = 0.005). No differences in the frequency or immunophenotype of OmpC-specific T cells were found between healthy and CD patients. However in patients with CD, multiplex PCR of sorted OmpC-specific T cells revealed significantly higher expression of the costimulatory molecule CD226 (p = 0.03) compared to healthy controls, with a concomitant trend towards decreased expression of the coinhibitory receptor TIGIT (p = 0.1) in CD patients.

Conclusions: Ompc-specific T cells with a gut-tropic, Th17-like phenotype can be found in the peripheral blood, and may have an imbalance in CD226 and TIGIT expression to favor inflammation in CD.

OR.41. PD-L1 Mediated Regulation of NKT Cells by Colonic CD90$^+$ M**yofibroblasts/fibroblasts in Crohn's** Disease

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Background: Crohn's Disease (CD) is a chronic inflammatory disease linked to an abnormal increase of Th1 type cytokines. CD90$^+$ stromal fibroblasts/myofibroblasts (CMFs) are abundant non-professional antigen presenting cells in normal colonic mucosa. Normal (N) CMFs suppress IFN-g production by activated CD4$^+$ T helper (Th)1 cells in PD-L1-dependent manner. Interestingly, PD-L1 expression is reduced in CMF derived from the CD colitis. Resident NKT cells are present in limited number in normal colonic mucosa. By contrast in CD, NKT is increased and an abnormal increase in IFN-g production by NKT suggests to critically contribute to the immunopathogenesis of CD. The regulation of NKT activity during colonic homeostasis and in CD is unknown. We hypothesized that under homeostasis CMFs suppress NKT responses via PD-L1 mediated interactions and this control is disrupted in CD.

Results: N-CMFs suppress activated NKT Tbet transcription factor expression, and resultant IFN-g production while upregulating GATA3 and IL-13 expression. Blocking PD-L1 by specific siRNA in CMFs or by use of blocking Abs in the CMF: NKT co-cultures reversed these processes. By contrast, CD-CMFs with lower surface expression of PD-L1 had reduced ability to suppress Tbet mediated IFN-g production or to induce GATA-3 dependent IL-13 production.

Conclusion: Our data suggest that under colonic homeostasis CMF may contribute to the control of NKT mediated inflammatory responses via PD-L1 mediated increase in tolerogenic IL-13 production. This control is reduced or lost in Crohn's disease, favoring pathological increase in "Th1 type" IFN-g production by NKTs.

T.71. RORC Antagonist Inhibits IL-17 Production in Gut Commensal-specific T Cells and Biopsies From **Crohn's Disease Patients**

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Increasing evidence suggests that Crohn's disease (CD) results from an aberrant immune response to commensal microorganisms. Since deregulated pathways include Th17 responses, the inhibition of RORC represents a potential therapeutic strategy. Our aim was to determine the *ex vivo* effect of RORC antagonism on blood and intestinal samples from CD patients. Peripheral blood mononuclear cells (PBMCs) from CD patients and controls were cultured for 7 days with commensal microbial proteins (ASCA, FliC, FrvX) in the presence of a RORC antagonist. In addition, intestinal biopsies from active CD were cultured with a RORC antagonist for 16 h. Supernatants and RNA isolated from cells and biopsies were analysed to determine the effect of the RORC antagonist (1 μM) on protein and mRNA

levels of RORC targets. Stimulation with commensal microbial proteins induced a significantly higher increase in IL-17A production in CD patients (n=6) compared to controls (n=6). The RORC antagonist specifically inhibited transcription of Th17-related genes in bacterial antigen-stimulated PBMCs from CD patients (n=3). Remarkably, culture of biopsies from CD patients (n=10) with the RORC inhibitor significantly ($p<0.05$) decreased transcription of IL17A, IL17F, IL26 and S100A8, whereas expression of Th1 genes TBX21 and IFNG did not change; supporting the target specificity of the compound. We provide evidence supporting a role for RORC in increased gut commensal-specific-Th17 responses in CD. Importantly, we demonstrate that blocking RORC in inflamed mucosa specifically modulates expression of a subset of IL-17 dependent genes. Therefore, RORC antagonists could represent a highly specific therapeutic approach to CD treatment.

T.72. IL-7 Receptor Blockade Prevents Intestinal Human T Cells Infiltration by Modulation of Alpha4-Beta7 Integrin Expression

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Interleukin 7 is an essential cytokine sustaining T lymphocyte proliferation, survival and homeostasis. Almost all T lymphocytes express the IL-7 receptor (IL-7R), with an exception for natural regulatory T cells (Treg), constituting an opportunity to selectively target effectors (Teff) while sparing Treg. IL-7R-positive cells were found accumulated in inflammatory lesions of inflammatory bowel diseases.

In this study, we observed that IL-7 is a potent inducer of α4β7 integrin expression in human, but not in mouse T cells, by acting at transcriptional and traductional levels. Addition of an IL-7R antagonist mAb prevented α4β7 overexpression. α4β7 integrin being the main intestinal lymphocyte homing receptor, we used NOD SCID gamma$^{-/-}$ mice engrafted with human PBMC to study intestinal xeno-GVHD (graft versus host disease). Treatment of recipient mice with IL-7R antagonist mAb delayed GVHD and extended survival ($p<0.01$), without reducing immune reconstitution. Treatment also decreased expression of α4β7^{+}on human T lymphocytes. Histological analyses revealed that anti-IL7R mAb specifically prevented human T cell infiltration in the colon but did not prevent cell infiltration in other target tissue of GVHD (e.g. liver and lung). Finally, we developed a novel acute colitis model in humanized mice reconstituted with human hematopoietic stem cells by intrarectal administration of TNBS/Ethanol and observed similarly that anti-IL7R mAb reduced weight loss and prevented diarrhea.

These results indicate that IL-7R blockade prevents specifically intestinal human T cell infiltration by modulating level of α4β7 integrin expression and could offer novel therapeutic option in inflammatory bowel diseases.

T.73. AHR Activation is Protective Against Colitis Driven by T Cells in Humanized Mice

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Inflammatory Bowel Diseases (IBD) are a group of inflammatory conditions thought to result from defects in the regulation of the mucosal immune response to commensal microorganisms. Existing IBD therapies are largely based on the broad suppression of the inflammatory response, resulting in variable clinical benefit and untoward side effects. A more direct *in vivo* approach leading to the induction of regulatory T cells (Tregs) that promote immune tolerance is considered a potential therapeutic approach for IBD. We tested whether AHR activation using the non-toxic agonist 2-(1'H-indole-3'-carbonyl)-thiazole-4-carboxylic acid methyl ester (ITE) would induces suppressive human regulatory T cells *in vitro* and *in vivo.* We found that ITE suppressed responder T cell proliferation through a mechanism mediated by CD39 and granzyme B. We then evaluated the translational potential of these findings we developed a humanized mouse model of the well-known 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced murine

model of IBD that is largely mediated by T cells. This model relies on reconstitution of immunodeficient NSG mice lacking murine major histocompatibility complex class II and instead expressing human leukocyte angtigen-DR1 (NSGAb^{o}DR1 mice) with purified human CD4^{+} T cells isolated from HLA-DR1^{+} donors. We found that AHR activation by ITE administration led to a significant amelioration of TNBS-induced colitis in NSGAb^{o}DR1 mice reconstituted with human CD4^{+} T cells. Collectively, these results describe the development of a new experimental model to investigate the immune response in IBD, and identify the non-toxic AHR agonist ITE as a potential therapy for expanding human Tregs and promoting immune tolerance in the intestine.

T.74. Over-expression of CD200 Protects Mice From Dextran Sodium Sulfate Induced Colitis

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Background and aim: CD200:CD200 receptor (CD200R) interactions cause potent immunosuppression and inhibition of autoimmune inflammation. We investigated the effect of "knockout"of CD200 or CD200R, or over-expression of CD200, on susceptibility to dextran sodium sulfate (DSS) - induced colitis, a mouse model of inflammatory bowel disease (IBD).

Methods: Acute or chronic colitis was induced by administration of dextran sodium sulfate (DSS) in four groups of age-matched C57BL/6 female mice: (1) CD200-transgenic mice (CD200^{tg}); (2) wild-type (WT) mice; (3) CD200 receptor 1-deficient (CD200R1KO) mice; and (4) CD200-deficient (CD200KO) mice. The extent of colitis was determined using a histological scoring system. Colon tissues were collected for quantitative RT-PCR and Immunohistochemical staining. Supernatants from colonic explant cultures and mononuclear cells isolated from colonic tissue were used for ELISA.

Results: CD200KO and CD200R1KO mice showed greater sensitivity to acute colitis than WT mice, with accelerated loss of body weight, significantly higher histological scores, more severe infiltration of macrophages, neutrophils and CD3^{+} cells, and greater expression of macrophage-derived inflammatory cytokines, whose production was inhibited *in vitro* (in WT/CD200KO mouse cells) by CD200. In contrast, CD200^{tg} mice showed less sensitivity to DSS compared with WT mice, with attenuation of all of the features seen in other groups. In a chronic colitis model, greater infiltration of Foxp3^{+} regulatory T (Treg) cells was seen in the colon of CD200^{tg} mice compared to WT mice, and anti-CD25 mAb given to these mice attenuated protection.

Conclusions: The CD200:CD200R axis plays an immunoregulatory role in control of DSS induced colitis in mice.

T.75. Epigenetic Regulation of Gastrointestinal Macrophage Expression in Crohn's Disease

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Crohn's disease is a subtype of inflammatory bowel disease. Across alleles associated with immune-mediated disease ~60% map to immune cell enhancers. We investigated whether Crohn's risk polymorphisms are linked to macrophage enhancer positions in a tissue-specific manner. We predicted enrichment of macrophage enhancer sites in Crohn's genes compared to non-Crohn's genes and increased enrichment among gastrointestinal macrophages. NCBI human-mouse homology maps were used to match human Crohn's loci to homologous mouse genes. H3K4me1 macrophage enhancer regions from mouse large and small intestine, lung, microglia, and monocytes were provided by Lavin et al. (2014). One-sided Fisher's exact test and chi-squared tests determined enrichment significance. 1524 mouse Crohn's genes were identified. 40% of mouse Crohn's genes had H3K4me1 enhancer sites (median, 605 genes; range, 592-617 genes) and >90% (564) of those genes were shared among all macrophage populations. There was significant enrichment for H3K4me1 sites among Crohn's genes than non-Crohn's genes in all macrophage populations (median, 1.46 fold-enrichment; $p < 10^{-25}$). Large intestine macrophages had the highest

enrichment (1.485-fold) while lung macrophages had the lowest (1.426-fold). The difference in enrichment by tissue-type was not significant ($\chi^2 = 0.582$, $p = 0.965$). The enrichment of H3K4me1 enhancer sites in mouse Crohn's genes suggests a role for macrophage epigenetic regulation in Crohn's disease. The lack of relative enrichment among GI-specific macrophages in healthy subjects may indicate that Crohn's disease associated signals necessitate an inflammatory phenotype to modulate gene expression. Future studies should investigate the enrichment of active enhancers in Crohn's genes among inflammatory macrophages.

T.76. Lymphocytes Bearing the Gut-homing Integrin Alpha4/Beta7 Have a Distinctive Phenotype, Which Is Targeted by Vedolizumab in Inflammatory Bowel Disease

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Background: The humanized anti-integrin alpha4/beta7 antibody vedolizumab successfully treats both Crohn's disease and ulcerative colitis (UC) by blocking lymphocyte migration to the intestinal mucosa.

Methods: Peripheral blood mononuclear cells (PBMC) from inflammatory bowel disease (IBD) patients and healthy controls were evaluated by flow cytometry using vedolizumab as a fluorescent tag to compare the immunophenotype and function of alpha4/beta7 positive and negative cells in a variety of contexts and populations.

Results: In both B and T cells, naïve cells demonstrated somewhat uniform low-level expression of integrin alpha4/beta7, while memory cells demonstrated a bimodal pattern, with high-level expression in a minority and no expression in a majority of cells. This minority population of alpha4/beta7$^+$ cells was diminished in Helios$^+$, FOXP3$^+$ cells, but similar between Helios$^-$, FOXP3$^+$ and FOXP3$^-$ cells. Conversely, IFN-g and IL-17A-producing cells were a higher fraction of integrin$^+$ than integrin$^-$ T cells. By Stat phosphorylation, integrin$^+$ cells were significantly more responsive to IL-6, IL-7, and IL-21, but less responsive to IL-2.

Conclusions: Integrin alpha4/beta7 is expressed on a population of circulating lymphocytes enriched for cytokine-producing and IL-6, 7, or 21-responsive cells, but is less common on Helios$^+$, FOXP3$^+$ "natural" regulatory T cells (Tregs), or cells responsive to the cytokine IL-2, upon which Tregs depend. Thus, vedolizumab may function by selectively blocking the mucosal migration of pro-inflammatory cells, without impairing Treg migration, resulting in a higher intramucosal Treg to effector T cell ratio. Mucosal studies are underway to address this question.

T.77. Intravenous Immunoglobulin-activated IL-10 Producing Macrophages may be Useful to Treat Inflammatory Bowel Disease

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Inflammatory Bowel Disease (IBD) is a chronic inflammatory disease characterized by inflammation along the intestinal tract. Current treatment for IBD relies on non-specific immune suppression. However, up to 40% of people are predicted to become refractory to all available therapies so development of new therapeutic strategies to treat people with IBD is urgently needed. Macrophages initiate the innate immune response and contribute to the inflammation that characterizes IBD, but they also play an equally important role in turning off the inflammatory response. We have reported that macrophages stimulated with Intravenous Immunoglobulin (IVIg), pooled polyclonal IgGs isolated from the blood of more than 1000 donors, produce high levels of the anti-inflammatory cytokine, IL-10, in response to the inflammatory stimulus, lipopolysaccharide (LPS). To determine whether IVIg-activated macrophages can be used to treat intestinal inflammation, we adoptive transferred (IVIg$^+$LPS)-activated macrophages into mice or treated mice with IVIg during DSS-induced intestinal inflammation. Adoptive transfer of (IVIg$^+$LPS)-activated macrophages or IVIg treatment reduced clinical disease activity during DSS colitis and reduced

histological evidence of inflammation in mice. Moreover, macrophage depletion by clodronate-containing liposomes demonstrated that macrophages were required for the anti-inflammatory effect of IVIg treatment. In conclusion, IVIg activated macrophages have potent anti-inflammatory activity that can be used to reduce intestinal inflammation *in vivo*. Adoptive transfer of *in vitro*-derived (IVIg^{+}LPS)-activated macrophages or activating macrophages with IVIg *in situ* may provide novel effective therapies to treat intestinal inflammation in people with IBD.

T.78. The Fcγ Receptor IIA (FcγRIIa) Gene Variant Increases Macrophage Inflammatory Responses and Reduces Antibody-mediated Anti-inflammatory Macrophage Activation

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Inflammatory Bowel Disease (IBD) is an immune-mediated disease characterized by inflammation along the gastrointestinal tract. Anti-TNFα antibodies have revolutionized IBD treatment but 10% of patients are unresponsive to therapy and we cannot predict which patients will respond. A gene variant in the FcγRIIa, an antibody (IgG) receptor expressed on macrophages, predisposes people to develop IBD and has been linked to a failure to respond to anti-TNFα therapy. We have reported that macrophages treated with IgGs produce large amounts of the anti-inflammatory cytokine, IL-10, in response to inflammatory stimuli. Based on this, we asked whether the FcγRIIa risk variant acts by reducing anti-inflammatory Fcγ receptor-mediated macrophage activation. Healthy control study participants were genotyped for the FcγRIIa gene variant and their peripheral blood monocyte-derived macrophages (MDMs) were stimulated with lipopolysaccharide (LPS), pooled IgGs, or both, in the presence or absence of blocking antibodies to FcγRs. Macrophages from participants with the FcgRIIa risk variant produced more pro-inflammatory cytokines in response to LPS and were less able to reduce pro-inflammatory cytokine production when treated with IgGs. FcγRI, FcγRIIb and FcγRIII were required to limit immune responses. Intriguingly, the FcγRIIa susceptibility gene variant changes this receptor from a low to a high affinity receptor. Thus, our data are consistent with a model in which the high affinity FcγRIIa risk variant sequesters antibodies from the FcγRs that mediate anti-inflammatory macrophage activation. In future studies, we will investigate whether the FcγRIIa gene variant can be used to predict whether a patient will respond to anti-TNFα therapy.

T.79. Activated B Cell Receptor Signaling May be Regulated by CEACAM1

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Background & Aim: It has been recently shown that CEACAM1 expression in T cells may regulate immune responses in the gut. In addition to T cells, B cells are also one of the major populations in the gut-associated lymphoid tissues that orchestrate mucosal homeostasis. However the role of CEACAM1 in B cells has not been elucidated.

Methods & Results: FACS analysis of the lymphocyte subsets isolated from lymphoid tissues such as spleen, mesenteric lymph nodes and Peyer's patches of wild type C57BL6 mice revealed that CEACAM1 expression on B cell surface was more than that of T cells. Bone marrow analysis showed that CEACAM1 expression was increased during B cell maturation. When isolated B cells were stimulated with either LPS, anti-CD40, or anti-μ antibodies (Abs) in the presence of agonistic anti-CEACAM1 Ab, the increased IL-4 and IL-5 production by the activation via B cell receptor (BCR) signaling was specifically diminished by CEACAM1 signaling rather than B cell activation via TLR4 or CD40 signaling. Confocal microscopy revealed aggregated and co-localized CEACAM1 expression with the BCR expression when B cells were activated with anti-μ. Overexpression of CEACAM1 in a murine B cell line, A20, showed less expression of activation markers such as CD69, CD80, CD86, MHC-I and -II on the cell surface. This

was associated with less Ca^{2+}influx and suppressed cytokine production by the overexpression of CEACAM1 after BCR signal activation.

Conclusion: These results suggest that CEACAM1 may regulate B cell activation specifically via BCR signaling.

T.80. Identification of a New Subtype of Latency-associated Peptide (LAP)-**expressing γδ T cells**

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γδ T cells are a subset of lymphocytes specialized in protecting the host against pathogens and tumors. Here we describe a subset of regulatory γδ T cells that express the latency-associated peptide (LAP), a membrane-bound TGF-β1. Thymic $CD27^+IFN\text{-}\gamma^+CCR9^+\alpha4\beta7^+TCR\gamma\delta^+$ cells migrate to the periphery, particularly to Peyer's patches (PP) and small intestine lamina propria, where they up-regulate LAP, down-regulate IFN-γ via ATF-3 expression and acquire a regulatory phenotype. $TCR\gamma\delta^+LAP^+$ cells express antigen presentation molecules and function as antigen presenting cells that induce $CD4^+Foxp3^+$ regulatory T (Treg) cells though $TCR\gamma\delta^+LAP^+$ cells do not themselves express Foxp3. Moreover, in a T cell model of colitis induced by $CD4^+CD45RB^{high}$ cell transfer into immunodeficient mice (a rodent model for Crohn's disease) or in an innate immune-mediated model of colitis induced by oral administration of the chemical compound dextran sodium sulfate (DSS), transfer of $TCR\gamma\delta^+LAP^+$ cells ameliorated wasting disease by promoting the induction of Foxp3 Treg cells. Furthermore, Oral tolerance, a physiologic process that helps maintain gut homeostasis to the daily challenge of microbiota and dietary antigens, was impaired in γδ T cell deficient mice, probably due to a reduction in the $Foxp3^+$ and LAP^+ Treg cell population in both PP and spleen. Thus, identification of $TCR\gamma\delta^+LAP^+$ regulatory cells provides an avenue for understanding immune regulation and biologic processes linked to intestinal function and disease.

Innate Immunity

OR.29. The Transcriptional Landscape of Human Innate T Cells

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A substantial portion (10-20%) of the human T cell repertoire recognizes non-peptide antigens and is not restricted by MHC class I or II. Rather, these cells use a set of conserved TCRs to see a limited array of antigens such as lipids and small organic metabolites. Prominent examples of such cells include invariant natural killer T (iNKT) cells, mucosal-associated invariant T (MAIT) cells, and γδ T cells. Given their innate-like role as first-responders, we refer to these cell populations collectively as innate T cells (ITCs). ITCs are activated very early during immune responses, secreting diverse cytokines and engaging in cytotoxicity. In doing so, they play important roles in host defense, cancer, metabolic disease, autoimmunity, and allergy. The transcriptional programming underlying human ITCs' contributions to immunity has yet to be ascertained. To determine the transcriptional basis for the effector roles of human ITCs, we undertook low-input, high-depth RNAseq and flow-cytometric immunophenotyping of ITCs as well as adaptive and innate comparator cell populations. Through a series of bioinformatic analyses, we found that ITCs maintain transcriptional programming from their adaptive immune roots, yet additionally co-opt innate transcriptional modules associated with both lymphoid and unexpectedly, myeloid lineages. Different ITC populations share an innate activation scheme, but have divergent developmental requirements, tissue localization, and specific effector mechanisms. Inter-ITC comparison revealed shared and unique transcriptional programs that reflect their functional roles. Together, these studies define human ITCs as a distinct arm of the immune system that concomitantly utilizes both adaptive and innate transcriptional modules.

F.45. Modulation of Acute and Chronic Inflammation by the Mineralocorticoid Receptor in Animal Models

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The mineralocorticoid receptor (MR) is a ligand dependent transcription factor. MR has been conventionally related with the control of water and electrolyte homeostasis to keep blood pressure through aldosterone activation. However, MR is also expressed in cells of the immune system, where it responds to stimulation or antagonism, controlling immune cell function. We previously showed that dendritic cells (DCs) express and respond to MR stimulation with aldosterone by secreting proinflammatory cytokines that controls Th17 polarization. Currently, we are evaluating whether aldosterone induces a pro-inflammatory state in DCs by promoting overexpression of Toll like receptors (TLR). We found that aldosterone treated DCs express higher levels *tlr4* than MRKO DCs or DCs treated with MR antagonists. Also these cells express higher levels of CD86 and secreted higher amounts of IL-6 than controls. Finally in sub-lethal model of endotoxic shock, we found that pre-treated mice die earlier after LPS challenge in contrast to vehicle, mainly due to a multi-organic failure. Additionally, in a model of chronic inflammation, such as EAE, we observed that myeloid MR conditional knockout mice (MyMRKO) mice developed higher clinical EAE scores compared to control. MyMRKO showed a higher percentage of $CD45^+$ leukocytes infiltrating the central nervous system (CNS), principally $CD45^+CD8^+$. *In vitro* co-cultures showed higher levels of IL17A and IFNγ secretion from $CD8^+$cells stimulated with MyMRKO DCs than control. In conclusion, our data suggest that aldosterone and its receptor play an important role controlling the function of immune cells in acute and chronic diseases.

F.56. A New Function for DNA: Immunoregulation

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Soluble DNA circulates in the peripheral blood of healthy subjects, and, at increased concentrations, of pregnant women and patients affected by tumors and vasculitis. Since the discovery of intracellular DNA sensors, inducing inflammatory responses after binding to DNA, free DNA has been included among danger signals. However, this view does not explain the presence of abundant circulating DNA could in conditions characterized by tolerance (pregnancy) or immunodeficiency (tumor). In order to verify whether DNA mediates is also an immunoregulating activity, two different 20 base-pair long oligonucleotides (poly-AT and poly-CG) were synthesized and tested for their capacity to: 1) bind MHC class II molecules; 2) exert immunoregulating activity. The oligos binding to PMJ2-PC macrophages was inhibited by anti-MHC monoclonal antibodies (mAb). The specific binding of DNA to MHC class II molecules was confirmed by western blot analysis. Proliferation of OVA-specific splenocytes was inhibited by incubation with the oligos; moreover, fluorescent oligos, injected intravenously to NZB/W F1 mice, bound only on MHC class II positive lymphocytes. Finally, pre-nephritic NZB/W F1 mice, administered weekly with either of the two oligos, showed delayed onset of protenuria and improved survival with respect to untreated animals, a phenomenon associated with increased frequencies of Breg and Treg. These data demonstrate for the first time that free DNA mediates immunoregulating activities.

F.57. Human Aortic Endothelial Cells as Important Immune Cells

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Vascular endothelial cells have many important physiological functions. However, their role as important immune cells has not been fully appreciated. Endothelial cells are the first line of cells to encounter pathogen-associated molecular patterns and endogenous danger signals in circulation. We utilized human primary aortic endothelial cells

(HAECs) and a newly developed HAEC cell line to examine innate immune functions *in vitro*. The HAEC cell line was generated by immortalization with the reverse transcriptase component of telomerase (TERT) and the E6/E7 genes of human papillomavirus and was found to be phenotypically and functionally similar to primary cells. HAECs expressed CD31, VE-Cadherin, von Willebrandt factor, and endothelial nitric oxide synthetase; internalized acetylated LDL, and formed tubes. Additional phenotypic analysis revealed that HAECs express high levels of LPS receptor TLR4 and co-receptor CD14; and intermediate levels of MHC-II and co-stimulatory molecule CD40. Stimulation of HAECs with LPS induced expression of IL-8 and IL-6 cytokines and caused upregulation of VCAM-1 and ICAM-1 in a dose-dependent manner. HAECs secreted IL-8 in response to stimulation with TNFα and IL-1β cytokines, but not with IFNγ, IL-2, and IL-6. Altogether, this data demonstrates that HAECs express numerous surface immune molecules and strongly respond to LPS and some inflammatory cytokines. Immune activation of HAECs may result in HAEC dysfunction, causing cardiovascular and systemic pathologies. Results of this study confirm HAECs as important immune cells and may aid in elucidating the role of HAECs in pathogenesis of systemic immune-mediated diseases.

F.58. Interleukin-17 Promotes Neutrophil-mediated Immunity by Activating Microvascular Pericytes and Not Endothelium

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A classical hallmark of acute inflammation is neutrophil infiltration of tissues, a multi-step process that involves sequential cell-cell interactions of circulating leukocytes with interleukin (IL)-1- or tumor necrosis factor-α (TNF)-activated microvascular endothelial cells (ECs) and pericytes (PCs) that form the wall of the postcapillary venules. The initially infiltrating cells accumulate perivascularly in close proximity to PCs. IL-17, a pro-inflammatory cytokine that acts on target cells via a heterodimeric receptor formed by IL-17RA and IL-17RC subunits, also promotes neutrophilic inflammation but its effects on vascular cells are less clear. We report here that both cultured human ECs and PCs strongly express IL-17RC and, while neither cell type expresses much IL-17RA, PCs express significantly more than ECs. Neither IL-17RA nor IL-17RC expression is altered by inflammatory cytokines on either cell type. IL-17, alone or synergistically with TNF, significantly alters inflammatory gene expression in cultured human PCs but not ECs. RNA-seq analysis identifies many IL-17-induced transcripts in PCs encoding proteins known to stimulate neutrophil-mediated immune responses. Conditioned media from IL-17-activated PCs, but not ECs, induce pertussis toxin-sensitive neutrophil polarization, likely mediated by PC-secreted chemokines, and also stimulate neutrophil production of pro-inflammatory molecules, including TNF, IL-1α, IL-1β, and IL-8. Furthermore, PCs but not ECs can prolong neutrophil survival through IL-17-induced production of G-CSF and GM-CSF. We conclude both that PCs, not ECs, are the major target of IL-17 within the microvessel wall and that IL-17-activated PCs can modulate neutrophil functions within the perivascular tissue space.

F.59. Regional Microglia Heterogeneity in Healthy Aging and Alzheimer's Disease

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Recent genome wide association studies implicated the innate immune system in the pathogenesis of late onset Alzheimer's disease. The resident immune cells of the brain, called microglia, belong to the innate arm of immunity. Microglia are believed to be intimately involved in the disease pathogenesis of AD but the exact nature of their contribution has not been characterized yet. Similarly unexplored is the notion that differences might exist in susceptibility to aging between cerebral white and grey matter microglia. Therefore, we set out to systematically

investigate the phenotypic differences between white and grey matter microglia in the aged brain and how this is affected by AD.

We performed RNA sequencing of 22 paired (white and grey matter) microglia samples prepared from healthy aged and Alzheimer's disease human brains.

Gene ontology analysis of the resulting RNAseq dataset revealed that GO terms, which were significantly enriched in differentially expressed genes between white and grey matter microglia included (but were not limited to) processes such as cell-matrix adhesion (GO:0007160; p = 0.0003), intrinsic component of plasma membrane (GO:0031226; p = 0.0007), negative regulation of cell proliferation (GO:0008285; p = 0.0008) and cytokine production (GO:0001816; p = 0.004). Furthermore, we found that AD pathology in itself can significantly amplify already existing differences between white and grey matter microglia. Our results show that the aged human white and grey matter harbor phenotypically dissimilar microglia populations that respond to the presence of pathology in a region specific manner - and thus might represent distinct therapeutic targets.

F.60. Complement Component C1q is a Key Negative Regulator of Osteoarthritis Pathogenesis in Mice

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Osteoarthritis, the most common form of arthritis in the world, is characterized by articular cartilage breakdown in synovial joints. Although there is evidence of low-grade inflammatory responses in osteoarthritis, the precise mechanisms by which inflammation contributes to pathogenesis remain unclear. Previous studies from our laboratory have shown that osteoarthritis involves dysregulated complement activity. The upstream complement component, C1q, is known to regulate inflammation via mechanisms involving apoptotic cell clearance and macrophage activation. To test the hypothesis that C1q downregulates inflammation and attenuates osteoarthritis progression, we surgically-induced osteoarthritis by destabilization of the medial meniscus in C1q-deficient (*C1q*$^{-/-}$) and wildtype (WT) mice. Consistent with the hypothesis, we found that *C1q*$^{-/-}$ *mice develop* exacerbated cartilage damage, osteophyte formation, and synovitis relative to WT controls. To pinpoint the mechanisms by which C1q limits osteoarthritis pathology, we investigated the effects of C1q on *in vitro* differentiated human macrophages using a combination of multiplexing technologies including NanoString-based mRNA quantification, bead-based cytokine profiling, and phospho-immunoreceptor antibody arrays. We found that macrophages differentiated in C1q-depleted serum exhibited an enhanced pro-inflammatory and activated phenotype. Upon stimulation with cartilage debris, these macrophages secreted increased levels of pro-inflammatory (e.g. IL1β) and reduced anti-inflammatory (e.g. IL10) cytokines compared to macrophages grown in the presence of C1q. Finally, we found that C1q elicits its immunoregulatory role on macrophages via phosphorylation/activation of the ITIM-containing receptor, leukocyte associated immunoglobulin like receptor 1 (LAIR1). Together, our data suggest that C1q regulates inflammation and limits cartilage damage in osteoarthritis in part by modulation of macrophage activation/polarization.

F.61. Chemical and Genetic Approaches to Study CD33 Fu**nction in Alzheimer's Processes**

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Recent GWAS have identified common variants in the CD33 locus, a myeloid specific immune receptor, as a risk factor for Alzheimer's disease (AD). The risk allele is associated with increased expression of CD33, which contains intracellular inhibitory motifs and an extracellular sialic acid binding domain, leading to decreased myeloid cell function (i.e. amyloid uptake). Little is known, however, about the downstream biochemical mechanisms and molecular players involved in this process. Here we use chemical and genetic methods to dissect the molecular

circuitry surrounding CD33 function and propose a potential therapeutic strategy to improve myeloid function by perturbing this pathway.

F.62. MicroRNA-101a Regulates Microglial Development and Inflammation

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Backgrounds: Microglia are the resident immune cells in central nervous system (CNS), belong to the mononuclear phagocyte lineage. Microglia have a crucial roles in CNS development and homeostasis, and are expected to be a therapeutic target for neurodegenerative diseases or neuroinflammatory diseases. microRNAs are small non-coding RNAs that function as guide molecules in RNA silencing. Certain microRNAs were reported to regulate differentiation and activation of microglia. Previously, we reported that lineage-negative bone marrow cells co-cultured with astrocyte differentiated into microglia-like (ML) cells. It was suggested that some secreted factors including microRNA enriched in CNS may play a role in this process. We tried to identify microRNA associated with the development of microglia using *in-vitro* co-culture model.

Methods: Murine lineage-negative bone marrow cells-astrocytes co-culture was treated with microRNA inhibitors or mimics for 7 days. We analyzed the numbers, shapes, cytokine production and mRNA expression in the presence of microRNA inhibitors or mimics.

Results: Using a panel of microRNA inhibitor library, we identified several microRNA inhibitors which changed the numbers or shapes of ML cells. Among them, miR-101a inhibitor decreased the number of ML cells, while miR-101a mimic increased them. miR-101a significantly increased the secretion of IL-6, and decreased the secretion of IL-1β from ML cells. These results suggest that miR-101a regulate microglial inflammation through several diverse pathways.

Conclusion: miR-101a promoted the differentiation of bone marrow cells into ML cells, and modulated inflammation.

F.63. Microarray and Whole-exome Sequencing Analysis of Familial **Behçet's** Disease Patients

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Behçet's disease (BD), a chronic systemic inflammatory disorder, is characterized by recurrent oral and genital mucous ulcers, uveitis, and skin lesions. We performed DNA microarray analysis of peripheral blood mononuclear cell (PBMC) mRNA from 41 Japanese BD patients and revealed elevated levels of interleukin (IL) 23 receptor (*IL23R*) mRNA in many BD patients. DNA sequencing around a SNV (Rs12119179) tightly linked to BD revealed an elevated frequency of the C genotype, consistent with a previous report that *IL23R* is a susceptibility locus for BD. Notably, four of these BD patients are members of familial BD; a whole-exome sequencing (WES) of these BD patients identified 19 novel single-nucleotide variations (SNVs) specific to these patients. They include heterozygous SNVs in the genes encoding IL-1 receptor-associated kinase 4 (*IRAK4*), nucleotide-binding oligomerization domain (NOD)-like receptor family pyrin domain-containing 14 (*NRP14*) and melanoma antigen-encoding gene E2 (*MAGEE2*); *IRAK4* harbors a missense mutation, whereas *NRP14* and *MAGEE2* harbor nonsense mutations. These SNVs may serve as genetic markers that characterize BD.

F.64. Inhibition of TLR7 and TLR8 Stimulation by Exosomal micro-RNA Using a Novel Antago-miR Cocktail Suppresses Inflammation in a Human-mouse Chimeric Model of Lupus

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Toll-like receptor (TLR)7 and TLR8 are innate immune system receptors that bind to single-stranded RNA sequences. We have previously demonstrated that TLR7 and TLR8 are significantly up-regulated in peripheral blood mononuclear cells (PBMCs) of patients with systemic lupus erythematosus (SLE) and induced with estrogen treatment. While the conventional role of TLR7 and TLR8 involves surveilling for RNA of viral origin, recent work has identified specific micro-RNAs (miRs) capable of activating these receptors that are packaged and secreted in exosomes. In this study, we examined the potential therapeutic strategy of antagonizing TLR7 and TLR8 inflammatory pathways by blocking miRs known to stimulate these receptors. A human-mouse chimeric model of SLE was created by adoptively transferring PBMCs from active SLE patients into immunodeficient NOD-scid IL-2rγ(null) mouse recipients. To block miR-induced inflammation through TLR7 and TLR8, PBMCs were transfected with a cocktail of locked nucleic acid miR antagonists or a nonsense control. While human T cells ($CD4^+$ and $CD8^+$), B cells, monocytes, and NK cells were all successfully recovered from whole blood of chimeric mice at similar levels, expression of human IL-2, IL-6, IL-10, and TNF-α was reduced with miR inhibition when compared to control treatment. Histopathological examination by H&E showed a robust inflammatory response in the small intestine, liver, and kidney with control treatment, which was markedly reduced with miR inhibition. Moreover, immunohistochemical analysis confirmed human $CD3^+$ T cells within the infiltrates. These results establish a novel chimeric model to study SLE and to further therapeutically develop miR-antagonists inhibiting TLR7 and TLR8 inflammatory signaling.

Organ Transplantation

OR.06. IL2 and IL-15 Rescue TEMRA CD8 T Cells From Mitochondrial Dysfunction Associated Senescence Through a p38MAPK Dependent Pathway and Foster Their Pathogenic Potential

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We recently showed that an accumulation of highly differentiated TEMRA CD8 T cells in kidney transplant recipients with a stable graft function is associated with an increased risk of kidney graft dysfunction. Because CD8 T cell response regulation is important for the rational design of immune-based strategies to treat transplant recipients, we aimed to identify signaling pathways involved in regulation of the survival and the activation of TEMRA CD8 T cells.

Cytokines (IL-2/7/15) and TCR stimulation were added alone or in combination to purified naïve ($CD45RA^+CD28^+$), TEMRA ($CD45RA^+CD28^-$) and EM early ($CD45RA^-CD28^+$) CD8 T cells. Survival (DAPI), proliferation (Cell Fluorescent Dye), activation (CD25 and CD69) were monitored. Signaling pathways were screened using phospho-mAbs. Finally, modification of mitochondrial membrane potential was assessed using MitoTracker Red and JC-1.

TEMRA CD8 efficiently responds to short-term stimulation with IL-2 and IL-15 as shown by the phosphorylation of STAT5 and S6 Kinase. IL-2 and IL-15 are able to prevent TEMRA CD8 cell death, by preventing the depolarization of the mitochondrial membrane potential. Whereas inhibition of mTOR does not alter the pro-survival signal from IL-2 or IL-15, inhibition of p38MAPK and Erk blunts this beneficial signal. In combination with TCR signals, IL-2 and IL-15 are able to efficiently activate TEMRA CD8 as shown by cell proliferation and upregulation of CD25 and CD69.

In contrast to their view as senescent cells, we provide evidences that TEMRA CD8 shares similar regulation of survival and function with EM cells and strengthen the need to re-assess their pathogenic role.

F.65. Identification and Validation of Non-HLA Antibodies in Cardiac Transplant Recipients

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Rejection in HLA matched donors and the discovery that antibodies directed against proteins expressed by endothelial cells (EC) are associated with an increased risk of transplant rejection emphasizes the importance of further characterization of non-HLA antibodies (Ab) in allograft rejection. Currently, rejection is diagnosed by biopsy findings. A non-invasive screen to detect early signs of allograft rejection due to non-HLA Ab and a better understanding of non-HLA Ab is greatly needed. We investigated the relevance of non-HLA Ab in 168 cardiac allograft recipients at UCLA transplanted in 2001-2008. Pre and post-transplant sera were tested for non-HLA Ab by flow cytometric endothelial cell cross match on a panel of primary human aortic EC (HAEC). Rejection was classified by pathological examination of the biopsy according to ISHLT diagnostic criteria. We found that 37/168 (22%) patients were diagnosed with AMR. Of these, 22 (60%) developed HLA DSA. In the subgroup of 37 patients, 30% of them presented with antibodies directed to non-HLA antigens. This study revealed an association between the presence of non-HLA Ab and risk of rejection. Protoarray analysis of 9000 human protein targets (Invitrogen) from 18 cardiac transplant recipient samples was used to identify non-HLA proteins that included 22 membrane proteins and 10 autoantigens. Non-HLA targets are being validated with a multiplex bead array platform testing both novel targets identified and previously published non-HLA targets.

F.66. Kidney Allotransplantation Induces the Differentiation of Antigen-experienced CD8 T Cells With Enhanced Metabolic Profiles

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Naïve and memory CD8 T cells have been shown to exhibit different metabolic profiles, especially upon antigen stimulation. In this report, we aimed to characterize the use of ATP upon antigen-stimulation and to identify the bio-energetic processes of ATP production in CD8 lymphocytes in healthy volunteers and in kidney transplant recipients.

ATP level of CD8 subsets (naïve; effector memory, EM; TEMRA) from healthy volunteers (HV) and patients with stable kidney graft (TX) was quantified before and after polyclonal stimulation (PMA&Iono or aCD3 +/- IL-2 or IL-15). Mitochondria polarization was assessed using MitoTrackerRed and JC-1. CD25 and CD69 expression was monitored after 2 days of culture with the aforementioned stimuli in the presence of inhibitor of glycolysis (2-DG) or glutaminolysis (DON).

Antigen-experienced CD8 (EM and TEMRA) exhibit a greater ATP reservoir as compared to naïve CD8 in HV whereas CD8 from TX patients exhibit similar level of ATP across the different subsets. Of interest, antigen-experienced CD8 from TX are able to reconstitute more efficiently their ATP pool as compared to those of HV upon polyclonal stimulation. Antigen-experienced CD8 from TX exhibit a greater amount of well-polarized mitochondria as compared to those of HV. Finally, we report that CD8 TEMRA preferentially use glutamine-based metabolism whereas CD8 EM preferentially use glycolysis.

Kidney transplantation results in the differentiation of antigen-experienced CD8 T cells with a metabolic machinery fitted to sustain strong stimulation. Metabolic interferences can efficiently control pathogenic CD8 T cells from TX but need to be adjusted according to the immune challenge.

F.67. Systemic Alloreactive T Cell Responses Following Lung Transplantation

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Background: Relative to other solid organs, rejection is highly prevalent following lung transplantation, and is a major barrier to long-term survival. Because regulatory T cells (Treg) limit rejection, we hypothesized that donor-reactive Treg increase after transplantation with development of partial tolerance and decrease relative to conventional $CD4^+$ (Tconv) and $CD8^+$ T cells during acute cellular rejection.

Methods: We prospectively collected 177 PBMC specimens from 39 lung transplant recipients at the time of transplantation and during bronchoscopic assessments for acute cellular rejection. We quantified Treg, $CD4^+$ Tconv, and $CD8^+$ T cells proliferating in response to donor-derived cells. We used generalized estimating equation-adjusted regression and t-tests to compare donor-reactive T cell frequencies with HLA mismatches, donor-specific antibodies (DSA), and acute cellular rejection pathology.

Results: Although donor-reactive T cell frequencies were largely stable following transplantation in individual recipients, increased donor-reactive $CD4^+$ Tconv frequencies were associated with higher numbers of HLA mismatches ($P = 0.01$), and pre-transplant DSA were associated with increased Treg frequencies at time of transplantation ($P = 0.03$). Donor-reactive Treg, $CD4^+$ Tconv, and $CD8^+$ T cell frequencies increased during A2-grade rejection compared with no rejection ($P \leq 0.01$).

Conclusions: Contrary to prediction, overall proportions of donor-reactive Treg are similar before and after transplantation and increase during A2-grade rejection. We found no evidence of donor-reactive lymphocyte deletion following lung transplantation, contrasting with reports in the liver transplant setting. The high proportions of donor-reactive Treg baseline and during acute rejection have implications for therapeutic manipulation of T cell populations.

F.68. Donor and Recipient Contributions to Innate and Adaptive Immune Signaling in Orthotopic Liver Transplantation Ischemia Reperfusion Injury

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Orthotopic liver transplant (OLT) success is hampered by cellular damage elicited by ischemia reperfusion injury (IRI) which lowers allograft survivals. Seminal observations in murine models indicate that OLT-IRI is mediated by the complex interplay between the innate and adaptive arms of the immune system. To understand the contributions of donor and recipient innate and adaptive immune signaling in OLT-IRI, we collected blood from the portal vein before (PV; recipient contribution only) and after being flushed through the donor liver (LF; recipient and donor contribution) during reperfusion. Biopsies were collected post-reperfusion and graded for IRI by histopathology (n=50; 52%=IRI^+ (26); 48%=IRI^- (24)). PV blood from IRI^+ patients showed increases in IL-3 and IL-8 that remained high in LF samples. However, LF from IRI^+ patients also showed significant increases in IL-2, IL-12 and IL-1Ra that were not seen in PV blood samples. These results indicate that innate cytokines (IL-2 and IL-8) are contributed by both donor and recipient, whereas early increased levels of cytokines with a strong potential to shape the adaptive immune response (IL-2 and IL-12) come initially from the injured donor tissue. Additionally, high levels of IL-1Ra could strengthen a conversion to the adaptive system in IRI^+ patients by dampening the innate response. Notably, IL-3 and IL-8 were also increased in IRI^+ patient's pre-operative circulating blood samples and therefore could potentially be used as a non-invasive predictor of future IRI severity in OLT recipients. Taken together these results could lead to more targeted therapeutic strategies to reduce IRI in OLT.

F.69. Targeting of Antibody-conjugated Polymeric Nanoparticles to Vascular Endothelium During *Ex Vivo* Normothermic Perfusion of Human Kidney

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A shortage of transplantable organs remains the most significant issue facing patients with end stage kidney failure. Recently, normothermic *ex vivo* perfusion (EVNP) has been shown to restore renal function of marginal kidneys, thereby potentially expanding the pool of available organs. However, these 'extended criteria' kidneys will undoubtedly hasten many new immunosuppression challenges and necessitate increasing therapeutic sophistication. One approach involves delivering therapeutics during EVNP. To take full advantage of this short (typically ~1 hr) therapeutic window, we hypothesize that antibody-conjugated polymeric nanoparticle drug carriers can provide rapid and complete coverage of renal endothelium and further act as sustained delivery depots to provide a prolonged therapeutic effect even after transplantation. Here we develop an antibody-conjugated nanoparticle platform capable of targeting vascular CD31 in human kidneys undergoing EVNP. We utilized *in vitro* culture of human endothelial cells to quantitatively establish the targeting kinetics of anti-CD31 nanoparticles under both static and flow conditions. These assays demonstrate that achieving robust nanoparticle targeting is not instantaneous, but can require several hours to build to significant levels when using therapeutically relevant doses. Guided by our *in vitro* work, we administered escalating doses of CD31-targeted (or isotype) nanoparticles within human kidneys undergoing EVNP and evaluated targeting specificity and kinetics via quantitative imaging of wedge biopsies sampled at various time points. Our results demonstrate the viability of utilizing CD31-targeting to deliver nanoparticles within a human kidney undergoing EVNP and also solidify the critical importance of accurately characterized association kinetics for the appropriate use of molecularly targeted nanoparticles.

Reproductive Immunology

F.70. DC-10 are Central Determinants in IL-10- and HLA-G-mediated Tolerance

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DC-10, a subset of human tolerogenic dendritic cells, are key players in promoting T regulatory type 1 (Tr1) cells, well known to participate in the immune homeostasis and in conferring peripheral tolerance. DC-10, present *in vivo* and inducible *in vitro* in the presence of IL-10, promote Tr1 cells *via* the IL-10-induced HLA-G/ILT4 pathway. HLA-G is a non-classical HLA class I molecule expressed by immune cells with well-recognized immune-modulatory functions. Here, we defined the role of IL-10 and HLA-G in DC-10-mediated tolerance *in vitro*. Being cells that naturally express HLA-G and secrete IL-10, we hypothesized that DC-10 might represent one of the major subsets of antigen-presenting cells present in the decidua during pregnancy.

Here we demonstrated that HLA-G expression on DC-10 is donor-dependent, and high HLA-G on DC-10 potentiates the IL-10-mediated induction of $CD49b^+LAG\text{-}3^+$ Tr1 cells. DC-10 accumulate in decidua in the first trimester of pregnancy; and a low percentage of DC-10 is detected in decidua of women with recurrent miscarriage. In addition to DC-10, in decidua we detected a subpopulation of regulatory HLA-G-expressing $CD4^+$ T cells, which can be either recruited from the circulation or induced in situ by DC-10.

These findings define the important contribution of IL-10 and HLA-G in promoting tolerance *via* DC-10, demonstrate for the first time the presence of DC-10 in an immune-privileged site such as the fetal-maternal interface, and indicate that DC-10 contribute to induce a tolerogenic microenvironment being cells that naturally express IL-10 and HLA-G, and that can induce IL-10 and HLA-G on neighboring cells.

F.71. Is Human Pregnancy Inherently Inflammatory or Anti-Inflammatory?
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Linkages between maternal health and subsequent development of allergic, autoimmune or other chronic inflammatory phenotypes in children are complex and controversial. Much is known of the immunology of the maternal-fetal interface, but basal innate immune status *in vivo* of women during and immediately following healthy pregnancy is largely unstudied. Whether a bias exists during healthy pregnancy towards inflammatory phenotypes (transiently enhancing host defense) or anti-inflammatory phenotypes (reducing potential responses to the fetus) needs to be determined. Here, in a longitudinal study of 250 healthy women giving birth to full term healthy infants, systemic innate immune status was examined during the $2^{nd}/3^{rd}$ trimester and again one and, in a smaller subgroup, three years postpartum. Following REB approval and informed consent, pro and anti-inflammatory plasma biomarkers were quantified. Constitutive CCL2, CXCL10 and TNFa levels were sharply reduced ($p<0.003$ to 0.0001) during pregnancy. Several anti-inflammatory biomarkers were elevated (sTNFRI, sTNFRII, IL-1Ra, all $p<0.0001$). Plasma IL-10, evident in >85% of the population, was not altered during/post pregnancy. Kinetic studies revealed that pro-inflammatory biomarker expression (CXCL10, CCL2, CXCL8, IL-18, TNFa) was independent of gestational age. Conversely, the intensity of anti-inflammatory responses increased with increasing gestational age (Spearman $p<0.0003$). In summary, among women experiencing healthy full term pregnancies, basal systemic immunity is characterized by an increasingly intense bias towards an anti-inflammatory innate immune phenotype that is resolved by one year postpartum. Support: CRC, CIHR, AllerGen NCE

Therapeutics/Pharmacology

OR.53. Alefacept Augments PD-1^+ CD4 Memory T Cells of Recent-onset T1D Subjects in an FcR-Dependent Manner
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In those newly diagnosed with type 1 diabetes (T1D), treatment with alefacept (LFA3-Fc fusion protein that targets CD2) preserved endogenous insulin production with effects lasting over a year off therapy. Immunological changes observed in the alefacept arm included depletion of CD2hi CD4 and CD8 memory T cells and $CD56^{hi}$ NK cells, preservation of Tregs, and an increase in PD-1^+ CD4 memory cells in peripheral blood. To investigate the mechanisms of alefacept, we designed *in vitro* studies. Culture of PBMC from 8 healthy and 5 T1D subjects with alefacept reduced the proportion of $CD2^{hi}$ cells (memory T, NK) by an average of 96% within 24 hours, with concomitant activation of $CD56^{lo}CD16^{hi}$ NK cells, while $CD25^{hi}CD2^{lo}$ Tregs did not change. The frequency of PD-1^+KLRG1^+ cells within the CD4 memory population increased with alefacept 2.4 fold and positively correlated with $CD56^{lo}CD16^{hi}$ NK cell activation. However, this was not observed when PBMC were depleted of PD-1^+ cells prior to culture, when alefacept was cultured with isolated CD4 T cells, or when FcR was blocked. Together, these data suggest preferential survival and/or expansion of pre-existing PD-1^+KLRG1^+ T cells in the presence of alefacept that likely requires FcR. Thus, in addition to depletion of CD2hi cells, our data support a possible agonistic role for alefacept resulting in increased PD-1^+ memory T cells. Future *in vitro* and *ex vivo* studies will further define underlying mechanisms of increased PD-1^+ cells and functional characterization of these cells. Better understanding the effects of alefacept will lead to improved treatments for T1D.

OR.54. Inhibition of the Potassium Channel Kv1.3 by Dalazatide, a Potential Immunotherapy Strategy for

Systemic Lupus Erythematosus

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The production of autoantibodies and immune complex deposition are hallmarks of systemic lupus erythematosus (SLE). Autoreactive effector memory T cells (T_{EM}), however, are also implicated in the immunopathogenesis of SLE. The production of inflammatory cytokines by T_{EM} cells requires expression of Kv1.3, a potassium channel regulating intracellular calcium levels. Treatment with dalazatide (ShK-186), a specific, highly potent peptide inhibitor of Kv1.3, has recently shown clinical benefits in treating patients with plaque psoriasis in a Phase 1 trial. In the study presented here, we characterized the expression of Kv1.3 and evaluated the effect of dalazatide on the production of inflammatory cytokines by phorbol myristate acetate (PMA)-activated T cells from the peripheral blood of pediatric and adult patients with active or inactive SLE. Kv1.3 expression by $CD8^+$ T_{EM} cells from patients with active SLE was significantly higher when compared to T cells samples from patients with inactive SLE or healthy controls. There was no significant difference in the expression of Kv1.3 by $CD4^+$ T_{EM} cells from patient or healthy control groups. Dalazatide at concentrations of 10 pM to 1 nM inhibited proinflammatory cytokine (IFN-γ, TNF-α, IL-17) production by PMA and ionomycin-activated $CD4^+$ and $CD8^+$ T_{EM} cells from SLE patients in a dose-dependent manner. Notably, IFN-γ and TNF-α expressing $CD4^+$ T_{EM} cells from patients with active SLE were more sensitive to the dalazatide-mediated cytokine inhibition than T cells from patients with inactive disease. The results of this initial study warrant further exploration of Kv1.3 as a target for dalazatide immunotherapy against SLE.

OR.55. Development of a MALT1 Inhibitor for the Treatment of Autoimmune Disease

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The human paracaspase MALT1 plays a central role in activation of NF-κB signaling downstream both B cell and T cell receptor and is thus an attractive target for therapeutic immunomodulation in autoimmune diseases. The mechanisms of MALT1 promoted NF-kB activation is based on both its scaffold and proteolytic functions.

Surprisingly, spontaneous development of destructive autoimmune inflammation has been reported in the genetically modified knock-in (ki) mice expressing a proteolytic inactive form of MALT1. In these models, inactivation of proteolytic activity of MALT1 results in decreased numbers of regulatory T cells and excessive production of interferon gamma (IFN-ɣ) by hyperactivated autoreactive $CD4^+$ T cells.

To address these issues, we explored the effect of MALT1 *in vitro* inhibition on human T cell subpopulations, B cells and human dendritic cells using allosteric MALT1 inhibitors.

We showed that whilst allosteric inhibition of MALT1 decreased *de novo* differentiation of T regulatory cells *in vitro*, it did not result in a complete dysfunction of thymic derived human Tregs. Moreover, MALT1 pharmacological inhibition decreased the activation and proliferation of effector Th1 and Th17 cells as well as their cytokine production. MALT1 inhibitors also decreased T cell induced B cell activation *in vitro*.

These results increase our understanding of the mechanisms and functional consequences of MALT1 inhibition. Our findings also suggest that MALT1 inhibition could be an attractive target for the treatment of T and B cell driven autoimmune inflammatory diseases.

W.83. Chronic Myelogenous Leukemia Treatment. A Review

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Background: Chronic myelogeous leukemia(CML) treatment is a frequently updated field. This is a review of current recommendations on CML treatment. Three of tyrosine kinase inhibitors(TKIs) are recommended as first line therapy(FLT): imatinib, dasatinib, nilotinib. DASISION study compared dasatinib 100mg,daily and imatinib400mg,daily as FLT for CML and showed a complete cytogenic response(CCyR) 77(dosatinib) vs 67(imatinib) by 12 months($p<0.05$). ENESTnd study compared nilotinib 300mg,twice daily and imatinib 400,daily and showed CCyR 87(nilotinib) vs 77(imatinib) by 24 months($p<0.05$). Evidence on long-term outcomes with these TKIs is lacking. The change of therapy is required in two circumstances: FLT side effects or resistance to FLT. In first case any of the approved TKIs can be used as second line therapy(SLT). If resistance is the reason for SLT, then there is no place for imatinib, only second-generation TKIs should be used. There are no large studies comparing different TKIs in SLT, this is why choice of SLT is often guided by the side-effects profile and patients characteristics. BCR-ABL-kinase-domain mutations should also be taken into account. T3151-mutation makes patients resistant to bosutinib, dastinib, nilotinib. The PACE trial suggests that pronatinib is effective in patients with a high level of TKI resistance, including those with the T1351-mutation. There are no evidence-based recommendations on third line treatment today.

Conclusion: When SLT fails, a trial of potaninib is appropriate and stem cell transplant is often employed. Recently there has been a shift in CML-treatment goal from remissions with life long therapy to a treatment-free remission. Treatment discontinuation studies are being conducted.

F.72. Immunosuppresive Effects of the Methanolic Extract of *Chrysophyllum cainito* Leaves on Macrophage Functions

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Actually, few studies have showed the pharmacological properties of *Chrysophyllum cainito*, including on the immune system. Then, the aim of this work was to evaluate the immunomodulatory effect of *C. cainito* leaves over the macrophages functions. Peritoneal murine macrophages isolated from Balb/c mice were treated with the methanolic extract from *C. cainito* leaves and stimulated with lipopolysaccharides. The effect on the phagocytosis was evaluated by flow cytometry assay. The nitric oxide and hydrogen peroxide production was measured by the Griess reagent and phenol red reaction, respectively. Levels of IL-6 and TNF-α was measured using an ELISA kit. The viability of macrophages and Vero cells were evaluated by the MTT method. All the experiments were done in triplicate and the results were expressed in mean ± SD. Data were analyzed by using one-way ANOVA with Dunnett *post hoc* tests and levels of $p<0.05$ were used as criterion of statistical significance. The results exposed that the methanolic extract from *C. cainito* leaves significantly inhibited the phagocytosis, IL-6 and TNF-α production, as well as decreased the nitric oxide and hydrogen peroxide production release by the macrophages, in a concentration dependent manner. The viability assays did not show cytotoxic effect over the cells. Those results suggest that *C. cainito* leaves possesses an immunosuppressive effect, without affect the cell viability. Many metabolites are present in the *C. cainito* leaves, whereby is possible that these molecules are implicated in the immunosuppressive effects. However, more phytochemical and pharmacological studies are necessary.

F.73. Targeting the PDE8A-Raf-1 Kinase Signaling Complex to Treat Autoimmune Inflammation

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The levels of cAMP are regulated by phosphodiesterases (PDE), which are targets for the treatment of inflammatory disorders. We previously showed that PDE8A regulates T cell motility. We report here that PDE8A exerts its control

of T cell function through the Raf-1 kinase signaling pathway. To mimic T cell migration under physiologic conditions, we performed flow chamber assays. The highly PDE8-selective enzymatic inhibitor PF-04957325 significantly suppresses rolling and adhesion of *in vivo* MOG_{35-55} activated inflammatory $CD4^+$ T effector (Teff) cells while interacting with inflamed brain endothelial cells under shear flow conditions. Recently, PDE8A was shown to associate with Raf-1 creating a compartment of low cAMP around Raf-1 thereby protecting it from protein kinase A mediated inhibitory phosphorylation. To test this in Teff cells, we used a cell permeable peptide (DP) that disrupts the PDE8A-Raf-1 signaling complex. Disruption of the PDE8A-Raf-1 complex by DP significantly reduces adhesion of Teff cells to endothelial cells. We further observed that disrupting PDE8A-Raf-1 through DP specifically reduces adhesion, spreading and locomotion of Teff cells while interacting with the vascular adhesion molecule ICAM-1. Our investigation of the effect of PDE8 inhibitor on chronic and relapsing-remitting experimental autoimmune encephalomyelitis (EAE) *in vivo* indicate suppression of clinical and histopathological signs of disease. Collectively, our studies demonstrate that PDE8A inhibition by enzymatic inhibitors or PDE8A-Raf-1 kinase signaling complex disruptors significantly decreases Teff cell adhesion and migration on endothelial cells, and represents a novel approach to treat autoimmune inflammation *in vivo*.

F.74. An IL-7 Receptor Blocking Antibody Increases the Ratio of Tregs to Effector Memory T Cells (T_{em}) and Increases PD-1 Expression on T Cell Subsets in the Peripheral Blood of Patients with Type 1 Diabetes

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IL-7 receptor-a (IL-7R) signaling is crucial for T cell development and survival, particularly after encounter with antigen. RN168 (PF-06342674) is a human IgG1 antibody that blocks IL-7 binding to IL-7R. In the ongoing B4351003 clinical study, thirty-seven (30 active + 7 placebo) adult subjects diagnosed with type 1 diabetes (T1D) within 2 years of study entry received multiple subcutaneous doses of either placebo or RN168 (1, 3, 8 mg/kg q2w or 6 mg/kg q1w) for 12 weeks with follow-up for an additional 6 weeks. A dose-dependent inhibition of *ex vivo* IL-7-induced STAT5 phosphorylation and a decrease in Bcl-2 expression was observed in peripheral blood $CD3^+$ T cells. $CD4^+$ and $CD8^+$ CCR7-/CD45RA- effector memory T cells (T_{EM}) were reduced as much as ~65% from baseline (at the 1 and 3 mg/kg dose), resulting in an increase in the relative ratio of $CD4^+$ $Foxp3^+$ Tregs to T_{EM} cell populations. RN168 treatment appeared to have a smaller effect on naïve T cells (maximum of ~35% decrease at 3 mg/kg dose). A trend towards increased PD-1 expression in $CD4^+$ and $CD8^+$ T cells provided evidence of a polarization to an exhausted or tolerized phenotype. The marked similarity of T cell modulation by RN168 to that observed in the alefacept trial (TiDal) suggests these pharmacodynamic effects may result in clinically relevant immunomodulatory activity in recent-onset T1D.

F.75. A PLGA-based Microparticle System for *In Vivo* Tolerization of Immune Cells and Reversal of Type 1 Diabetes in NOD Mice

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Current paradigms for treatment of autoimmune diabetes (T1D) are inadequate. To address this, we are developing a multi-component, synthetic microparticle-based 'anti-vaccine' using biodegradable materials with encapsulated factor. In combination, this MP formulation provides targeted antigen delivery to antigen-presenting cells, and controlled-release delivery of recruiting and tolerance-promoting factors to create an immune-modulating microenvironment localized subcutaneously at the injection site. Specifically, we have developed poly lactide-co-

glycolide (PLGA) microparticles (MPs) encapsulating immunomodulatory agents (vitamin D3, transforming growth factor-beta 1, and granulocyte macrophage colony stimulating factor) as well as the denatured auto-antigen, insulin. We have demonstrated the *in vitro* ability of MP formulation to induce suppressive immune cell phenotypes in dendritic cells, which can subsequently inhibit allogenic T cell proliferation and induce regulatory, FoxP3-expressing T cells in a mixed lymphocyte coupling. Notably, this MP vaccine prevents diabetes development in pre-diabetic non-obese diabetic (NOD) mice to the level of 60%. Further, administration of this MP anti-vaccine to recent onset diabetic NOD mice, restores normoglycemia in 20% of treated mice. *In vivo* cellular mechanisms appear to involve transient increases in regulatory T cells and IL-10-secreting B cells. Additionally, we have investigated the safety, biodistribution and toxicology of this formulation in efforts to translate this therapy to the clinic.

F.76. Manipulate the Gut Microbiome via Fecal microRNA

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The human gut harbors trillions of microorganisms, which include millions of genes. These microbes, as well as their metabolites, are involved in the maintenance of health and their alternation has been linked to diseases such as colitis, obesity, diabetes, cardiovascular diseases, autism and cancer. While the microbiota is host-specific, the mechanism of this specificity and how it is regulated are largely unclear. In our study, we identified abundant of extracellular microRNAs (miRNAs) presented in human and mouse feces. By using mice that are deficient of miRNA processing enzyme, Dicer, in specific cells, we identified the source of fecal miRNAs to be intestinal epithelial cells (IEC) and Paneth and goblet cells. Functionally, we found *in vitro* that these miRNAs were able to enter bacteria and regulate bacterial gene expression and thus affect the growth of bacteria. Furthermore, we found *in vivo* that mice with IEC generated miRNA loss had imbalanced microbiota and were more susceptible to colitis. Transferring fecal miRNAs from wild-type mice to the IEC-miRNA deficient mice restored the microbiota and rescued colitis. In conclusion, our findings identified miRNA-mediated bacterial gene expression regulation as a novel strategy for the manipulation of microbiome.

F.77. Phase I Clinical Trial Results of *Epigallocatechin Gallate* in HIV-1-infected Subjects

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Epigallocatechin gallate (EGCG), green tea catechin, inhibits HIV-1 gp120 binding to CD4 cells (IC_{50} of 4.5-12μM). We conducted the first Phase I clinical trial to assess the safety, tolerability and pharmacokinetics (PK) of Polyphenon E (standardized EGCG oral formulation) in HIV-1-infected individuals in a multi-center, placebo-controlled, dose-blinded, dose escalation, monotherapy study. PolyphenonE was administered orally for 14 days (800mg, 1200mg and 1600mg bid daily) in HIV-1-infected clinically stable adults not on concomitant antiretroviral therapy. Safety assessments were conducted for 21 days. 23 subjects (58% male, 68% black, 27% white, 5% other) were enrolled, median age of 28 years (20-59). Baseline HIV RNA was 90,663 (±14,738) copies/ml and $CD4^+$ cell count 540 (± 229)c/mm^3.Adherence was >85%. No major toxicities nor serious clinical or laboratory adverse events (AEs) were reported. Six reported AEs were Grade 1 (1 at 800mg, 2 at 1200mg and 3 at 1600mg). There was no change in HIV RNA levels over 14 days (p>.05). The PK data (nonparametric statistics), suggest a dose-proportional trend in AUC median over 12-hour interval AUC_{12H} for subjects in 800mg and 1200mg groups and greater than dose-proportional trend in AUC_{12H} transitioning from 1200 to 1600mg group. One subject on active drug achieved EGCG >4.5μM (2063ng/mL) over dosing interval. PolyphenonE was well tolerated, safe and acceptable in HIV-1-infected individuals. These data plus future studies using higher doses for virologic and immunologic effect will assist in determination of appropriate doses of PolyphenonE that are safe and useful for HIV treatment.

F.78. Prophylaxis and Treatment in Successful Hysterectomy of Hereditary Angioedema Patient

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Hereditary Angioedema (HAE) is a rare, autosomal dominant disease characterized by unpredictable swelling without pruritus or urticaria. These potentially fatal attacks can often be triggered by stress, trauma, or inflammation. We present a 48-year-old female with Type 1 HAE and fibroids requiring hysterectomy. Despite prophylaxis with plasma-derived C1-INH. She required treatment with recombinant C1-INH to successfully treat edema that developed after surgery.

Case Presentation: We present a 48-year-old female with HAE and leiomyomata. Lab results show low C1-INH level of 6 mg/dL (reference range 11-26 mg/dL), low C1-INH function level of 28 (>=68), and low C4 of 3 mg/dL (16-47 mg/dL). She had successful surgeries on her hand with mild to moderate swelling perioperatively. Past minor attacks were successfully treated acutely with icatibant, self-adminstered subcutaneously. The patient was on intravenous human C1-INH prophylaxis in the past with success. To prepare for surgery, she continued prophylactic infusions of human C1-INH regularly before surgery. The treatment plan also included instructions for the anesthesiologist to administer recombinant C1-INH during the procedure in the event of angioedema. We administered 3500 Units of recombinant C1-INH for swelling on postoperative day 2, and the patient reported complete resolution within 90 minutes.

Conclusion: Guidelines stress the need for an acute treatment plan since prophylaxis may not always be effective. Although a prophylactic C1-INH regimen was instituted for the prevention of perioperative swelling, our patient still experienced breakthrough angioedema. The combination of acute and prophylactic treatment plans allowed for a successful procedure with minimal complications from HAE attacks.

F.79. Antigen-specific Immune Tolerance Induction by Single Cycle Low Dose Methotrexate Appears to Induce Tolerogenic $CD4^+$ T Cells and Dendritic Cells in the Spleen

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Low dose methotrexate induction treatment that is administered alongside and within the first week of therapy has been shown in mice to induce long-term immune tolerance to continually administered enzyme-replacement therapies and antibody therapies. Importantly, a low dose induction regimen of methotrexate has been incorporated into a clinical protocol that includes Rituximab and has reproducibly achieved immune tolerance to alglucosidase alfa in high-risk infantile Pompe patients. Murine studies suggest the use of methotrexate alone would be effective in achieving immune tolerance in a clinical setting. To further assess the potential translatability of this approach from mouse to man, we are investigating the mechanism by which such a brief, low dose treatment of methotrexate can induce immune tolerance by studying the immune modulatory effects of this novel regimen. Here, wild type C57BL/6 mice were given single cycle low dose methotrexate concurrent with alglucosidase alfa. The behavior of $CD4^+$ T cell and dendritic cell subsets were examined in spleen by flow cytometry. Consistent with earlier studies that demonstrated enhanced regulatory B cell induction, here we identify evidence of additional tolerogenic mediators in our methotrexate induction treatment which appears to involve IL-10 expressing $CD4^+$ T cells but not $Foxp3^+$ or anergic $CD4^+$ T cells and the dendritic cell subsets CD11b and CD8α. These tolerogenic subsets share homology with humans which suggest a similar mechanism of immune tolerance induction may be conserved and supports the further investigation of methotrexate induction treatment alone in patients.

F.80. Intravenous Administration of Spleen Cells Coupled with Heterobifunctional Crosslinker SMCC-Conjugated Antigens Stimulate Potent Antigen-specific Immune Response in Mice Even Though a Minimal

Amount of Antigens is Delivered

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In the present study, we report our recently developed new approach to inducing antigen-specific immune response. We use two nucleophilic substitution "click" chemistry reactionss to successfully couple protein antigens or peptides to mouse immune cells by a heterobifunctional crosslinker, succinimidyl-4-(N-maleimidomethyl cyclohexane)-1-carboxylate (SMCC) or sulfo-SMCC. SMCC and its water-soluble analog sulfo-SMCC contain N-hydroxysuccinimide (NHS) ester and maleimide groups, which allow stable covalent conjugation of amine- and sulfhydryl-containing molecules in trans. Protein coupling to cells relies on the free sulfhydryls (thiols) on cell surfaces and the free amines on protein antigens. Although the amount of protein coupled to cells is limited due to the limited number of cell surface thiols, the injection of spleen cells coupled with antigenic proteins, such as keyhole limpet hemocyanin (KLH) or ovalbumin (OVA), induces a potent antigen-specific immune response in vivo, which is even stronger than that induced by the injection of a large dose of protein plus adjuvants. Further studies show that antigen-coupled spleen cell treatment leads to augmented antigen-specific IFN-γ-producing T cells in both $CD4^+$ and $CD8^+$ T cells. We also demonstrate that the infusion of melanoma antigen-coupled spleen cells elicits strong immunity against tumor in a melanoma mouse model. Our study provides a unique antigen delivery method that efficiently distributes antigens to the entire immune system, subsequently eliciting a potent antigen-specific immune response with enhanced IFN-γ production. The findings in the present study suggest that this antigen-cell coupling strategy could be employed in immunotherapy for cancers, infectious diseases as well as immune-mediated disorders.

Transplantation

OR.13. A New Window into the Human Alloresponse via High-Throughput T Cell Receptor Sequencing

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Characterizing the human T cell alloresponse presents a great challenge due to the presumed large size and diversity of this repertoire. We have recently developed and validated a method of identifying the repertoire of biologically-relevant (in transplant recipients) alloreactive T cells for any responder-stimulator pair by combining *in vitro* mixed lymphocyte reactions with high-throughput T cell receptor (TCR) sequencing. Here we use this tool to characterize the normal human alloresponse, providing the first quantification of alloreactive T cell repertoire diversity. Clonality measures diversity, accounting for both clone number and frequency. While the healthy adult CD4 T cell pool is markedly more diverse than that of CD8 T cells (mean clonality ± SD: CD4 0.057±0.02, CD8 0.17±0.08, $n = 5$, $p = 0.01$); that difference is reduced between CD4 and CD8 alloreactive T cells (0.14 ± 0.04; 0.17 ± 0.05, $n = 7$, $p = 0.08$). The diversity of the alloreactive repertoire appears stable over time and we observe a significant increase in diversity with increased HLA mismatches between the responder and stimulator. Furthermore, quantification of repertoire divergence suggests that the alloreactive repertoires generated by the same responder to two different stimulators are highly divergent from each other; the alloresponse also strongly diverges from the unstimulated responder T cell population, confirming that the alloresponse does not simply reflect the dominant clones in circulation. We are presently developing computational models that utilize this sequencing approach to tackle the challenge of quantifying CD4 and CD8 alloreactive clone frequency.

OR.30. A New Population of $IFNG^+IL10^+IL34^+$ Secreting Human $CD8^+CD45RC_{Low}$ Tregs Efficiently Inhibits Anti-Donor Immune Response In Transplantation

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We previously reported the suppressive properties of rat $CD8^+CD45RC_{low}$ T cells. To date, human counterparts have never been studied for their relevance as regulatory cells, we thus investigated their properties. Compared to $CD45RC^{high}$ subset, $CD45RC_{low}$ cells expressed lower level of CD127 and higher levels of PD1, CD122, Foxp3, GITR and HLADR, as well as IL34, IL10, TGFb1 and IFNg. By sorting the $CD8^+CD45RC_{low}$ based on the expression of IFNg and IL10, we demonstrated that $IFNg^+IL10^+$ secreting $CD8^+CD45RC_{low}$ T cells inhibited more efficiently allogeneic responses *in vitro* than classical $CD4^+CD25^{hi}CD127^-$ Tregs. Further phenotypic analysis revealed that Foxp3, IL34, TGFb1 and GITR expression were restricted to $IL10^+IFNg^+$Tregs. We confirmed the involvement of IL10, IFNg and IL34 cytokines in Tregs suppressive function by adding blocking antibodies to the co-culture assay. We identified their mechanisms of action as mediated by IL-2 deprivation, and preferential contact with pDCs, but not cytotoxicity. Finally, we observed that these Tregs can be efficiently expanded up to 1000 fold in 14 days. Following expansion, Tregs were enriched in $Foxp3^+$ $IL34^+$ $IL10^+$ and $IFNg^+$ cells and possessed a strong suppressive function, without any change in cytotoxicity. Indeed, transfer of expanded Tregs significantly delayed in a dose dependant manner GVH development and allogeneic skin graft rejection in humanized mice infused with human PBMCs. We identified and characterized a new natural regulatory T cell population efficiently inhibiting anti-donor immune response.

OR.40. Endosome-based Activation of Non-canonical NF-**κB Signaling** by Membrane Attack Complexes

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Using sera from transplant patients with high-titer panel reactive antibody (PRA), we previously described a model for studying the effects of alloantibody binding and complement activation on human endothelial cells (EC). With this model we found that complement membrane attack complexes (MAC) induced rapid post-translational stabilization of NF-κB-inducing kinase (NIK), the activator of non-canonical NF-κB signaling, which was required for inflammatory changes in cultured human EC and for vasculopathic changes in human coronary artery segments implanted into immunodeficient mouse hosts. To elucidate the mechanism of NIK stabilization, we performed a genome-wide siRNA screen and discovered a novel, TRAF3-independent signaling mechanism in which MAC induces NIK stabilization on endosomes. MAC is internalized by EC in a clathrin-, AP2-, and dynamin-dependent mechanism into $Rab5^+$ endosomes. Activated Akt is recruited in a Rab5-dependent manner to the same endosomes which then bind and stabilize NIK and subsequently recruit and activate IKK-α, the catalytic activator of p52/RelB complexes, the non-canonical form of NF-κB. Pharmacological inhibition of MAC endocytosis blocked this pathway both in cultured EC and in human coronary artery xenografts. These data reveal a novel endosome-based signaling cascade for activating non-canonical NF-κB and identify new targets for inhibiting complement-mediated tissue injury.

F.82. Short Term anti-CD45RC MAbs Treatment Induces Transplantation Tolerance Associated With Potent Regulatory Cells Induction

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Success in transplantation relies on immunosuppressive therapies to inhibit allograft rejection. However, it's necessary to develop therapies leading to more specific immunosuppression. We previously described in rats that CD40Ig treatment prolongs allograft survival through the induction of $CD8^+CD45RC_{low}$ T cells (Guillonneau, J. Clin. Invest., 2007). CD45RC molecule can be used to define two different subpopulations among T cells. Th1 T cells express high level of CD45RC ($CD45RC^{high}$), while Th2 and regulatory T cells (Tregs) express low level of CD45RC ($CD45RC_{low}$). Here, we evaluated the effect of a short term targeting of $CD45RC^{high}$cells (i.e. potentially effector T cells) on allograft survival and tolerance induction.

We demonstrated that short treatment with anti-CD45RC mAbs induces allograft tolerance in a heart allograft rat model. We showed that tolerance induction was associated with an increase of $CD45RC_{low}$ Tregs and transitory $CD45RC^{high}$ downregulation. We showed the absence of humoral anti-donor immune responses. Rats immunized with cognate antigen at early time point during anti-CD45RC mAb treatment or at late time point 120 days after treatment were able to mount efficient antibody responses, demonstrating a specific immunosuppression. We demonstrated *in vitro* the potentiation of $CD45RC_{low}$ Tregs from tolerant anti-CD45RC-treated recipients compared to those obtained from naïve rats. Finally, we showed *in vivo* that adoptive transfer of total splenocytes or purified Tregs $CD45RC_{low}$from tolerant anti-CD45RC-treated recipients to newly grafted irradiated recipients induces in turn long-term allograft survival.

Our results highlight the potential of anti-CD45RC mAbs as a new innovative therapy in transplantation to induce specific immune tolerance.

F.83. Contributions of Naïve, Effector and Memory T Cells in Mixed Lymphocyte Reactions

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It has been proposed that primary *in vitro* T cell alloresponses to allogeneic cells (MLR) is rendered possible by the high precursor frequency of naïve T cells capable of recognizing an allogeneic MHC molecule. On the other hand, it is possible that the MLR results from the presence of preexisting alloreactive memory T cells (TMEM) in naïve mice (endogenous TMEM). To address this question, we isolated naïve T cells and TMEM ($CD4^+$ and $CD8^+$) from unmanipulated BALB/c ($H\text{-}2^d$) mice and tested their ability to mount a direct inflammatory response (using IL-2 and gIFN-ELISPOT assays) *in vitro* after exposure to irradiated fully allogeneic B6 ($H\text{-}2^b$) spleen cells. The presence of gIFN producing spots (100-200 spots/million T cells) was detected with $CD8^+$ endogenous TMEM but not naïve T cells. Alternatively, a few IL-2 producing spots were detected after 4 days culture only with both naïve and endogenous $CD4^+$ TMEM. Next, the same MLR assays were performed with T cell subsets from BALB/c mice collected 10 days (effector response) or 6 weeks (memory response) after B6 skin transplantation. Again, no response was recorded with naïve T cells. In contrast, both T effector cells and TMEMs produced gIFN but displayed different kinetics of activation upon alloantigen presentation.

F.84. Analysis of Acute Skin Rejection in NonHuman Primate Models of Face and Hand Allotransplantation

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Purpose: Almost 85% of patients develop acute rejection (AR) of the skin within a year after hand or face transplantation (i.e. vascularized composite allografts, VCAs) and require treatment to prevent allograft loss. However, the mechanisms underlying AR remain poorly defined.

Methods: 6 cynomolgus monkeys received hand (n=2) and face VCAs from MHCmismatched donors after induction with antithymocyte globulin and postoperative maintenance on triple immunosuppression (tacrolimus, mycophenolate mofetil, methylprednisolone) for up to 120 days. Protocol biopsies of VCA and host skin were performed at 30 day intervals for flow cytometric analysis of resident skin leukocyte populations. Further biopsies were obtained for histopathology during clinical AR and steroid treatment was administered.

Results: Flow cytometric analysis revealed near complete turnover of passenger donor skinresident leukocytes within the VCA to hostorigin cells by 30 days after transplantation. Interestingly, this coincided with the first episode of AR in those animals with a complete MHC mismatch, although no AR developed in those animals that were haplomatched, despite the same immunosuppressive regimen. All but one episode of AR were successfully treated with steroids; no

alloantibodies were detected in all cases. Histological grading of AR was Banff I to II with corresponding higher ratios of CD8:CD4 T cells.

Conclusions: Here we show a clinically appropriate model for studying AR in VCA with implications on longterm management and tolerance induction. Sharing of haplotype appears to confer additional protection against AR by mechanisms that are currently being investigated in our laboratory.

F.85. C1INH Ameliorates Complement Deposition and Leukocyte Infiltration in a Murine Model of Antibody Mediated Rejection

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Upon transplantation, donor tissue may be recognized as foreign by the recipient immune system, resulting in the production of donor specific antibodies (DSA), which induce endothelial cell activation and proliferation, as well as classical complement activation and leukocyte recruitment. These processes define the histopatholgical diagnostic criteria ($C4d^+/Mac2^+$ biopsies) in cardiac antibody-mediated rejection (AMR). C1-esterase inhibitor (C1INH) is a pleiotropic protease inhibitor with two functional domains. The C-terminal serpin domain of C1INH inhibits classical complement activation, while sialyl LewisX (SLe^X) motifs in the highly-glycosylated N-terminal domain block leukocyte/endothelial interactions through selectin binding. As C1INH blocks processes found in AMR, we hypothesized administration of C1INH in a murine model of AMR would reduce complement activation and leukocyte recruitment. We heterotopically transplanted MHC-mismatched BALB/c hearts into Rag1KO.C57BL/6 recipients, and administered DSA (15µg each of H2-D^d & H2-K^d) or isotype control (IC, 30µg mIgG2a), ± C1INH (Berinert ®,15u) intravenously twice a week. After 4 weeks, transplanted hearts were harvested, fixed, embedded, and stained for endothelial swelling and myocardial injury (H&E), complement deposition (C4d), and myeloid infiltration (Mac2), and scored by a pathologist (according to ISHLT guidelines). We found hearts from DSA treated mice had endothelial cell swelling, and C4d and Mac2 positivity, whereas IC treated mice did not. Furthermore, C1INH-treated DSA mice had significantly reduced C4d and Mac2 staining when compared to mice receiving DSA alone. These data demonstrate intravenous administration of C1INH diminishes complement activation and leukocyte recruitment, and suggest C1INH may be a useful therapeutic for treating pathologies associated with AMR.

F.86. Effect of Infusion of mAbs to TNFRSF25 on Graft Rejection in Allo-immune Mice Receiving Autologous Marrow Transplantation

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Rapamycin used alone fails to prolong BALB/c skin grafts in C57BL/6 mice. Immune ablation by busulphan/cyclophosphamide, followed by autologous CD45-congenic marrow reconstitution. (BMTx), promotes graft survival and allows cessation of immunosuppression. Tregs of host and donor origin µare implicated in augmented survival. We reported that heteroantisera to mouse TNFRSF25, a molecule expressed on Tregs, further enhanced graft survival. We used mAbs to TNFRSF25 to study allograft survival in hyperimmune mice which had previously rejected skin grafts, following re-transplantation and autologous marrow infusion.

C57BL/6 mice which had rejected BALB/c skin grafts, received fresh BALB/c skin grafts with rapamycin (1mg/Kg/36hr) for 4 days, then busulphan/cyclophospamide for 6 days followed by infusion of $5x10^6$ cells of T-depleted CD45.1 BL/6 bone marrow. Controls received busulphan/cyclophospamide with no marrow infusion. 3d after BMTx (14d post-skin transplantation) all mice received rapamycin for a further 21d (to 35d post-transplant). In some studies mice also received mAbs (20µg/iv/mouse) to TNFRSF25 at 96hr intervals for 8doses.

Immunosuppression was withdrawn at 35d post-transplant. Graft survival was monitored throughout, and MLCs measured with splenocytes from individual mice at 50d and 80d.

After BMTx, graft survival in pre-immunized mice was ~25% at 80d. These mice showed attenuated MLC responses relative to controls (no BMTx-100% rejection at 18d post transplantation), increased Tregs, and markedly diminished (>100fold) serum anti-donor IgG. Infusion of anti-TNFRSF25mAb increased survival to ≥75% at day 80 with increased graft-infiltrating Tregs. These mAbs also expanded murine and human Tregs *in vitro* which attenuated MLCs using fresh PBL.

Conclusion: Anti-TNFRSF25 mAbs increase skin allograft survival in pre-immunized mice.

F.87. Development of a Dual-functional Magnetic Resonance (MR) Contrast Agent Beneficial for Cell Transplantation

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Cell transplantation such as bone marrow, stem cells and islets are believed to be effective in treating diseases. However, graft tracking and immune rejection are the two primary issues. To achieve the first goal, a non-invasive and quantitative *in vivo* imaging system could be very helpful. The combination of superparamagnetic iron oxide nanoparticles (SPION) together with magnetic resonance imaging (MRI) has been used for *in-vivo* imaging of grafted cells in a real-time manner. Here we attempt to further equip the SPION with the luciferase and/or green fluorescence protein (GFP), and apply in transplantation. First, we found that HSPIO encapsulated with a quaternized chitosan, N-[(2-hydroxy-3-trimethylammonium) propyl] chitosan chloride, bear a better potential to deliver gene expression. The relaxivity of such HSPIO showed no effects on T1 and T2 signals in MRI, indicating that the interaction or intracellular uptake of such HSPIO led to the expression of luciferase and/or GFP, which enables the optical imaging. Next, the results of Prussian blue staining and luciferase expression showed that the HSPIO labeled cell efficiency were dose- and time-dependently increased. The transmission electron microscopy (TEM) analysis confirmed the intracellular location of iron particles. The T2-weighted images present iron content-dependent changes. Finally, after correlating both values from the MRI signal changes and the Prussian blue positive cells, we have found a positive correlation between the plotted graphs of iron content versus MR signal changes. These findings provide evidence beneficial for future application of SPIO in cell tracking.

Other

OR.35. $CD11b^{+}Gr\text{-}1^{+}$ Myeloid-Derived Suppressor Cells Reduce Atherosclerotic Lesion Development in LDLr Deficient Mice

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Objective: Atherosclerosis is a chronic inflammatory disease in which accumulation of lipoproteins in the arterial wall activates the immune system causing abnormal vascular remodelling and vessel occlusion. Understanding innate and adaptive immune responses involved in atherosclerosis is essential for the development of novel therapies that aim to restore immune homeostasis. Myeloid-derived suppressor cells (MDSCs) form a heterogeneous population of cells composed of early myeloid progenitor cells and immature myeloid cells, which strongly suppress pro-inflammatory immune cells in inflammatory diseases. Currently, it is unknown whether MDSCs contribute to atherosclerosis. Here we investigated whether and how MDSCs contribute to the development of atherosclerosis.

Methods and Results: We show that MDSCs arise in the bone marrow of LDLr$^{-/-}$ mice during atherosclerosis and strongly suppress proliferation of T cells. Adoptive transfer of MDSCs into LDLr$^{-/-}$ mice fed a Western-type diet ameliorates atherosclerosis and reduces the amount of adventitial T cells. More specifically, MDSCs suppress Th1 and Th17 cells and reduce circulating pro-atherogenic B2 cells. Mechanistically, we show that MDSCs from atherosclerotic mice suppress T cells in an IFN-γ and NO-dependent manner.

Conclusion: This study demonstrates that MDSCs develop during atherosclerosis and represent a novel therapeutic strategy to reduce atherosclerosis via suppression of pro-inflammatory immune responses.

OR.42. Characterization of Hematopoietically-derived CNS Macrophages Following Bone Marrow Transplantation

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Macrophages of the central nervous system (CNS) consist of a heterogeneous population, including resident microglia and hematopoietically-derived CNS macrophages. However, unlike other tissue-specific macrophages, post-fetal microglial populations have been observed to undergo self-renewal in the brain parenchyma with limited contribution from hematopoietically-derived monocytes in the absence of an inflammatory state. We hypothesize that in a normal, non-disease-state there will be a minimal, but observable, contribution of cells to the CNS that are of hematopoietic origin. To further investigate the phenotype of these CNS infiltrating macrophages, recipient C57BL/6 mice were treated with a non-inflammatory myeloablative busulfan regimen followed by a reconstitution with green fluorescent protein (GFP)-expressing bone marrow cells. Analysis by flow cytometry in chimeric mice demonstrated that while 1-5% of cells expressing a CNS macrophage phenotype (CD11b^{+}/CD45^{mid}) were GFP^{+} at 7 post-BMT, no further increase was observed at later time points. Interestingly, an increase in MHC class II expression was only detected in the hematopoietically-derived GFP^{+}CD11b^{+}/CD45^{mid} population, suggesting that these cells may have a different function than resident microglia at these time points. Additionally, immunofluorescence (IF) microscopy identified Iba-1^{+}/GFP^{+} CNS macrophages at ~ 8 weeks post-BMT, however they did not demonstrate a ramified morphology until ~ 13 weeks post-BMT. Finally, IF microscopy confirmed the presence to GFP^{+}/Iba1^{+}/MHC class II+ cells. These data suggest a possible continuum of cells that originate as hematopoietically-derived monocytes into CNS macrophages, with potential immune surveillance function.

OR.51. Cbl-b Deficiency Renders T Cells Resistant to PD-L1/PD-1 Mediated Suppression

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The blockade of the PD-L1/PD-1 pathway has become a significant focus in cancer immunotherapy. Cbl-b is an E3 ubiquitin ligase that negatively regulates T cell activation. Cbl-b deficient (-/-) mice demonstrate robust anti-tumor immunity and T cell resistance to multiple immune regulatory mechanisms. Thus, there is a great interest in manipulating Cbl-b for cancer immunotherapy. Here, we report for the first time that Cbl-b$^{-/-}$ T cells are resistant to PD-L1/PD-1 immune regulation.

We now demonstrate that, in contrast to wild-type (WT) T cells, *in vitro* proliferative responses of Cbl-b$^{-/-}$ CD4^{+} and CD8^{+} T cells are not suppressed by a recombinant PD-L1 fusion protein (PD-L1 Ig) (% suppression CD4^{+}: WT 40%, Cbl-b 0%, $p<0.01$; CD8^{+}: WT 32%, Cbl-b 5%, $p<0.05$). Moreover, IFN-γ production of Cbl-b$^{-/-}$ CD4^{+} T cells is significantly less suppressed by PD-L1 Ig than that of WT (% Suppression: WT 68%, Cbl-b 20%, $p<0.0001$). To confirm this PD-L1 resistance *in vivo*, we used a model of B16 melanoma in which liver metastases develop only when PD-L1/PD-1 immune regulation is functional. WT mice develop numerous liver metastases which are significantly reduced by anti-PD-1 antibody treatment. Strikingly, Cbl-b$^{-/-}$ mice develop only rare liver metastases even in the absence of anti-PD-1.

In sum, we report for the first time that Cbl-b$^{-/-}$ T cells are resistant *in vitro* and *in vivo* to suppression by PD-L1/PD-1. Our finding broadens our understanding of Cbl-b's role in immune regulation and suggests a new mechanism by which manipulation of Cbl-b may lead to enhanced anti-tumor T cell responses.

T.34. Development and Validation of a Flow Cytometry Assay to Analyse Circulating Endothelial Cells as Biomarker for Cardiovascular Diseases

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Increased numbers of circulating endothelial cells (CECs) and reduced numbers of endothelial progenitor cells (EPCs) are found in many cardiovascular diseases. Endothelial dysfunction, the impaired NO-mediated vasodilation, is a major issue because established pharmacological treatments are not effective. However, the direct pharmacological stimulation of NO synthesis may be a therapeutical option. Robust minimally invasive techniques for diagnosis of endothelial dysfunction and therapy decision in a clinical setting are still missing. Our purpose was to develop a multiparameter flow-cytometry (FC) method to measure circulating endothelial cells and the vasodilator-induced eNOS activity as potential biomarkers of endothelial dysfunction.

Methods: CECs were identified as DNA^{+}, CD45^{-}, CD31^{+}, CD146^{+}; EPCs as CD45^{dim}, CD34^{br}, CD133^{+}, CD31^{+}, FSC$^{low/int}$, SSC^{low}. eNOS stimulation was quantified on the basis of the phosphorylation at Ser-1177. The validation experiments were conducted using primary endothelial cells spiked into blood samples from healthy individuals.

Results: The quantification and functional assay method for CEC and EPC is highly sensitive and reproducible, with variance below 20% and a CEC recovery rate of approx. 100%. CEC and EPC counts in healthy individuals are in agreement with published data (CEC: 9±4.9 cell/ml, EPC: 541.4±204.0 cell/ml). The highest eNOS phosphorylation was induced by Acetylcholine after pre-stimulation of β1-integrins in all endothelial cells tested.

Conclusions: A robust and precise FC assay combining the quantification of CEC and EPCs and a eNOS-stimulation test has been established. This assay is currently being evaluated in patients with heart failure, diabetic nephropathy, and arterial hypertension for its use in early clinical trials.

T.35. A Framework for Meta-analysis of Cytometry Data

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The amount of publicly available immunological data, particularly flow and mass cytometry data, is beginning to explode. In addition, computational tools are now emerging to enable improved statistical and visual analysis of such data. Meta-analysis of large-scale flow experiments will allow scientists to leverage the wealth of information embedded in these datasets. However, flow cytometry experiments greatly differ with regards to gating procedure, diversity in antibodies and fluorescent dyes, and other confounding variables that makes meta-analysis very challenging. We are developing a pipeline to systematically analyze flow cytometry datasets collected across multiple studies from Immport (https://immport.niaid.nih.gov). We have collected and preprocessed over 6000 raw flow data files to minimize technical variation. Further, the preprocessed data were clustered and a subset of clusters associated with the phenotype of interest was identified based on statistical methods. These clusters from different datasets were then mapped onto a common reference of cell populations to establish a complete picture of all cell types associated with the phenotype of interest. To validate our pipeline, we analyzed data from healthy subjects in Immport to discover cell types associated with gender and age. Preliminary analysis on two datasets identified 12 clusters associated to age and 1 associated to gender (FDR< 0.01). This result suggests our pipeline has the

potential to become a new unbiased and time-efficient approach for meta-analysis in the immunology field and demonstrates the importance of publicly available data.

T.42. Acute Treatment of Hereditary Angioedema in a 2-Year-Old

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Rationale: Hereditary angioedema (HAE) is a rare autosomal dominant disease, where patients develop non-urticarial and non-pruritic swelling. Despite new treatment modalities in the past 7 years, a large unmet need for approved acute treatment persists in the pediatric population.

Methods: 500 Units of intravenous plasma derived C1inh was used to treat an HAE attack in a 2-year-old child.

Results: A 2-year-old Caucasian female with type 1 HAE began experiencing recurrent abdominal swelling at 3 months of age. She was treated ineffectively for food allergy and acid reflux despite genetic evidence of HAE at birth. At 13 months of age she was hospitalized for one month with septal orbital cellulitis, which was later deemed to be a progression of an HAE attack. She continued to have severely depressed C4 and low C1inh levels and function. Finally at age 2 she received her first infusion of C1inh for a severe abdominal swelling episode. Her visual analog score (VAS) decreased from 8 to 2 within 45 minutes after infusion, as she started feeling better and tolerated oral fluids. There were no adverse effects from the treatment and she was discharged home.

Conclusions:We believe this is the youngest case of HAE in the U.S. treated with on demand therapy. Treatment with C1inh intravenously appears to be a safe, efficient and cost-effective mode of acute therapy in children with HAE.

F.19. Blockade of Tim-1 and Tim-4 Enhances Atherosclerosis in LDL Receptor-deficient Mice

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Objective: T cell immunoglobulin and mucin domain (Tim) proteins are expressed by numerous immune cells, recognize phosphatidylserine (PS) on apoptotic cells and function as costimulators or coinhibitors. Tim-1 is expressed by activated T cells but is also found on dendritic cells and B cells. Tim-4, present on macrophages and dendritic cells, plays a critical role in apoptotic cell clearance, regulates the number of PS-expressing activated T cells and is genetically associated with low LDL and triglyceride levels. Since these functions of Tim-1 and Tim-4 could affect atherosclerosis, their modulation has potential therapeutic value in cardiovascular disease.

Approach and Results: *ldlr*$^{-/-}$ mice were fed a high-fat diet for 4 weeks while being treated with control (rat IgG1) or anti-Tim-1 (3D10) or -Tim-4 (21H12) mAbs that block PS recognition and phagocytosis. Both anti-Tim-1 and anti-Tim-4 treatments enhance atherosclerosis by 45% compared with controls by impairment of efferocytosis and increasing aortic CD4^{+}T cells. Consistently, anti-Tim-4-treated mice show increased percentages of activated T cells and 'late' apoptotic cells in the circulation. Moreover, *in vitro* blockade of Tim-4 inhibited efferocytosis of oxLDL-induced apoptotic macrophages. Whereas anti-Tim-4 treatment increased Th1 and Th2 responses, anti-Tim-1 induced Th2 responses but dramatically reduced the percentage of Tregs. Finally, combined blockade of Tim-1 and Tim-4 increased atherosclerotic lesion size by 59%.

Conclusion: Blockade of Tim-4 aggravates atherosclerosis likely by prevention of phagocytosis of PS-expressing apoptotic cells and activated T cells by Tim-4-expressing cells, whereas Tim-1-associated effects on atherosclerosis are related to changes in Th1/Th2 balance and reduced circulating Tregs.

F.21. Novel Approaches for *Ex Vivo* Isolation of Human Tr1 Cells

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Regulatory $CD4^+$ T cells (Tregs) have a major role in maintaining tolerance to self and benign foreign antigens. Animal studies have demonstrated that different types of 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 found that expanded mouse Tr1 cells, but not $FOXP3^+$ Tregs, secreted high levels of IL-10 and potently suppressed inflammasome activation and IL-1β production. To test whether this difference between $FOXP3^+$ Tregs and Tr1 cells holds true in humans, we optimized protocols to obtain *ex vivo* human Tr1 cells. We first attempted to FACS-sort *ex vivo* $CD49b^+LAG\text{-}3^+$ cells, reported to contain Tr1 cells. Within *ex vivo* $CD4^+$ T cells we found CD49b expression on ~8% of cells but negligible LAG-3 expression. However, stimulation with aCD3/CD28 for 48h resulted in ~20% $CD49b^+LAG\text{-}3^+$ cells. We then compared the phenotype and function of two putative Tr1 cell populations: (1) $CD4^+CD49b^+LAG\text{-}3^+$ cells post 48h activation; and (2) $CD4^+IL\text{-}10^+IFN\text{-}\gamma^-$ T cells, sorted using cytokine capture, post 16h activation. After 12d expansion both populations had high (~8000pg/mL) IL-10 secretion and were $IFN\text{-}\gamma^+IL\text{-}2^-$ $IL\text{-}4^-$, consistent with a Tr1 cell phenotype. Interestingly, $CD49b^+LAG\text{-}3^+$ cells secreted 14X more TNF-α than $IL\text{-}10^+IFN\text{-}\gamma^-$ T cells, suggesting they may be distinct subsets. Experiments are ongoing to determine the ability of these cells to suppress the inflammasome in comparison to $FOXP3^+$ Tregs and to optimize *in vitro* expansion protocols that ensure Tr1 cell phenotype and function is retained.

F.55. Developing a Third Party HPV-specific T Cell Bank for use as an Immunotherapeutic Strategy for Immune Compromised Patients with HPV-associated Diseases

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Immune-compromised patients, including those with primary immune deficiencies and recipients of stem cell transplants, are at risk for persistent HPV infections and HPV-associated malignancies. Vaccine studies suggest that cytotoxic T cell responses against HPV antigens correlate with virus control. Adoptive transfer of (donor) HPV-specific T cells may be efficacious for immune-deficient patients with HPV-associated diseases.

HPV E6 and E7 are attractive targets for T cells as they play critical roles in malignant transformation. We hypothesized that strategies for *ex vivo* priming and expansion of either antigen-inexperienced and/or anergic T cells would generate HPV-specific T cells irrespective of donor source.

We evaluated the feasibility of generating HPV-specific T cells from peripheral blood of HPV-primed (n=2) and non-primed healthy donors (n=12) using GMP-compliant methodologies. Antigen presenting cells pulsed with HPV antigens in combination with different cytokines were used to stimulate T cells.

We successfully generated T cells targeting HPV antigens from 8/14 donors. T cells expanded with a median 142-fold expansion after 23-25 days. The resultant product specifically recognized HPV E6 or E7 proteins by IFN-γ ELISpot (mean 109.7 SFC/1-2x10^5 cells (range 12.5-334), compared to mean 6.1 SFC/1-2x10^5 cells, (range 0-23.5) for irrelevant antigen). Evaluated lines were comprised of 26±0.02% $CD8^+$ and 59±0.08% $CD4^+$ T cells. Current efforts are focused on developing a third-party T cell bank and demonstrating functional activity against HPV-expressing targets.

In summary, expansion of HPV-specific T cells is feasible from vaccinated and unvaccinated healthy donors, and may be used as an "off the shelf" immunotherapy for HPV-associated diseases.

F.81. Therapeutic Potential of Immunoproteasome Inhibition in Duchenne Muscular Dystrophy

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DMD arose from mutation of dystrophin protein determining loss of muscle force and inflammation. Emerging data from several laboratories demonstrated that immunoproteasomes exert a plethora of functions, in addition to the best-known immunological one. As they were found in skeletal muscles as regulators of skeletal muscle differentiation, they were characterized in dystrophic mice, where their increased expression was related to the oxidative stress of dystrophic muscles. Taken together with the evidence that the transformation of the standard proteasome in the immunoproteasome is regulated by the action of different cytokines that are normally up-regulated into dystrophic environment, we suggested that the immunoproteasome could exert an important role in modulating the development of muscle and in controlling the inflammation in DMD patients. According to the fact that the inhibition of the immunoproteasome could be theoretically driven by steroids, the main therapy for DMD, we employed a highly specific inhibitor, known to modulate cytokine production and to ameliorate the pathological phenotype of autoimmune diseases. This way, we decreased the rate of inflammation into dystrophic muscles by reducing activated lymphocytes but allowing the development of myogenic regulatory T cells. More importantly, we demonstrate that by interfering with MHC-I peptide presentation pathway, we acted specifically against anti-dystrophin activated lymphocytes. Auto-reactive lymphocytes arose from rare revertant dytrophin expressing fibers which prime T cell response in the periphery, impeding the success of gene therapy. Taking into account these data, we suggested that the immunoproteasome could be a feasible pharmacological target for DMD.

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