Allergy/Asthma

OR.17. Small-molecule Inhibition of Stat3 Prevents House-Dust-Mite (HDM)-Induced Airway Inflammation by Blocking Lung Production of Th17 and Th2 Cytokines

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Alternative treatments are needed for Th17-mediated asthma. Signal transducer and activator of transcription 3 (Stat3) drives Th17 differentiation and inflammation mediated by Th17 and Th2 cells. We hypothesized that C188-9, a small-molecule probe that inhibits Stat3 activation, can prevent HDM-induced airway inflammation by blocking lung production of Th17- and Th2-type cytokines. C57BL/6 mice were treated for 15 days: 10 mice (control) daily received vehicle 1 (PBS; 50 ul) intranasally (IN) and vehicle 2 (10% DMSO + 50% PEG400 in 5% dextrose; 200 ul) intraperitoneally (IP); 10 mice daily received HDM (40 ug in vehicle 1) IN and vehicle 2 IP; and 10 mice daily received HDM IN and C188-9 (50 mg/kg in vehicle 2) IP. Sections of left lung were stained with Alcian Blue/PAS and examined microscopically. Total (t) Stat3, Stat3 phosphorylated on Y705 (pStat3), IL-4, IL-5, IL-13, and IL-17 levels were measured in right lung protein extracts using Luminex beads. HDM inhalation increased goblet cell numbers 35-fold, epithelia thickness 5-fold, and subepithelial smooth muscle thickness 3-fold ($p<0.0001$ for each), which was accompanied by a 7-fold increase in the pStat3-to-tStat3 ratio, a 3-fold increase in IL-17, a 100-fold increase in IL-4, a 4-fold increase in IL-5, and a 2-fold increase in IL-13 ($p<0.05$ for each). C188-9 administration normalized each endpoint. Stat3 activation is critical for HDM-induced Th2- and Th17-mediated airway inflammation, which can be prevented by treatment with C188-9. Studies are underway to determine the effect of C188-9 treatment on Th2 and Th17 subsets within the lung and peripheral blood.

OR.24. High Molecular Weight Hyaluronan Promotes Immune Tolerance to Airway Allergens

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High-molecular weight hyaluronan (HMW-HA), is an anti-inflammatory polymer typically found in healing tissues. We have asked whether intra-nasal treatment with HMW-HA could promote immune tolerance to airway allergens in a mouse model of asthma. We administered crosslinked HMW-HA together with ovalbumin (OVA) to mice previously sensitized to OVA. This treatment suppressed airway hypersensitivity and eosinophilia in an allergen-specific manner. These effects were CD44 and IL-10 dependent and were not seen with HA fragments or other polymers tested. HMW-HA treatment promoted an immature phenotype in dendritic cells (DC) from pulmonary lymph nodes, but not the spleen, and resulted in increased numbers of IL-10 producing OVA-specific T-cells. These effects of HMW-HA treatment were durable for up to one month after treatment. We conclude that HMW-HA provides tissue integrity signals that alter the phenotype of local DC in ways that promote immune tolerance. We propose that HMW-HA is a highly innovative approach to immune modulation with great potential as a treatment for allergic sinusitis and asthma.

OR.30. *In vitro* Evaluation of Intestinal Epithelial TLR Activation in Preventing Food Allergic Responses

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Changes in the microbiota composition are associated with food allergy, but the mechanisms by which the microbiota interacts with the gut mucosal immune system are not known. IEC express Toll-like receptors (TLRs) under inflammatory conditions, which may interact with microbiota-derived antigens. To study the effects of epithelial TLR ligation in preventing an allergic effector response, T84 cell monolayers were co-cultured with PBMC from healthy controls or subjects with IgE-mediated food allergy. PBMC were CD3/28-activated and IEC were apically exposed TLR2 (Pam3CSK4), TLR4 (LPS) or TLR9 (CpG DNA) ligands. PBMC from food allergic subjects showed a more potent reduction in the TER (65.6±12.2% vs. 35.5±3.0% of initial TER) and increased the permeability of T84 cell monolayers (103.6±26.8 vs. 250.6±61.7 pmol/[h x cm^2] 4kDa FITC-dextran flux, $P<0.05$) compared to PBMC from healthy subjects. Apical exposure of IEC to CpG DNA, but not Pam3CSK4 or LPS, prevented PBMC-induced disruption in epithelial barrier function when using PBMC from healthy and food allergic subjects. Apical TLR9 activation on IEC increased the IFN-g/IL-13 (110.9±20.5 in controls vs. 256.4±68.9 in CpG DNA stimulated IEC,

P<0.05) and IL-10/IL-13 ratio (20.1±3.6 in controls vs. 36.9±9.1 in CpG DNA stimulated IEC, P<0.05), while suppressing TNF-a production by PBMC from food allergic subjects (2410.8±513.3 pg/mL in controls vs. 2092.0±497.7 pg/mL in CpG DNA stimulated IEC, P<0.05) in the T84/PBMC co-culture model. These data suggest that CpG DNA may prevent an allergic effector response. The IEC/PBMC co-culture model may be a relevant model to study host-microbiota interactions.

W.53. IL-21 Suppresses the Development and Functions of Th2 cells in Human and Mouse
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Background: T helper (Th) 2 cells, which produce IL-4, IL-5, and IL-13, control immunity to all forms of allergic inflammatory responses. Previous studies demonstrated that IL-21 reduces allergic symptoms in the murine models of allergic rhinitis and immediate hypersensitivity reaction in skin. Moreover, IL-21 inhibits IL-4-induced IgE secretion by B cells. However, whether or not IL-21 directly affects Th2 cells which leads to reduced allergic symptoms is unclear at this time. **Methods**: we investigated the effects of IL-21 on the differentiation of naïve Th cells to Th2 cells and on the regulation of Th2 effector functions *in vitro* and *in vivo* using ovalbumin (OVA) specific Th2 models. In addition, we examined the effects of IL-21 on T cells of allergic patients. **Results:** IL-21 reduced Th2 cell differentiation and IL-4, IL-5, and IL-13 production of already polarized Th2 cells by downregulation of transcription factor GATA-3. IL-21 also induced apoptosis of Th2 cells with decreased anti-apoptotic factor Bcl-2. Intranasal administration of IL-21 at the beginning of OVA sensitization or before OVA challenge decreased Th2 cytokines in the bronchoalveolar lavage fluid (BALF) of OVA/alum immunized allergic mice. In addition, the inhibitory effects of IL-21 on Th2 effector functions can also be found in allergic subjects. **Conclusions:** IL-21 can suppress Th2 cell development and cytokine production of polarized Th2 cells and induce Th2 cell apoptosis. Hence, the administration of IL-21 may be considered for use as a preventive and therapeutic approach when dealing with Th2 mediated allergic diseases.

W.54. *Ascaris suum*-derived Products Modulate the Chronic Lung Allergic Inflammation in Mice
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Previously, we have demonstrated that PAS-1 (Protein from *Ascaris suum* 1) down-modulate the mouse acute lung allergic inflammation. However, the anti-inflammatory role of PAS-1 in a chronic allergic inflammation model has not been reported. Here, we investigated the role of PAS-1 in the airway remodeling and lung angiogenesis induced by APAS-3 (Allergenic Protein from *A. suum* 3). BALB/c mice were sensitized with OVA (50 µg), APAS-3 (50 µg) and/or PAS-1 (300 µg) and 1mg alum by intraperitoneal (days 0 and 7) and subcutaneous (days 14 and 21) routes and, then, challenged three times/week for 2 months. Two days later, we measured serum IgE and IgG1, airway cellular infiltrate in bronchoalveolar lavage (BAL), cytokine production (IL-4, -5, -13, -10, TGF-b, IFN-g, VEGF, eotaxin) in BAL and metalloproteinase (MMP-2, MMP-9, ADAM-33) activity and expression in BAL cells. We found that APAS-3 increased IgG1 and IgE production, eosinophilic infiltrate, Th2 cytokines and TGF-b production, which were decreased in APAS-3+PAS-1- and PAS-1-immunized mice. Furthermore, APAS-3^{+}PAS-1 or PAS-1 induced IL-10 and IFN-g production and decreased metalloproteinase expression, indicating that PAS-1 ameliorates the airway remodeling. Besides, the production of VEGF and IL-13 was also decreased in APAS-3^{+}PAS-1-immunized mice, suggesting that PAS-1 may impair the formation of lung blood vessels. In conclusion, our data reveal that these two proteins present opposite actions in the chronic inflammatory response: APAS-3 induces airway remodeling and angiogenesis and PAS-1 down-modulates the chronic lung inflammation. These findings may support the use of PAS-1 as a potential therapeutic strategy for allergic disorders.

T.101. A Regulatory CD9⁺ B cell Subset Controls HDM-induced Allergic Airway Inflammation

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Background: Multiple regulatory B cells have been recently identified in rodents and humans. A specific subset of regulatory IL-10 producing B cells has been extensively studied in autoimmune and inflammatory pathologies. In allergic diseases observations that expansion of Bregs are necessary for allergen tolerance make us suggest that development of asthma should be associated with a specific defect in the regulatory B cells compartment Objective: To investigate this point we identify, quantify and characterize regulatory B-cell subpopulation in a house dust mite (HDM) model of asthma. Methods: IL-10–producing B cells from the spleens and lungs of allergic mice were quantified, phenotyped by microarray analysis and flow cytometry, isolated, and transferred to HDM-treated mice, and their ability to constrain allergic airway inflammation was studied. Results: IL10⁺ B cells comprises approximately 1,6% (+/-0,22) and 3,14% (+/-0,4) of total B cells in spleen and lung of control mice respectively but only 0,97% (+/-0,05) and 2,1% (+/-0,3) of total B cells in spleen and lung of asthmatic mice ($P<0.001$). To characterize them we sorted spleen IL-10 producing B cells by secretion assay and perform whole-genome microarray expression analysis. Mice IL-10⁺ cells expressed high surface CD9 levels. This marker alone permitted us to isolate highly enriched Bregs able to suppress allergic airway inflammation after adoptive transfer. Conclusions: Our data show that development of asthma is associated with a decreased of IL-10 regulatory B cells in Balb/c mice. This population is enriched within the CD9⁺ compartment and transfer of CD9⁺ B cells subset was able to control allergic inflammation.

T.102. *in vitro* Response of Peripheral Blood Lymphocytes Taken from Allergic Rhinitis Patients on Nickel and Chromium Salts

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The blast transformation (BT) reaction was used for evaluation of *in vitro* response of peripheral blood lymphocytes. Control samples were taken from eight healthy donors. Number of treated lymphocytes was 2.0×10^6 cells/L. Concentrations of 5, 25, 50 and 100 μM nickel chloride and 10, 50, 100 and 500 μM chromium chloride were used. AR patients were classified as following: 1) seasonal AR (n=10); 2) year-round AR (n=10); 3) seasonal AR with bronchial asthma (BA) (n=10); 4) year-round AR with BA (n=10). After third day of cultivation in the medium with phytohaemagglutinin (PHA), percent of blast-transformed lymphocytes was counted. Concentrations of 5 and 25 μM nickel chloride did not affect BT of donor lymphocytes, 50 μM stimulated BT, while 100 μM inhibited it. Similar trend was detected for patients of 1st, 2nd and 3rd group; 50 μM nickel chloride had dramatic stimulating effect among patients of 4th group. 100 μM nickel chloride had inhibiting effect in all groups of patients. Chromium chloride in concentration of 10 μM affected BT in donor blood similarly to PHA; 50 and 100 μM stimulated BT, while 500 μM inhibited it. Similar trend was observed for patients of 1st and 2nd group; however blast percentage was lower in comparison with donors. Dramatic stimulating effect of 50 and 100 μM chromium chloride was detected for patients of 3rd group. The same concentrations stimulated BT in patients of 4th group; however stimulating effect was less expressive. Concentration of 500 μM inhibited BT in all groups of patients.

Autoimmune Neurologic Disease

OR.4. Cbl-b Deficiency Results in Abnormalities in Response to the Multiple Sclerosis Therapeutic Agent FTY720 and in T Cell Sphingosine-1-Phosphate Receptor 1 Function

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FTY720 is a sphingosine-1-phosphate receptor 1 ($S1P_1$) modulator used to treat Multiple Sclerosis (MS). While FTY720 is postulated to mediate its effect by enhancing T cell $S1P_1$ internalization and degradation, the mechanisms underlying the

therapeutic effect of FTY720 in MS are not fully understood. Clarifying these underlying mechanisms may allow treatment to be individualized to avoid adverse side-effects. To investigate the functional effects of FTY720 we utilized Cbl-b-deficient (Cbl-b$^{-/-}$) mice. Cbl-b is an E3 ubiquitin ligase that regulates the T cell PI3K-Akt pathway and its absence leads to resistance to regulatory T cells (Tregs) and autoimmunity in mice. Given that MS has been associated with both SNPs in the *CBLB* gene and resistance to Tregs, Cbl-b$^{-/-}$ mice represent an important model for studying MS. We now report that Cbl-b$^{-/-}$ T cells demonstrate multiple functional abnormalities in S1P$_1$. These include significantly less lymphopenia induced by the S1P lyase inhibitor, THI, (Cbl-b$^{-/-}$ blood CD4$^+$ T cells decreased 19% vs 59% in wild type (WT) at 48 hrs), suggesting abnormal regulation of Cbl-b$^{-/-}$ T cell S1P$_1$ in response to the endogenous ligand S1P. Paradoxically, Cbl-b$^{-/-}$ T cells demonstrate enhanced lymphopenia induced by the therapeutic agent FTY720 (Cbl-b$^{-/-}$ blood CD4$^+$ T cells decreased 51% vs 26% in WT at 120 hrs). These results for the first time identify a critical role for Cbl-b in regulating expression and turnover of T cell S1P$_1$ and suggest that SNPs in the *CBLB* gene may associate with a differential response to FTY720 in MS patients.

OR.12. Human B cell and Glial Cell Interactions: Implications to the Compartmentalized CNS Inflammation of MS

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B cells play an important role in multiple sclerosis (MS) both within the periphery and the central nervous system (CNS), where their presence may be fostered including formation of B cell rich 'follicle-like' structures (FLS). The presence of FLS is linked with areas of sub-pial cortical injury, demyelination, a gradient of microglial activation and astrogliosis. We showed that MS B cells can produce abnormally elevated levels of pro-inflammatory cytokines (TNFa, LT, IL-6), while IL-10 expressing B regulatory (Breg) cells are deficient. We hypothesized that glial cells within the MS CNS may foster B cell responses that may, in turn, contribute to propagation of disease-relevant glial cell responses. We first examined how functional properties of MS patient and healthy control (HC) B cells may be influenced by soluble products secreted by human glial cells that were cultured under either basal conditions, or following pro-inflammatory activation. Soluble products of activated astrocytes significantly promote survival ($n=6$; $p<0.01$), and upregulate CD86 co-stimulatory molecule expression ($P<0.001$), of MS and HC B cells. Similar results are obtained following B cell exposure to products of pro-inflammatory (M1-polarized) but not M2-polarized microglia. We next tested the capacity of distinct B cell subsets soluble factors to impact microglia responses. Interestingly, soluble factors of Breg cells, but not effector B cells, increased the expression of inhibitory molecules CD47, TREM2 and Cx3Cr1, in human microglia. Our results point to a potential interaction between distinct human B cell subsets and glial cells, which may influence the propagation of CNS-compartmentalized inflammation in MS.

OR.13. Podoplanin is Expressed in Multiple Sclerosis Lesions and Perivascular Infiltrates and Regulates T Cell Proliferation and Th17 Differentiation

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Ectopic lymphoid follicles form in the subarachnoid layer of the meninges of multiple sclerosis (MS) patients. These follicles are associated with subpial cortical demyelination and neurodegeneration, as well as faster progression to clinical disability. Recent work in EAE mice suggests that the transmembrane glycoprotein podoplanin (PDPN) is specifically expressed on Th17 cells and is necessary for ectopic lymphoid follicle formation. Consistent with these findings, we have identified PDPN+ cells in MS meningeal infiltrates and perivascular lesions, but not in healthy adjacent white matter, suggesting a role in disease. In vitro, PDPN is specifically expressed on a sub-population of CD4 T cells under Th17 polarizing conditions. Although they are differentiated under Th17 conditions, PDPN+ cells do not express IL-17 or IFN-gamma. Stimulation of these cells with CLEC-2, a ligand for PDPN, promotes proliferation and shifts in vitro cultures away from a Th17 phenotype. Taken together, these results suggest that CLEC-2 signaling through PDPN results in enhanced T cell proliferation and decreased Th17 differentiation. In addition, we have previously shown that salt promotes Th17 induction and may play a role in MS. Addition of salt to Th17 cultures decreased PDPN expression, further suggesting a reciprocal relationship between IL-17 and PDPN. We speculate that CLEC-2 expressed on neutrophils and dendritic cells may signal through PDPN to regulate inflammation associated with Th17 cells. This work introduces a potential new therapeutic option for treating MS by targeting a molecule associated with meningeal follicle formation and Th17 cells.

OR.34. Transcriptional Profiling of Central Nervous System-Infiltrating CD4+ T Cells Identifies a Lipid-Metabolizing Enzyme as a Novel Therapeutic Target in Experimental Autoimmune Encephalomyelitis

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CD4 T lymphocytes have key roles in the pathology of multiple sclerosis (MS), but the mechanisms that govern T cell effector functions within the central nervous system (CNS) are poorly understood. To define potential novel regulators of pathogenic CNS inflammation, we performed transcriptional profiling of memory phenotype CD4 T cells FACS-sorted from CNS and lymphoid tissues of mice with experimental autoimmune encephalomyelitis (EAE) induced by immunization with myelin oligodendrocyte glycoprotein (MOG) peptide amino acids 35–35 (MOG_{35-55}). We found that mRNA for diacylglycerol O-acyltransferase-1 (DGAT1), a lipid-synthesizing enzyme, was highly upregulated by CNS-infiltrating memory CD4 T cells from mice with EAE. Mice deficient in DGAT1 developed less severe clinical disease and had significantly fewer mononuclear cell inflammatory foci within the CNS than their wild-type (WT) counterparts, consistent with a critical role for this enzyme in this autoimmune demyelinating disease model. In addition, administration of a selective DGAT1 inhibitor attenuated MOG_{35-55}-induced EAE. When compared to control animals, lymphocytes from DGAT1-deficient mice, as well as those from WT EAE mice treated with the DGAT1 inhibitor, displayed reduced MOG_{35-55} recall proliferation and cytokine responses. Collectively, our results demonstrate the ability of CNS-specific CD4 T cell expression profiling to define novel factors of therapeutic relevance in EAE. Through the use of genetic and pharmacologic approaches, we identify DGAT1 as a regulator of immunopathology and T cell effector function in EAE. DGAT1-targeted therapies may therefore represent a novel treatment strategy in MS.

W.1. Inhibiting TWEAK (TNF-like Weak Inducer of Apoptosis) Signaling Ameliorates Blood Brain Barrier (BBB) Integrity and Neuronal Damage in Neuropsychiatric Lupus Prone MRL/lpr Mice

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Neuropsychiatric disease (NPSLE) is one of the most common manifestations of human lupus, but remains poorly understood. TWEAK is a cytokine member of the TNF superfamily; its sole receptor, Fn14, is expressed in brain endothelial cells, astrocytes, microglia and neurons. In vitro, TWEAK signaling promotes expression of MCP-1, IL-6 and IL-8, decreases ZO-1 expression, and increases the permeability of human brain microvascular endothelial cells. We recently showed that Fn14 knockout (KO) in the MRL/lpr lupus strain of mice led to markedly attenuated neuropsychiatric disease, as revealed by significant reduction in depressive-like behavior and improved cognitive function. The purpose of the present study was to determine the mechanisms by which TWEAK signaling is instrumental in the pathogenesis of neuropsychiatric lupus. We found that Fn14KO mice had improved BBB integrity as shown by decreased fibronectin and IgG deposition, and reduced VCAM and ICAM expression in the brain. Furthermore, Fn14KO mice displayed fewer cellular infiltrates in the choroid plexus, and reduced neuron apoptosis and hippocampal gliosis. Interestingly, there were no differences in neurogenesis and microglia activation, or in circulating autoantibody titers, between Fn14KO and Fn14WT mice. Interestingly, in a pilot study using intracerebroventricular injection, TWEAK-treated B6 mice displayed a trend towards lower motor activity, cognitive dysfunction and increased depressive-like behavior, with cellular infiltrates in the lateral ventricles. Our studies indicate that TWEAK/Fn14 interactions play an important local role in the pathogenesis of NPSLE by improving BBB integrity and reducing neuronal damage, suggesting a novel target for the disease.

W.27. Adenosine A_{2A} Receptors Contribute to Local Immune Regulation after Neonatal Hypoxic Ischemic Brain Injury

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Inflammation influences outcome after brain hypoxic ischemia (HI), and adenosine affects both HI and immune regulation. Interestingly, the unselective adenosine receptor antagonist caffeine has shown neuroprotective properties when clinically tested for apneas in preterm infants. Here, we investigated the influence of adenosine A_{2A}receptor deficiency ($A_{2A}R^{-/-}$) and the effect of caffeine treatment in a neonatal brain HI model.The Vannucci brain hypoxic ischemia model was applied on ten-day-old mice. Infarction size and behavioural outcomes were evaluated. Local immune responses were studied by flow cytometry of brain infiltrating cells. $A_{2A}R^{-/-}$ mice developed significant, 29% larger tissue loss and performed worse in beam walking tests compared to WT. $CD11b^{+}CD86^{+}$ increased in the $A_{2A}R^{-/-}$. $A_{2A}R^{-/-}$ mice also had significantly diminished $Ly6G^{+}CD11b^{+}$ myeloid derived suppressor cell count compared to WT. $CD4^{+}FoxP3^{+}$ Treg numbers were increased in the $A_{2A}R^{-/-}$, but no alterations in $CD4^{+}$ and $CD8^{+}$ T-lymphocytes were observed. Principal Component Analysis discriminated between genotype and injury effects, and the immune populations associated to each factor respectively. Caffeine 5mg/kg given directly after HI significantly decreased atrophy by 44% compared to PBS treated animals, with no alterations in immune responses 72h and two weeks after the lesion. The $A_{2A}R^{-/-}$ displayed altered post-insult activation of anti-inflammatory MDSCs and Tregs, paralleled by higher activation of innate cells, a phenotype associated with increased injury. In contrast, caffeine treatment was beneficial for outcome. In conclusion, A_{2A} receptor deletion is detrimental and induces immune alterations after HI brain injury, whereas short-term antagonism may have potential for clinical use.

W.46. STAT4 is Dispensable for Th17 Generation but Essential for GM-CSF Production by CD4 T Cells During EAE

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Th1 and Th17 effector CD4 T cell subsets are critical for eliciting a robust immune response during experimental autoimmune encephalomyelitis (EAE), a mouse model of autoimmune demyelinating multiple sclerosis (MS). Recent genome wide association studies have identified a susceptibility variant for MS in the STAT4 gene locus. STAT4 is an important Th1 transcription factor that, when activated by IL-12, results in the production of the hallmark Th1 cytokine IFNγ. Genetic deletion of STAT4 protects mice from EAE; in contrast, deletion or inhibition of IL-12 and/or IFNγ does not ameliorate disease, revealing gaps in our current understanding of STAT4 during EAE. In order to study the CD4 T cell intrinsic role of STAT4 during EAE, we utilize mixed bone marrow chimeric mice. As expected, the ability of CD4 T cells to produce IFNγ is compromised in the absence of STAT4; however, we find that $STAT4^{-/-}$ CD4 T cells maintain multiple key properties of "pathogenic" Th17 cells, including the production of IL-17A and activation of STAT3 via IL-23 signaling. Nevertheless, induction of EAE by passive transfer of Th17 enriched $STAT4^{-/-}$ CD4 T cells is not able to initiate disease, implying an important unknown function for STAT4 in Th17 cells. Upon further investigation, we find that the production of the IL-23-associated cytokine GM-CSF, a critical component in EAE pathogenesis, to be defective in $STAT4^{-/-}$ CD4 T cells. Therefore, we posit that STAT4 mediated regulation of GM-CSF is a key factor in driving EAE pathogenesis.

W.48. A Commensal Symbiosis Factor Prevents Murine CNS Demyelination via TLR2 and Induce $CD39^{+}$ Regulatory T Cell Phenotypes in Humans

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While the etiology of human multiple sclerosis remains elusive, our published preclinical studies have revealed the link between gut commensal microbiota and immune-mediated CNS demyelination. We have reported that polysaccharide A (PSA), a symbiosis factor derived from the gut commensal strain *Bacteroides fragilis*, protects against murine experimental autoimmune encephalomyelitis (EAE) both prophylactically and therapeutically. The regulatory function of PSA depends on TLR2. Deficiency in TLR2 signaling abrogated PSA protection against EAE. TLR2 mediated anatomically localized induction of $CD39^{+}CD4$ T cells by PSA. The $CD39^{+}CD4$ T cells express multiple suppressive molecules. In human PBMC, PSA induced the $CD39^{+}Foxp3^{+}$ Tregs and IL-10 release *in vitro*. CD39 defect has been related to RRMS. We hypothesize that *B. fragilis* PSA plays a critical role in sustain the pro- versus anti-inflammation balance during CNS-targeting autoimmunity. PSA significantly reduced murine EAE severity, restricted lymphocyte infiltration and demyelination in the CNS. Transcriptionally, PSA suppressed inflammatory cytokines and chemokines but reciprocally boosted the ectonucleotidase CD39 in the disease-state CNS. Ablation of TLR2 negated the protective function of PSA. PSA preferentially expanded $CD39^{+}CD4$ T cells at CLN and MLN during EAE. $CD39^{+}CD4$ T cells, as opposed to $CD39^{-}CD4$ T cells, exhibited elevated level of IL-10, TGFβ, CTLA-4

and CD25. CD39 expression was enhanced in human FoxP3⁺Tregs when stimulated *in vitro* with PSA. Our results suggest that the commensal antigen PSA could limit CNS autoimmunity and enhance the regulatory profile of T cells in mice and human. TLR2 signaling and CD39⁺ T cell subset mediate the regulatory capacity of PSA.

T.41. Cutting Edge of New Discovery of Pharmacological and Pathophysiological Associations Related to Alzheimer's, Parkinson's Disease, Type 2 Diabetes Mellitus and Cushing's syndrome

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Background: Parkinson's disease and Alzheimer's disease both are the two well known devastating diseases in the central nervous system whereas type 2 Diabetes Mellitus and Cushing's syndrome are notorious diseases in the peripheral endocrine system. While scientists have been evaluating the underlying mechanisms within the four diseases, their close connections are just beginning to float up to surface. Methods: A comprehensive review of all current studies, publications and clinical trials including all four diseases was conducted. Results: On the basis of recently older drugs approved for new indication such as "Bromocriptine" for type 2 Diabetes Mellitus, "Mifepristone" for Cushing's syndrome with other multiple clinical trials, it has accelerated uncovering the correlations among four diseases. In this review, the overlapping treatment and pathophysiology linkages are discussed. These four chain reactions:"Mitochondrial dysfunction, "endoplasmic reticulum stress", "inflammation cascade", and "metabolism impairment", are all involved to either central neuron cell apoptosis or peripheral cell insulin resistance. In addition, elevation of hypothalamic-pituitary-adrenal axis and glucocorticoid secretion also impose on this vicious cycle that leads to development of the illness. We illustrated the pathophysiology figure and pharmacology table in terms of four diseases. Conclusion: Alzheimer's disease, Parkinson's disease, Type 2 Diabetes Mellitus and Cushing's syndrome can share same mechanism and one single drug may be used for all four diseases.

T.44. Beneficial Effect of Tolerogenic Dendritic Cells Loaded with Myelin Peptides in Experimental Autoimmune Encephalomyelitis

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Objective: To evaluate the effect of tolerogenic dendritic cells (tolDCs) loaded with myelin oligodendrocyte glycoprotein $(MOG)_{40-55}$ peptide as a therapy in experimental autoimmune encephalomyelitis (EAE), animal model of multiple sclerosis (MS). Methods: Bone marrow (BM) cells from C57BL/6 mice donors were cultured in presence of GM-CSF, LPS, vitamin D3 (VitD3) as tolerogenic agent and MOG_{40-55}. A total of 1·10x6 generated tolDC-MOG cells or PBS (sham control) were administrated preventively (on days -2 and +5 post-immunization, pi), pre-clinically (on days +5 and +9 pi) or therapeutically (on days +18 and 25pi) on C57BL/6 mice immunized with MOG_{40-55}. Clinical and immunological parameters were evaluated. Results: TolDCs differentiated in presence of VitD3 displayed a semi-mature phenotype (low levels of MHC class II, CD40 and CD86) and poor stimulatory ability, exhibiting a 68.50±9.52% reduction of allogeneic proliferative response compared to mature DCs. *In vivo*, 75% of mice receiving tolDC-MOG preventively were resistant to EAE induction compared with control group (25% vs 100% of incidence, respectively, p=0.021). In contrast to controls, tolDC-MOG treated mice showed no proliferative response against MOG_{40-55} (4.27±2.65 vs 0.97±0.42, p=0.003) as well as increased levels of Treg cells (9.78±3.56 vs 38.10±10.85, p=0.002). Preliminary data of mice treated pre-clinically and therapeutically with tolDC-MOG showed a reduction of EAE severity. Conclusion: Our results show that tolDC-MOG differentiated in presence of VitD3 induce in vivo a Treg response capable to ameliorate EAE disease, suggesting that tolDCs loaded with myelin peptides might be a potential therapy for MS.

T.45. Neither Increased TCR Affinity nor Epitope Spread Govern Secondary Progression in EAE

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The nonobese diabetic (NOD) mouse model of demyelinating disease is clinically relevant in its MHC-class II associated proclivity to autoimmunity and its tendency to follow a secondary progressive disease course resembling that experienced by the majority of MS patients. We investigate the cellular mechanisms that govern the progression from acute symptoms into

disease remission, relapse and chronicity. The micropipette adhesion frequency assay was used to obtain a sensitive and physiologically relevant two-dimensional measurement of T-cell receptor affinity and frequency for myelin as the low T-cell affinity does not allow the use of peptide:MHC tetramers. We found the highest affinity polyclonal MOG-reactive cells infiltrate the central nervous system (CNS) during acute disease. Affinities during remission, relapse and chronicity are not significantly different from each other but are significantly lower than affinities measured during acute disease. The majority of CNS-infiltrating CD4 T cells are MOG-reactive at these later time points arguing against epitope spread as an explanation for disease progression. Additionally, time points at which mice were actively symptomatic (acute, relapse and chronic) were characterized by an infiltration of Th17s in the CNS while remission time points showed an enrichment of cells producing IFN-γ in the CNS. The frequency of regulatory T cells (Tregs) was significantly higher in the CNS during remission than during acute disease. The results of this study indicate a changing cytokine profile of MOG-reactive CD4 T cells, rather than epitope spread or increased TCR affinity, governs the transition from acute disease through remission, relapse and chronic disease states.

T.46. Tetramer and Crossreactivity Studies Suggest Molecular Mimicry Between Influenza Hemagglutinin and Hypocretin (HCRT) Epitopes in Narcolepsy

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Narcolepsy is a sleep disorder characterized by excessive daytime sleepiness, sleep paralysis, and sudden loss of muscle tone (cataplexy). The cause of narcolepsy in most cases is the loss of hypothalamic neurons that produce hypocretin (HCRT), or orexin, a neuroptide that regulates sleep and wakefulness. Epidemiological studies have associated infections with narcolepsy, and recently the 2009 H1N1 influenza A strain (pH1N1) was associated with increased narcolepsy risk. We found increased levels of circulating HCRT-reactive T cells in narcolepsy patients who had received the seasonal influenza vaccine (containing pH1N1). Stimulation of CD4+ T cells from narcoleptic patients with a pH1N1-specific hemagglutinin epitope with homology to narcolepsy-associated HCRT epitopes increased the frequency of HCRT-reactive T cells. We have used tetramer staining of short-term CD4+ T cell lines to test for cross reactivity between HCRT and hemaglutinin epitopes. Our results indicate that T cell responses induced by pathogens may be associated with CD4+ T cell reactivity to HCRT in narcoleptic patients. Molecular mimicry between HCRT and influenza virus may underlie the autoimmune response in at least some cases of narcolepsy.

T.47. A Unique Microbiome-Associated Lipid Plays a Putative Immunoregulatory Role in Multiple Sclerosis

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Although the microbiome has been implicated in the pathogenesis of autoimmunity, the mechanisms by which commensal flora communicate with the systemic immune system and regulate systemic autoimmunity remain unknown. We recently identified a unique Bacteroidetes-derived lipodipeptide, Lipid 654, which functions as a TLR2-agonist. In analyzing its potential to mediate the effect of the microbiome on systemic immunity, we found that Lipid 654 gains access to the human systemic circulation. Critically, however, we found that healthy individuals have a wide distribution of serum Lipid 654 levels while MS patients show serum Lipid 654 levels that are tightly clustered in the very low range. Based on recent concepts suggesting that microbiome-derived factors may mediate homeostatic immunoregulation, we postulated that Lipid 654 may be such a factor that is significantly diminished in MS. To test this postulate in a mouse model, we administered Lipid 654 to mice with EAE and found variable effects on disease. To understand this variability, we measured endogenous levels of serum Lipid 654 in C57BL/6 mice using MRM-mass spectrometry and found that Lipid 654 not only gains access to the systemic circulation in mice, but also demonstrates the same wide distribution of levels seen in healthy humans. Our identification of widely distributed endogenous levels of serum Lipid 654 in mice now suggests a new model for understanding the influence of the microbiome on the inherent variability in autoimmune susceptibility and thus for identifying the functional significance of low serum Lipid 654 in patients with MS.

T.48. Myelin Reactive T cells in the Gut Regulate Experimental Autoimmune Encephalomyelitis (EAE)

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Multiple sclerosis (MS) is a demyelinating autoimmune disease of the central nervous system (CNS). Recent increase in the number of MS patients should be attributed to the environmental changes rather than genetic changes. The intestine has lately received much attention as a potential location for the regulation of T cells. Prior studies showed that alterations of gut environment could lead to the amelioration of experimental autoimmune encephalomyelitis (EAE), a rodent model for MS. Here we investigated the characteristics of myelin reactive T cells in the gut and the molecular mechanism of the way they could influence on CNS autoimmunity using myelin oligodendrocyte glycoprotein (MOG) reactive T-cell receptor transgenic (2D2) mice. There were two distinct T- cell populations in the small intestinal intraepithelial lymphocytes (IEL) of 2D2 mice, which had the phenotypes of $CD2^{-}CD5^{-}$ natural IEL and $CD2^{+}CD5^{+}$ induced IEL respectively. Adoptively transferred 2D2-$CD4^{+}$induced IEL ameliorated EAE. The transferred IEL were found to migrate into the CNS and up-regulated several immune regulatory molecules such as Lag3. 2D2-$CD4^{+}$ induced IEL exhibited the suppressive activities on T cell proliferation in LAG3,CTLA-4 and TGFβ dependent manner *in vitro*. Furthermore, LAG3 blockade abolished the suppressive effect of EAE by transfer of 2D2-$CD4^{+}$ induced IEL *in vivo*. These findings suggested that gut is an important place to regulate systemic autoimmune responses through controlling auto-reactive T cells to regulatory phenotypes.

T.49. First-in-Human Phase 1 Study of Invariant NKT Cell Ligand OCH

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We have previously reported that OCH, a sphingosine-truncated analog of α-galactosylceramide (αGC), that selectively induces IL-4 production from invariant NKT (iNKT) cells, would suppress the development of autoimmune disease models, including experimental autoimmune encephalomyelitis (EAE) (Miyamoto et al. *Nature* 2001). Here we report on the results of the First-in-Human phase 1 study of OCH for healthy subjects, which we have recently conducted in the NCNP as an investigator initiated trial. The aim of the study was to evaluate safety and pharmacokinetics of a single oral administration of OCH, investigate alterations in immunological parameters and gene expression profiles. Fifteen healthy subjects were enrolled and allocated to 1 of 5 cohorts and given escalating doses of OCH. Mild leukopenia occurred in two subjects, but recovered without any treatments. Plasma concentration of OCH was much higher than anticipated based on preclinical study. Flow cytometer analysis of lymphocyte subsets revealed that GM-CSF producing fractions in CD4+ memory T cells and CD8+ T cells were reduced after administration of OCH in all cohorts. DNA microarray analysis revealed that expressions of some genes associated with autoimmune responses were decreased, and some immunoregulatory genes increased. The results were potentially interesting and allowed us to start an early phase 2 study for patients with multiple sclerosis in March 2014.

T.50. An Increased Proportion of IL-6-dependent Plasmablasts Characterizes Interferon beta-resistant Patients with Relapsing-remitting Multiple Sclerosis

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Patients with relapsing-remitting multiple sclerosis (RR-MS) respond differentially to disease-modifying drugs such as interferon beta (IFN-b), reflecting its heterogeneity in the immune-mediated pathogenesis. To gain a clue to understanding the heterogeneity of RR-MS involving B cell subsets, we here analyzed frequencies of plasmablasts (PB) in the blood of RR-MS. The pathogenic role of PBs, that produces pathogenic anti-aquaporin 4 autoantibodies in an IL-6 dependent manner, has been recently established in neuromyelitis optica (NMO) (Chihara et al. 2011). NMO does not respond to IFN-b but to anti-IL-6 receptor antibody tocilizumab (Araki et al. 2013). Flow cytometric analysis of peripheral blood showed that the RR-MS patients could be divided into PB-low (42/65) and PB-high MS (23/65) according to PB frequencies among total B cells. In the

patients who have experienced IFN-b treatment without severe adverse effects (36/65), PB-high MS (13/36) was enriched with IFN-b nonresponders (13/13) in comparison with PB-low patients (4/23), indicating the resemblance of PB-high MS to NMO. IL-6 receptor as well as gp130 was more abundantly expressed on PBs from PB-high MS than PB-low MS ($p < 0.05$). In vitro survival of PBs from PB-high MS was significantly prolonged with adding exogenous IL-6 ($p = 0.0142$), but was significantly decreased by adding anti-IL-6 receptor antibody ($p = 0.0078$). Gene expression profiling by nCounter® (NonoString Technologies) demonstrated the enhanced IL-6 signaling pathway in the PBs from PB-high MS ($n = 6$). Collectively, the pathology mediated by IL-6-dependent PBs may provide a mechanism of resistance to IFN-b in a subgroup of RR-MS.

T.51. Cgen-15001 a Novel Immunomodulatory Fc Fusion Protein of the B7 Family Induces Tolerance During Inflammatory Immune Response

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The present data show that Cgen-15001, a novel B7-like fusion protein, induces a tolerogenic phenotype in the PLP135-151-induced R-EAE model in SJL mice, the type-1 diabetes model in NOD mice, and the H-Y bone marrow transplantation model in female C57BL/6 mice. Cgen-15001 consists of the extracellular region of Cgen-15001T, a membrane protein predicted to be a member of the B7/CD28-family, fused to IgG Fc domain. Initial *in vitro* studies have shown that Cgen-15001 treatment of both mouse and human naive CD4+ T cells cultures decreased T cell activation as well as Th1 and Th17 differentiation, while promoting Th2 differentiation. Cgen-15001 treatment also significantly increased the differentiation of naive CD4+ T cells toward a Treg phenotype *in vitro*. Similar immunomodulatory effects of Cgen-15001 treatment on CD4+ T cells were confirmed in the R-EAE model, *i.e.*, a decrease in the Ag-specific Th1 and Th17 responses while increasing Th2 responses and iTreg counts. We have extended these studies to show that Cgen-15001 also inhibits T1D disease incidence in the NOD mice, and that the majority of mice treated with Cgen-15001 retained normoglycemic for 18 weeks after cessation of treatment. Additionally, the present data show that Cgen-15001 treatment resulted in bone marrow cell graft survival and increase in Treg cells, indicating donor specific tolerance induction. The prolonged therapeutic responses in models of autoimmunity and the prevention of graft rejection suggest specific immunomodulation and imply establishment of immune tolerance by Cgen-15001 treatment providing a potentially safe and effective approach for the treatment of autoimmunity.

T.52. Genetic Variants Associated with Multiple Sclerosis Result in Enhanced NFkB Signaling and Responses to Inflammatory Stimuli

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Autoimmune diseases are a heterogeneous collection of over 40 syndromes affecting ~7% of the population. Genome-wide association studies (GWAS) have found hundreds of variants associated with an increased risk of developing disease. In particular, variants in the NFκB signaling cascade appear to be the most common pathway across autoimmune diseases. NFκB is a central regulator of immune responses and is activated downstream of TCR, BCR, TLRs, CD40, TNF-α, and IL-1β suggesting it may be a critical node where genetic predisposition and environmental factors interact to promote disease. Naïve CD4 T cells from Multiple sclerosis (MS) patients exhibit constitutive activation of p50/p65 NFκB, leading us to hypothesize that genetic variants associated with MS result in increased NFκB signaling. To determine whether increased NFκB responses in MS may be genetically mediated, we investigated the impact of two MS-associated variants on p65 NFκB signaling. A polymorphism in an intron of the type I TNF-α receptor (TNFR1) results in a truncated splice variant with impaired trafficking to the cell membrane and intracellular accumulation. After stimulation with TNF-α, the risk variant results in increased IκBα degradation and nuclear localization of p65 NFκB. A second MS-associated variant proximal to NFκB1 results in increased p65 NFκB signaling to TNF-α, LPS, and PMA, suggesting that this variant broadly impacts NFκB signaling to multiple stimuli. Taken together, our results demonstrate that genetic polymorphisms associated with MS may alter NFκB signaling pathways resulting in increased constitutive NFκB activation and greater responsiveness to inflammatory stimuli.

T.53. The Wnt Pathway Downregulates APC-mediated Inflammation in MS

Igal Ifergan*, Rachael Terry and Stephen Miller. Northwestern University, Chicago, IL*

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-specific 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 signalling in APCs could play a central role in regulating the balance between inflammatory versus regulatory responses in the gut. Since Wnt proteins are essential for neurodevelopment and are also widely expressed in the brain, in adulthood, our goal was thus 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-1b, IL-6, IL-12 and IL-23 were reduced on these populations while phagocytic capacities were increased following Wnt activation. These data were confirmed using human monocytes. In addition, purified naïve MOG_{35-55}-specific T cells from 2D2 mice expressed less IL-17 and IFN-g when cultured with MOG_{35-55}-pulsed APCs treated with the Wnt agonist. Finally, treatment with Wnt agonist decreases the clinical severity of EAE. Altogether our data demonstrate that Wnt activation on APCs reduces the ability of T cells to mount an inflammatory response.

Autoimmune Rheumatologic Disease

1101B. Increased Interferon Activity Precedes SLE Classification but Follows Autoantibody Onset

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Determining processes that lead to clinical illness in systemic lupus erythematosus (SLE) years before diagnosis would provide insight into the fundamental origins of SLE pathogenesis. This study evaluates the temporal relationship between autoantibody production, interferon (IFN) levels and the onset of SLE. Serial sera from 56 pre-clinical SLE cases leading up to SLE classification (average timespan=4.3 years) and matched healthy controls were obtained from the Department of Defense Serum Repository. Sera samples were tested for autoantibodies (BioPlex2200), IFN-α activity, and soluble inflammatory mediators, including IFN associated chemokines and BLyS (B lymphocyte stimulator). Temporal relationships between IFN activity and autoantibody positivity were determined and confirmed using Path analysis. Serum IFN-α activity scores, as well as IFN-correlated ($p<0.001$) soluble mediators IFN-γ, IP-10, and BLyS levels increase in patients leading up to SLE disease classification ($p\leq0.01$). IFN activity positive individuals have an increased number of autoantibodies compared to IFN activity negative individuals ($p<0.05$). Autoantibodies accumulate prior to or concurrent with increases in IFN activity in 84% of IFN positive cases. Of the 3 models of IFN vs. autoantibody positivity determined via Path analysis (autoantibody 1st, IFN activity 1st, or concurrent), autoantibody positivity prior to IFN activity ranked first, irrespective of whether DNA- or RNA-binding autoantibodies were present, as assessed by both Akaike's information criterion (AIC) and root mean square error of approximation (RMSEA). That autoantibodies accumulate prior to the increases in IFN activity levels suggests that the elevated IFN activity observed in SLE patients is a consequence of pre-clinical immune system perturbations.

1109B. The High Number of Plasmacytoid Dendritic Cells and Their Altered Biology in Systemic Sclerosis Correlate with Low Expression of RUNX3

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Background*:* Systemic sclerosis (SSc) is an autoimmune disease with unknown pathogenesis manifested by inflammation, vasculopathy and fibrosis in skin and internal organs. Although majority of research is still focussed on fibroblast biology, type I IFN-signature found in SSc propelled us to study plasmacytoid dendritic cells (pDCs) in this disease. Methods: We enumerated and phenotyped pDCs from healthy individuals and SSc patients using flow cytometry. pDCs were isolated using magnetic beads for transcription factor and methylation status analyses using PCR-based assays, and SNP genotyping by sequencing. Runx3-/- mice were used for pDC enumeration and dendritic cells functional analysis. Results: SSc patients showed higher frequencies of circulating and bone marrow pDCs compared to healthy individuals, and they differentially expressed activation markers. Focussing on a possible differentiation defect, we performed transcription factors profiling and found RUNX3 to be downregulated in SSc pDCs. We identified a non-synonymous SNP and observed a higher methylation status that correlated with pDC number, RUNX3 level, and disease susceptibility. Furthermore, Runx3-/- mice showed a higher pDC frequency in the lymph nodes compared to wild-type, and their dendritic cells exhibited enhanced cytokine expression upon TLR stimulation recapitulating pDC biology in SSc patients. Conclusion: pDCs are extremely frequent in SSc patients likely attributed to RUNX3 downregulation. The presence of an associated SNP and higher methylation status suggest two causal pathways underlying low RUNX3 expression. In addition to our previous study showing SSc pDCs that highly secrete CXCL4 and IFN-alpha, this data further supports targeting pDC function as novel therapeutic target in SSc.

1110B. Low-dose Interleukin-2 Therapy in Active Systemic Lupus Erythematosus

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Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by the loss of tolerance to nuclear self-antigens, the production of pathogenic autoantibodies and damage to multiple organs. While available therapies, such as corticosteroids and immunosuppressive agents have improved the outcome of patients, there remains a significant unmet need for safe and more effective treatments. Low-dose Interleukin-2 (IL-2) therapy has recently been shown effective to treat autoimmune diseases without broad immunosuppression. To assess the safety and efficacy of low-dose IL-2 therapy in active SLE, we conducted a single-centre phase 2 study (ChiCTR-ONC-13003497) on SLE patients with refractory or relapse to glucocorticoid therapy. 19 patients with scores>=8 on the Safety of Estrogens in Lupus Erythematosus National Assessment (AELENA) version of the SLE Disease Activity Index (SLEDAI) received three courses of recombinant human IL-2 (1 million IU every second day for 2 weeks followed by a 2-week hiatus) with standard of care. The primary end point was the safety and response rate at week 10. 18 of 19 patients responded to the therapy, defined as a ≥4-point reduction in AELENA-SLEDAI score. Major clinical indicators were improved: reduced dsDNA autoantibody titres and 24-hour proteinuria and increased levels of the complement proteins C3 and C4. Immunological analysis demonstrated significant increase of Treg cells and decrease of effector helper T cells after the therapy. Our results showed low-dose IL-2 therapy in active SLE were safe and achieved satisfactory efficacy.

OR.1. Sub-phenotype Mapping in Systemic Lupus Erythematosus Identifies Multiple Novel Loci Associated with Circulating Interferon Alpha

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Systemic Lupus Erythematosus (SLE) is a chronic autoimmune disorder characterized by involvement of multiple organ systems, loss of tolerance to self-antigens and dysregulated interferon responses. SLE is both genetically and phenotypically heterogeneous, greatly reducing the power of overall case-control studies in SLE. Genetic studies focusing on pathogenic molecular SLE sub-phenotypes should greatly increase our power to detect SLE susceptibility loci. In this study, we genetically mapped the serum IFN-α trait in SLE patients to discover novel and important genetic variants involved in this critical pathogenic pathway. We used a case-case design to detect genetic influences on serum IFN-α in over 1800 SLE

cases, data from 400 European ancestry cases formed the discovery GWAS set, and 1443 cases from a large independent multi-ancestral replication cohort were used to validate associations. In meta-analysis, the top associations in European ancestry PRKG1 rs7897633 (P_{Meta}=2.75 x10^{-8}) and PNP rs1049564 (P_{Meta}=1.24 x10^{-7}) loci. These loci have not been previously identified in case-control SLE genetics studies and may play a critical role in the dysregulation of IFN-α. We also found evidence for cross-ancestral background associations in *ANKRD44* (rs4850410; P_{Meta} =1.3 × 10^{-6}), the intergenic SNP rs297573 (P_{Meta} =1.2 × 10^{-4}), and *CALD1* (rs6467557; P_{Meta} =2.9 × 10^{-5}) As case-control studies of complex heterogeneous diseases reach a limit of feasibility with respect to subject number and effect sizes detectable, the study of informative pathogenic subphenotypes becomes a highly attractive and efficient strategy for genetic discovery in complex human disease.

OR.2. A Novel Defect in ER-Golgi Transport Provides a Molecular Link Between ER Stress and the Generation of Autoimmunity

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ER stress is increasingly recognized as an important mechanism in the pathogenesis of both autoimmunity and interstitial lung disease. Here we report on a novel autoimmune syndrome characterized by interstitial lung disease, pulmonary hemorrhage and inflammatory arthritis. We performed whole exome sequencing in three separate families each manifesting an autosomal dominant pattern of disease. In our analysis we identified three distinct missense mutations each of which are predicted to cripple the same functional domain of a protein involved in ER-Golgi transport. Using primary cells from patients and in assays overexpressing the mutant protein in cell lines, we demonstrate that the mutations lead to an increase in ER stress and impaired autophagy. Importantly, both of these cellular defects have been described in a number of autoimmune syndromes. In conclusion, our data demonstrate a novel defect in ER-Golgi transport that provides a molecular link between ER stress and the generation of a rare monogenic syndrome of autoimmunity.

OR.19. Blocking KCa1.1 Channels Inhibits the Invasive Properties of Fibroblast-Like Synoviocytes and Reduces Disease Severity in Animal Models of Rheumatoid Arthritis

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Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease of largely unknown etiology that mainly affects diarthrodial joints, leading to joint destruction, pain, and decreased mobility. The fibroblast-like synoviocyte (FLS) is a resident synovial cell that has a central role in RA pathogenesis, including the formation of synovial hyperplasia and increased invasiveness, along with increased cytokine, growth factor, and protease release. These factors contribute to cartilage and bone degradation within the joint. Currently, no RA therapy has been developed to specifically target FLS. We have found that FLS from patients with RA and from rats with the pristane-induced arthritis model of RA have increased expression of KCa1.1 at their plasma membrane when compared with FLS from patients with osteoarthritis or from healthy rats, respectively. Selectively blocking the function of this channel reduces many of the pathogenic aspects of RA-FLS, including decreasing proliferation, invasiveness, and release of cytokines, chemokines, angiogenic factors, and proteases. Furthermore, decreasing the amount of KCa1.1expressed in FLS reduces their invasiveness. We have also found that inhibiting KCa1.1 with a selective small-molecule blocker after onset of clinical signs of arthritis significantly reduced disease severity in both the collagen-induced and pristane-induced rat models of RA, along with reducing the *ex vivo* invasiveness of FLS from blocker-treated animals. These studies indicate the importance of KCa1.1 as a novel target for RA and emphasize the potential efficacy of directly inhibiting FLS in reducing the severity of this debilitating disease.

OR.22. Treatment of Refractory Systemic Lupus Erythematosus with the Proteasome Inhibitor Bortezomib Results in Decreased Pathogenic and Protective Antibody Titres

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Refractory disease courses of SLE may be often caused by pathogenic autoantibodies secreted by long-lived plasma cells (PC), because these are resistant to conventional treatments including high dose glucocorticoids and cyclophosphamide. We previously reported that the proteasome inhibitor bortezomib can efficiently eliminate PC, including long-lived ones, decrease anti-dsDNA antibodies and ameliorate lupus-like disease in mouse models. Bortezomib has been approved for the treatment of multiple myeloma. After informed consent, we treated 5 patients suffering from severe refractory SLE with bortezomib. Bortezomib was used as an induction therapy with 2 to 6 cycles of 3 to 4 injections per cycle. The SLEDAI disease activity score decreased in all patients. Adverse events were mild and transient. To analyze the influence of proteasome inhibition on concentrations of total IgG, IgA and IgM, pathogenic anti-dsDNA versus protective antibodies against HBs antigen, tetanus toxoid and rubella, concentrations were determined by nephelometry, Farr-assay (anti-dsDNA), EIA or ELISA. Total IgG, IgA and IgM concentrations decreased, but mainly remained within normal limits. Anti-dsDNA was markedly deminished in all patients, in one patient it even disappeared. Also protective antibodies were decreased by bortezomib treatment, however, only by approximately 30 percent. Our results indicate that proteasome inhibitors may represent a promising treatment option in refractory antibody-mediated diseases like SLE. The doses of bortezomib used cannot eliminate all plasma cells, since antibodies against vaccines/viruses and ENA were usually much less affected than those against dsDNA. After bortezomib treatment vaccination titers should be monitored to perform booster vaccination when necessary.

OR.25. Monocytes but not Dendritic Cells from the Site of Autoimmune Inflammation Impair T Cell Regulation

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Ever since their discovery research has focused on whether deficiencies in FOXP3$^+$ regulatory T cells (Treg) underlie human autoimmune pathology. Recently however It has become clear that resistance of effector cells (Teff) to suppression contributes to disturbed immune regulation in autoimmune inflammation, especially at the site of inflammation. It remains unknown how resistance of T cells to suppression is induced. Here, we phenotyped APCs present at the site of autoimmune inflammation in patients with juvenile idiopathic arthritis (JIA) and investigated their role in inducing Teff resistance to suppression and T cell regulation in general. We observed a clear difference in the phenotype and composition of APCs in synovial fluid (SF) obtained from inflamed joints, compared to peripheral blood (PB). The majority of SF monocytes expressed high levels of CD16, most closely resembling the CD14+CD16+ intermediate PB monocyte subtype. Moreover, SF monocytes displayed clear pro-inflammatory characteristics with especially high TNF-α and IL-6 production directly *ex vivo*. Upon co-culture with PB effector cells, these SF monocytes and not inflammatory dendritic cells (DCs) induced impaired Treg mediated control of both CD4 and CD8 T cell proliferation and cytokine production even though DC were stronger inducers of T cell proliferation. Regulation was restored when TNF-α and/or IL-6 were blocked. These data shed new light on the role of monocytes in autoimmune pathology, indicating that monocytes support DC in driving T cell responses and actively contribute to the ongoing inflammation by interfering with T cell regulation by producing pro-inflammatory cytokines.

OR.28. Circulating Dendritic Cell Subsets in Human Lupus: Correlations with Circulating Cytokines and Disease Status

Mark Jensen[1], *Jessica Dorschner*[1], *Danielle Vsetecka*[1], *Gaby Daboul*[1], *Elizabeth Rosenzweig*[2] *and* ***Timothy Niewold***[1]. [1]*Mayo Clinic, Rochester, MN;* [2]*University of Chicago, Chicago, IL*

Systemic lupus erythematous (SLE) is a complex autoimmune disease that often affects multiple organs resulting in chronic morbidity. High production of type I interferon by TLR triggered plasmacytoid dendritic cells (PDCs) likely contributes to disease pathogenesis. Previously, we and others have linked numerous gene polymorphisms with blood IFN levels, disease severity, and clinical characteristics. Here we have studied expression of multiple inhibitory and stimulatory surface receptors on PDCs and MDCS from a large cohort of patients (n>80) and controls (n>30) and correlated them with IFN levels, clinical characteristics, and disease severity. From these studies we wish to uncover changes in expression levels of various markers on DCs that tie to specific SLE groups, disease mechanisms, and drug efficacy. Our results show discordant regulation of receptors between PDCs and MDCs in SLE groups, with high level expression of stimulatory receptors on MDCs in high IFN

patients. Additionally, PDCs from all SLE patients demonstrated low expression levels of key inhibitory receptors irrespective of IFN levels and disease status. These data suggest that PDCs are dysregulated in SLE patients even at early stages of disease and that distinct SLE groups may benefit from MDC targeted therapies.

OR.31. Altered Wiring of Signal Transduciton Networks in Human Systemic Lupus Erythematosus B Cell Subsets

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Autoantibody production is a hallmark of Systemic Lupus Erythematosus (SLE), supporting a central role for B cells in disease pathogenesis. Prior studies demonstrate augmented B cell receptor (BCR) signaling in SLE patients relative to healthy controls (HC). However, whether signaling aberrations are relevant only in mature B cells or whether they also play a role in immature B cells remains unclear. This is an important distinction because BCR signaling in mature naive B-cells leads to proliferation and survival but receptor editing, depletion or anergy in immature cells. We hypothesized that dysregulation of signaling networks could contribute to SLE pathogenesis. We used multiparameter phosphoflow cytometry to interrogate signaling in the basal state and in response to BCR stimulation in transitional and naïve B cells from 20 SLE patients and 20 healthy controls (HC). Both transitional and naïve B cells obtained from SLE patients with well-controlled disease have increased activation of the MAPK pathway in the basal state and in response to BCR stimulation. Phosphorylation of S6 was also elevated in the basal state in all SLE B cell subsets. However, surprisingly, S6 was *hypo*phosphorylated in response to BCR ligation in naïve mature but not transitional B cells. While S6 is a target of the PI3K/AKT/mTOR pathway after BCR ligation, it also receives inputs through growth hormone and cytokine receptors as well as the mTOR complex, which senses energy and nutritional levels. Current investigations are under way to identify the mechanisms mediating these observations and their relation to SLE pathogenesis.

OR.37. Genetic Risk Alleles Associated with Autoantibody Production and Responsible for Initial Breach in Tolerance to Self-Antigens in Normal Individuals

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Antinuclear antibodies (ANA) are serological hallmarks of SLE. Studies have shown that healthy individuals also develop ANA, raising questions on relevance of ANA positivity and autoimmune disease. We screened 2223 healthy controls (HC) and 143 SLE patients for ANA by ELISA and found that 26% HC are ANA positive, with 17% moderate (20-40 AU) and 9% high (>40 AU) ANA levels. To elucidate the autoantibody profiles associated with ANA in HC and SLE, we performed autoantigen arrays to profile over 90 autoAbs and found that ANA positive HC make significant amount of IgG and IgM autoAbs against many antigens with high frequency to non-nuclear antigens such as collagens and phospholipids. We noticed stronger autoAb signatures in females than males. We then genotyped 1583 HC with high and low ANA by Immunochip and did quantitative association test using ANA as dependent variable. Seven genomic loci showed suggestive genomewide association (p=1.00E-05) with ANA. Multiple independent association signals were observed in HLA class I, II and III region and the strongest association mapped to HLA-DR alleles where peak SNP rs9268832 in HLA-DRA gene reached p value 7.63606E-07. The impact of ANA risk alleles on HLA-DR gene expression was studied in B cells and macrophages from healthy donors by RNA-seq and results show that ANA risk haplotype is associated with up regulation of HLA-DRB1 gene which could be associated with initial breach in tolerance to self-antigens in general population.

OR.39. Auto-Antibodies in Necrotizing Autoimmune Myopathies : From Diagnosis to Pathogenesis

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Background and objective. Necrotizing autoimmune myopathies (NAM) are a newly recognized group of severe acquired myopathies, characterized by prominent myofiber necrosis without significant inflammation. NAM may be associated to auto-antibodies (aAbs) to signal recognition particle (SRP) or to the statins target 3-hydroxy-3-methylglutaryl-coenzyme A

reductase (HMGCR). We developed quantitative assays of anti-SRP and anti-HMGCR aAbs and investigated their possible pathogenic role. Methods. Recombinant human SRP or HMGCR was coupled to fluorescent beads and used to measure the levels of aAbs by Luminex. NAM patients were compared to different inflammatory/autoimmune diseases and healthy controls. Reactivity of anti-SRP Abs against muscle was determined. IgGs from aAb-positive patients were transferred to mice. Results. Both Luminex assays revealed sensitive and very specific. Titers of IgG aAbs correlated with creatine kinase levels. Anti-HMGCR positive patients were mostly females and only 40% had been exposed to statins. Incubation of muscle sections with anti-SRP Abs revealed a punctuated intracellular staining with peripheral reinforcement which disappeared upon preincubation with excess recombinant SRP54. Immunogold electron-microscopy revealed immunoreactivity to the endoplasmic reticulum. Anti-SRP positive plasma was toxic to myotubes in vitro. Injection of anti-SRP positive IgG to mice resulted in significant decrease in muscle strength. The effect was transient in immunocompetent mice (with production of anti-human IgG Abs) and prolonged in immunodeficient RagKO animals. Similar results were found after transfer of anti-HMGCR positive IgG or immunization with recombinant HMGCR. Conclusion. The results suggest a direct pathogenic role of anti-SRP and anti-HMGCR aAbs, and prompt to evaluate B-cell targeting therapies in NAM.

W.6. The B' Family of Protein Phosphatase 2a is Indispensable for the Stability of T Regulatory Cells

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Protein phosphatase 2a (PP2A) is a ubiquitously expressed enzyme that regulates a number of cellular processes, including cell cycle, apoptosis, migration and cellular metabolism. It is a heterotrimer, as it consists of 3 distinct subunits: a catalytic C subunit (PP2Ac), a scaffold A subunit (PP2Aa) and one of a number of regulatory B subunits that are categorized into three major families (B, B' and B''). We have previously demonstrated that PP2Ac levels and activity are increased in T cells from patients with Systemic Lupus Erythematosus (SLE) through both genetic and epigenetic mechanisms. Furthermore, we have shown that this aberrant regulation of PP2Ac contributes to the IL-2 defect exhibited by T cells from SLE patients. Here, we demonstrate both by pharmacologic inhibition of PP2A and by the use of a mouse model that PP2A activity is indispensable for the maintenance of T regulatory cells (Tregs). More specifically, the use of okadaic acid (OA), a PP2A-specific inhibitor, abrogates the generation of *in vitro* induced Tregs in the presence of TGF-β and IL-2. The Tregs generated in the presence of OA have lower expression of Foxp3 and are less suppressive. Furthermore, by using $PP2Aa^{E64G}$ mutant knockin mice that harbor a mutation in the scaffold A subunit that specifically abrogates the binding of B' family proteins to the catalytic-scaffold heterodimer, we show that the B' containing PP2A complexes promote the stability of Foxp3 Tregs both *in vitro* and *in vivo*. In conclusion, PP2A is a key cellular phosphatase that promotes Treg stability and function.

W.7. The E3 Ligase Cbl-b Modulates Peripheral Regulatory T cell Tolerance via $p27^{kip1}$ in Systemic Lupus Erythematosus Patients

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The interplay between effector and regulatory T cells (Tregs) is a key element among peripheral tolerance in Systemic Lupus Erythematosus (SLE). Resistance to suppression has been recently acknowledged as part of the defects shown by T cells from SLE patients. The E3 ligase Cbl-b has been shown to regulate T cell unresponsiveness in SLE. However its potential role in the modulation of peripheral Tregs tolerance has not been fully addressed. The aim of this study was to assess the expression of Cbl-b and its relationship to the resistance to suppression phenotype in SLE patients. We included 20 SLE patients (8 in remission and 12 with active untreated disease) and 20 healthy controls. PBMCs were isolated and effector ($CD4^+CD25^-$) and Tregs ($CD4^+CD25^+CD127^-$) were purified by magnetic selection. The expression of Cbl-b and $p27^{kip1}$ was analysed by Western blotting. Interaction between Cbl-b and $p27^{kip1}$ was addressed by immunoprecipitation. Proliferative responses were assessed in allogeneic and autologous cocultures by CFSE. We found diminished Cbl-b expression in Tregs from SLE patients in comparison to healthy controls (5.8±1.0 vs 10.8±1.7, p=0.021), which was associated with resistance to suppression in proliferation assays. Moreover, this phenomenon was related to deficient expression of the cell cycle regulator $p27^{kip1}$ in Tregs from SLE patients when compared to healthy controls. We found no significant differences regarding to

disease activity. Our data suggest that the ligase Cbl-b is able to regulate the interplay between effector and Tregs, particularly, the resistance to suppression via ubiquitination of $p27^{kip1}$ in SLE patients.

W.12. Combined Prevention of IL-7 and TSLP Signaling Results in a Strong Additive Reduction of Th17-Driven Immune Activation and Tissue Destruction in Experimental Arthritis

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Introduction. IL-7 and Thymic Stromal Lymphopoietin (TSLP) are increased in synovial fluid of rheumatoid arthritis (RA) patients and separately mediate critical proinflammatory effects in autoimmune arthritis models. IL-7 and TSLP are related cytokines involved in Tcell development and dendritic cell (DC) activation. We aimed to investigate if IL-7 and TSLP have an additive effect on *in vitro* immune cell activation and *in vivo* autoimmune arthritis. Methods. CD1c-expressing mDCs and CD4 T-cells were isolated from the circulation of RA patients and co-cultured for 6 days in presence of IL-7, TSLP or both. Proteoglycan induced arthritis was induced in wildtype (WT) and TSLPR knockout (TSLPR-/-) mice treated with PBS or antibody against the IL-7Ra. Clinical arthritis score and joint destruction were assessed and proinflammatory mediators were analyzed by multi-analyte profiling. Results. IL-7 and TSLP additively increased DC-induced Tcell proliferation and production of IL-17 and IL-22. In mice, clinical arthritis score were almost completely inhibited upon prevention of IL-7 and TSLP signalling, stronger than in TSLP-/- mice or anti-IL-7Ra WT mice. Anti-IL-7Ra treated TSLPR-/- mice hardly showed any joint destruction with superior inhibition compared to the other groups, which was associated with additive inhibition of multiple proinflammatory mediators including MIP-1a, IL-6 and IL-12. Discussion. IL-7 and TSLP additively enhance T-cell proliferation and pro-inflammatory cytokine production in a human *in vitro* and murine experimental arthritis models. Considering the interplay between IL-7 and TSLP, therapeutic strategies that target IL-7R preventing both TSLP and IL-7 signalling have strong therapeutic potential.

W.32. Altered Innate Lymphoid Cell Frequency in the Peripheral Blood of Systemic Sclerosis Patients

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Systemic Sclerosis (SSc), or scleroderma, is a disease characterized by wide-spread vascular damage, inflammation, and fibrosis. Although the etiology of SSc is unclear, numerous studies support an autoimmune process. We investigated whether the frequencies of recently described innate lymphoid populations were altered in the peripheral blood of scleroderma patients as compared to controls. Innate lymphoid cells (ILC) are a heterogeneous, but developmentally related group of lymphocytes that have emerged recently as important mediators of inflammation and immune homeostasis. ILC rapidly produce large amounts of T cell-associated cytokines in response to innate stimulation; they are antigen-independent and, thus, can straddle the divide between T cells and other innate cells. Considering this feature, they may play a role in the initiation and/or amplification of inflammation in SSc. Interestingly, there was an increase in the frequency of group 1 ILC (ILC1), whereas $NKp44^-$ ILC3 were decreased in frequency in SSc patients as compared to controls. Within the ILC1 subset, this increased frequency was present in a newly discovered $CD4^+$ subset, but not in $CD4^-$ ILC1. We also detected intracellular CD3e in both the $CD4^+$ and $CD4^-$ ILC1 subsets, but not in group 2 ILC (ILC2) or ILC3, possibly hinting at the interrelatedness of $CD4^+$ and $CD4^-$ ILC1. However, $CD4^+$ and $CD4^-$ ILC1 appear functionally divergent based on their IL-6R expression, cytokine production, and chemokine receptor expression. Our data suggest that $CD4^+$ ILC1 may play a role in the pathogenesis of SSc and further highlights the heterogeneity of the ILC1 population.

W.33. Autoimmunity Through Unbalanced T Cell Receptor Signaling

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Introduction: RasGRP1 is a RasGEF, an important regulator of lymphocyte receptor signaling, and thymocyte selection. Utilizing structural, biochemical and molecular biology, we elucidated how RasGRP1 activity is controlled by autoinhibition (Iwig et al.,*eLife* 2013). Furthermore, we showed how a point mutation of Rasgrp1 causes loss of RasGRP1 autoinhibition, abnormal mTor signaling, and autoimmune pathology in mice (Daley et al.,*eLife* 2013). Currently, we use our insights to understand how single nucleotide variants (SNVs) in RasGRP1, found through mining of public databases, lead to unbalanced T cell receptor signaling and may lead to disease in patients. Results: In our recently published work, we mutated key amino acids based on the structure of RasGRP1, reconstituted a T cell line with mutant or wildtype RasGRP1 and compared RasGRP1 activity. We established that intracellular calcium binding to EF-hand 1 promotes RasGRP1 activity, and identified 2 novel self-inhibitory mechanisms regulating RasGRP1, preventing either spontaneous interaction of RasGRP1 with Ras, or recruitment of RasGRP1 to the cell membrane. Using a similar approach, we are now characterizing patient SNVs, and find that these lead to aberrant RasGRP1 activity. We are elucidating how these SNVs lead to autoimmunity, and through analysis of Illumina immunochip and exome chip databases of a cohort of patients with Systemic Lupus Erythematosus (SLE), we are establishing the frequency of such SNVs in SLE. Conclusions: RasGRP1 displays autoinhibitory mechanisms to control Ras signaling in lymphocytes. We discuss how we are currently unraveling how aberrant RasGRP1 signaling through SNVs in RasGRP1 leads to development of diseases like SLE.

W.36. Routine Flow Cytometry-based Immunophenotyping of Patients Across a Range of Autoinflammatory and Autoimmune Disease Highlights Common Features

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Using a prospectively acquired library of flow cytometry-based immunophenotyping data obtained from 30 healthy volunteers and patients with systemic lupus erythematosus, inflammatory bowel disease, Behçet's syndrome, and ANCA-associated vasculitis, we sought to identify shared features of autoimmune and autoinflammatory disease. Patients were enrolled on the basis of flaring disease and minimal immunosuppression and were immunophenotyped using a broad panel of surface markers adapted from that proposed by the FITMaN meeting[1] with the addition of CXCR5 and PD-1 (circulating Tfh) and CD161 (capacity to produce IL-17).A partial least squares regression analysis using flow parameters as predictors and disease status (yes/no) as the response variable identified a single latent factor that was highly associated with flaring disease ($p=1.5x10^{-6}$, Wilcoxon). Examination of the flow parameters loading onto this factor suggests that differences in the population of non-switched memory B-cells (IgD+CD27+), and of HLA-DR+ and CD161+ effector memory (CCR7+CD45RA-) CD8+ T-cells constitute important components of a general autoimmune/autoinflammatory phenotype. Moreover, the factor loadings highlight previously unappreciated relationships between these populations, especially a strong reciprocal relationship between CD161+ and HLA-DR+ effector memory CD8+ T-cells. These findings demonstrate the ability of routine, standardized immunophenotyping to elicit common perturbations of immune subsets across a number of diseases, with broad clinical research applications including insight into mechanisms of autoimmunity and the identification of clinically useful biomarkers of disease activity.

W.47. A Novel Population of Inflammatory Effector B Cells is Enriched in a Subset of Systemic Lupus Erythematosus and Rheumatoid Arthritis Patients

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We previously demonstrated that antigen-presenting mouse B cells, primed by T_H1 and T_H2 cells, differentiate into distinct cytokine-secreting B effector (Beff) subsets. In order to generate and characterize human Beff cells we developed a novel human T_H/B allogenic co-culture system. After 5 days of co-culture, a significant fraction of the Be1 cells (Beff cells from T_H1 co-cultures), but not the Be2 cells (Beff cells from T_H2 co-cultures), expressed high levels of FcRL5 and CXCR3. However, the Be2 cells expressed high levels of CD23. Transcriptome analyses of the sorted Beff populations revealed increased expression of IFNγ, T-bet and IL12Rβ2 in Be1 cells compared to Be2 cells. Re-stimulation of purified Beff cells with exogenous sCD40L and IL-21, singly or in combination, induced Be1 cells, but not Be2 cells, to produce IP10. By contrast, restimulation of Beff cells with sCD40L and IL-4, singly or in combination, induced Be2 cells, but not Be1 cells, to produce macrophage-derived chemokine (MDC). Next, using biomarkers identified in the *in vitro* generated Be1 and Be2 populations,

we asked whether similar Beff subsets are present in the blood of healthy individuals or autoimmune patients. Be1-like cells which express high levels of CXCR3, T-bet and FcRL5 and low levels of CD23 were present in low numbers in the peripheral blood of healthy controls. Interestingly, this population was greatly expanded in the blood of SLE patients and a subset of RA patients, suggesting that Beff cells with the capacity to produce inflammatory cytokines and chemokines are increased in autoimmune disease.

W.49. Microscopic Gut Inflammation in Spondyloarthritis is a Prognostic Factor for Initiation of Anti-TNF Therapy in Daily Practice

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Background: Microscopic gut inflammation frequently occurs in spondyloarthritis (SpA), and is associated with a risk of evolution to Crohn's disease (CD) and ankylosing spondylitis (AS). Half of all SpA patients have gut inflammation, without associated gastro-intestinal symptoms. Two types of inflammation are seen: an acute and a chronic type, the latter resembling early CD. Objectives: Assessing the association between microscopic gut inflammation, and the independent decision of the rheumatologist to initiate anti-TNF therapy. Methods: 63 axial and/or peripheral SpA patients from The Ghent Inflammatory Arthritis and spoNdylitis cohorT (GIANT) underwent an ileocolonoscopy and were followed for 18 months. Results: Gut histology was normal in 34 patients, whereas microscopic gut inflammation was found in 29 (acute/chronic). During 18 months of follow up, 25 (39,7%) patients were given anti-TNF because of persistent high disease activity despite ≥ 2 NSAIDs. Of the 34 patients with normal gut histology, 9 (26,5%) had started TNF-blocking agents. In patients with gut inflammation at baseline, a significantly higher proportion (16/29 i.e. 55,2%) had been started on anti-TNF (p=0,038). Results were similar in chronic and acute inflammation. Patients with chronic gut inflammation also seemed more likely to respond to anti-TNF (80% significant ASDAS response versus 40% in the normal subset; p=0,170). Conclusion: SpA patients with microscopic gut inflammation at diagnosis were more likely to be given anti-TNF within the next 18 months. Additionally, SpA patients with chronic gut inflammation seemed more likely to respond well to anti-TNF, but further analysis with a larger group of patients is needed.

W.50. Ectopic Lymphoid Neogenesis is Specifically Associated with Activation of the IL-23/IL-17 Pathway in Rheumatoid Synovitis

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Objective: Ectopic lymphoid neogenesis (ELN) occurs in 30-50% of the rheumatoid synovial samples but its functional relevance remains unknown. As ELN correlates with the degree of tissue inflammation we investigated here whether ELN was associated with specific cytokine profiles. Patients and Methods: Paired synovial tissue (ST) (n=63) and fluid (SF) (n=44) was obtained from the inflamed knee joints of rheumatoid arthritis (RA) patients. Synovial inflammation and ELN was determined by immunohistology. CD21L was used as molecular marker of ELN. Cytokine expression was determined by ELISA and quantitative PCR in SF and ST, respectively. Results:48% of ST displayed ELN by histology. ELN+ samples had increased T and B lymphocyte infiltration (p<0.001) and CD21L expression (p=0.014). SF analysis showed higher expression of IL-23 (p=0.018) and IL-17F (p=0.028) in ELN+ versus ELN- samples, with a similar trend for IL-22 (p=0.070). Other cytokines, including IL-17A, IL-6, TNF, Th1 cytokines and Th2 cytokines, were not different. In ST, IL-23 (p=0.030) mRNA levels were increased in ELN+ samples. Moreover, CD21L expression as molecular marker of ELN correlated significantly with mRNA expression of IL-23 (r=0.70), IL-17F (r=0.42), IL-21 (r=0.30) and IL-22 (r=0.33), but not IL-17A, IL-6 and TNF. The strong correlation between CD21L and IL-23, IL-17F, IL-21 en IL-22 was confirmed in an independent RA ST sample set (n=36). Conclusion: Synovial ELN in RA is specifically associated with increased expression of IL-23/IL-17-related cytokines. Whether patients depicting synovial ELN respond differently to therapeutic targeting of this pathway remains to be determined.

W.55. A Comprehensive Approach to Identify Approved Drugs and Treatments for Repositioning as Therapies for Systemic Lupus Erythematosus (SLE)

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Development of new SLE treatments has been slow. To accelerate the pace, an evidence-based approach was developed to find new lupus therapies amongst 6800 compounds FDA-approved for human use. The Lupus Treatment List (LRxL) was constructed with intense input from the entire lupus community, including patients. All drugs widely used for lupus or known to be ind evelopment for lupus by Pharma/Biotech were excluded. Details of the project can be viewed at www.linkedin.com/in/lrxlstat. A novel evidence-based composite utility scoring system was developed to rank the identified drugs/therapies numerically by scientific rationale, experience in lupus mice/human cells, previous clinical experience in autoimmunity, drug properties and adverse event profile. Of the 154 therapies initially screened, more than 25 have an appropriate set of characteristics to consider for testing in clinical trials in lupus, including drugs targeting cellular metabolism, kinases, the immune system, HDACs, complement as well as cellular therapies & non-drug interventions. This approach has not only identified unique candidates that could be useful in SLE and possibly other autoimmune/inflammatory conditions, but has also yielded a rigorous evidence-based process by which therapies can be usefully rated for possible clinical application to treat these conditions, thereby mitigating risk in drug development.

T.1. Differential Cytokine and Regulatory Pathways in Patients with Primary and Secondary Sjögren's Syndrome

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The aim of this study was to characterize and compare the presence of diverse cytokines and regulatory T and B cells in minor salivary gland biopsies of patients with primary Sjögren's syndrome (pSS), secondary SS (sSS), and patients with connective tissue disease (CTD) without SS. We included 15 pSS, 24 sSS (6 scleroderma, 9 RA and 9 SLE) and 15 CTD without SS patients (4 scleroderma, 4 RA, 7 SLE). Once deparaffinized and rehydrated, the tissues were examined by an indirect immunoperoxidase technique and antibody staining (goat polyclonal anti-human IL-19, goat polyclonal anti-human IL-22 or mouse monoclonal anti-human IL-24). To determine the subpopulation of $CD4^+$/IL-$17A^+$, $CD25^+$/IL-4^+, $CD4^+$/IFN-gamma$^+$ expressing T cells, $CD25^+$/$Foxp3^+$ Treg cells and $CD20^+$/IL-10^+–producing B cell subset, a double-staining procedure was performed. We estimated the mean percentage of positively staining cells in two fields. The median disease duration was 10 years, 11 years and 6 years for the pSS, sSS and CTD without SS patients, respectively. $CD4^+$/IFN-gamma$^+$, $CD25^+$/IL-4^+ and IL-22 T cell percentages were elevated in both SS varieties, however the response prevailed in pSS. pSS patients had a high number of $CD4^+$/IL-$17A^+$ and IL-19^+ T cells and a lower prevalence of IL-24^+cells. The T and Breg subpopulations were also both increased in SS, but mainly in pSS. In conclusion a pro-inflammatory and regulatory balance coexists in both SS varieties, however a more striking process prevailed in pSS. The explanation of these differences may be related to disease activity, disease duration and treatment.

T.2. Antigen Presenting Cells are Decisive for Regulatory T Cell Function in Chronic Autoimmune Inflammation

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Autoimmune diseases are characterized by an imbalance between regulatory T cells (Treg) and effector T cells (Teff) both in terms of number and function. We have shown that regulation is impaired at the site of active autoimmune inflammation, but

there are contradictory results on resistant Teff populations versus defective Treg. In this study we investigated the role of APC in Treg mediated suppression in synovial fluid (SF) of juvenile idiopathic arthritis (JIA) patients. $CD4^+CD25^-$ or $CD8^+$ Teff cells from PB and SF of JIA patients were used in a Treg suppression assay and stimulated with either anti-CD3 plus autologous APC or anti-CD2/CD3/CD28 beads. We also performed cross-over suppression assays with syngeneic or allogeneic PB and SF cells. In the presence of SF-derived APC no Treg-mediated suppression of Teff cell proliferation occurred which, instead, partially occurred when APC were replaced by beads. The suppression of IFN-g, TNF-a, IL-10 and IL-6 in the supernatant confirmed these findings. When SF-derived Teff cells where crossed with PB-derived Treg (syngeneic or allogeneic) in absence of APC cells we observed good suppression, which was absent when SF-derived Treg were crossed with PB-derived Teff cells. These data show that APC derived from the SF of JIA patients are critical players in the induction of Treg-mediated suppression. Furthermore our data suggest that, in addition to resistance of Teff to suppression, Treg from synovial fluid are intrinsically affected, at least in APC-independent suppression. These data shed light on the hurdles of tolerance induction at the site of chronic inflammation.

T.3. CXCL10, TNFR2 and Galectin-9 Correlate with Disease Activity in Juvenile Dermatomyositis

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Background: Juvenile Dermatomyositis (JDM) is a systemic autoimmune disorder of unknown immunopathogenesis in which the immune system targets the microvasculature of skeletal muscles, skin and other organs. The current mainstay of therapy is a steroid regimen in combination with other immunosuppressive treatments. So far there are no validated markers for monitoring disease activity, which hampers a personalized treatment. Objectives: To identify a panel of mediators specifically related to disease activity in JDM. Methods: We performed a multiplex immunoassay for plasma levels of 45 proteins related to inflammation in 25 JDM patients in four clinically well defined groups, as determined by clinical activity and treatment. We compared them with age-matched controls consisting of 14 healthy children and 8 children with non-autoimmune muscle disease. Results: Cluster analysis of circulating mediators showed distinct profiles for JDM patients and controls based on a group of 10 mediators. Next to CXCL10, TNFR2 and galectin-9 were significantly increased in active JDM. The levels of these three mediators were tightly linked to disease activity and correlated to clinical scores (CMAS and PhyGloVAS). Conclusion: This study shows that CXCL10, TNFR2 and galectin-9 correspond to the disease status in JDM and thus could be helpful in monitoring disease activity and guiding treatment. Furthermore, they might provide new knowledge about the pathogenesis of this autoimmune disease.

T.4. Celastrol COntrols Autoimmune Arthritis by Modulating Pro-Inflammatory Cytokines, TH17/TREG Balance and Gene Expression

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Rheumatoid arthritis (RA) is a debilitating autoimmune disease characterized by chronic inflammation affecting the joints. RA pathogenesis involves various cellular and molecular mediators of inflammation and bone damage. Many conventionally used drugs are effective in suppressing arthritic inflammation, but they do not always offer protection against progressive joint damage. In addition, the long term use of such drugs is associated with severe adverse effects. Natural products offer promising alternative/adjunct therapeutic agents. Using the rat adjuvant-induced arthritis (AA) model of human RA, we demonstrated the anti-arthritic activity of Celastrol, a bioactive component of a traditional Chinese medicine Celastrus, and defined the immunological and molecular basis of its action. Celastrol treatment of arthritic rats reduced the levels of antigen-induced pro-inflammatory cytokines and chemokines, suppressed anti-CCP antibody production, and inhibited phophorylation of STAT3 and ERK. Celastrol's protective effects against bone and cartilage damage are mediated primarily via the inhibition of RANKL, and reduction in both osteoclast numbers and MMP-9 activity. Further, Celastrol treatment reduced the frequency of Th17 as well as altered the Th17/Treg ratio in the joints. Finally, microarray analysis of lymphoid cells of Celastrol-treated arthritic rats revealed at least 10 differentially-expressed genes related to immune regulation and inflammatory responses. Thus, Celastrol effectively controlled immune-mediated events leading to arthritic inflammation and bone damage. Celastrol

has mostly been used in studies on cancer treatment. Ours is the first report on its use for the treatment of arthritis. Our results suggest that Celastrol might offer a promising alternative/adjunct treatment for RA.

T.5. Circulating Plasmablasts Reveal Overactive Mucosal Immune Responses in Patients with SLE

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Autoreactive and protective antibodies are secreted by plasma cells and their immediate precursors, the plasmablasts, which circulate through the blood. Increased levels of plasmablasts are found in patients with active SLE, yet their composition and origin remained largely uncertain. We demonstrate here that ~75% of plasmablasts in SLE patients express markers of mucosal immune reactions, i.e. IgA, the chemokine receptor CCR10, the adhesion molecule beta7 integrin, or combinations thereof. In vitro, SLE plasmablasts migrated along gradients of the mucosal chemokine ligand of CCR10, CCL28, and secreted polymeric IgA. The plasmablast composition in the blood of SLE patients recapitulated the phenotype of plasmablasts induced by oral vaccination against cholera rather than by parenteral vaccination against tetanus. Our data indicate that most plasmablasts circulating in SLE blood are generated in mucosal immune responses. Levels of mucosal but not parenteral plasmablasts in SLE blood were statistically associated with blood cytokine levels, including IL-2, IL-6 and IL-17, all of which are known to enhance plasmablast differentiation. Consistent with the importance of IgG (auto-)antibodies in SLE pathogenesis, autoreactivity of blood plasmablasts was limited to the IgG subclass, and IgG^+ plasma cells dominated over IgA+ plasma cells in lupus kidney biopsies. The stable presence of non-autoreactive IgA+ plasmablasts beside autoreactive IgG+ plasmablasts in SLE blood reflects distinct pathways of abnormal B cell differentiation in the overly activated immune system in SLE, which both contribute to the plasmablast expansion in SLE. Finally, our data indicate the abnormally enhanced activity of mucosal immune responses in patients with SLE.

T.6. The DNA Methyltransferase Inhibitor Azacitidine Suppresses Autoimmune Symptoms of MRL/lpr Mice with the Increase of Foxp3 Positive Cells

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DNA globally hypomethylation of $CD4^+$ T lymphocytes has been the one of important pathogenesis of autoimmune diseases. In a mice model, syngeneic recipient mice were shown to develop autoimmune phenotypes when they received $CD4^+$ T cells treated with 5-azacytidine (5AzC). On the other hand, the administration of 5AzC was reported to increase the number of regulatory T cells (Tregs) in patients with myelodysplastic syndromes (MDS). Suppression of immunity to self-antigen by Tregs is now well established, and demethylation of the Treg-specific demethylated region (TSDR) has been elucidated to be important for 1) the development of Tregs from thymus, and 2) the maintenance of suppressive activity of Tregs. We analyzed effects of 5AzC in autoimmune-prone, MRL/lpr mice. 5AzC treatment suppressed splenomegaly, lymphadenopathy, nephritis and sialoadenitis in MRL/lpr mice with the improvement of phenotypes of immune cells. The number of Foxp3 positive cells was increased in the thymus, spleen and lymph node of MRL/lpr mice treated with 5AzC compared to those without treatment. However, in vitro and vivo, the suppressive activity and losing Foxp3 expression of these cells were same levels as those of MRL/lpr mice without treatment. 5AzC effectively prevents autoimmune symptoms of MRL/lpr mice as previously reported. The plasticity of increased Foxp3 positive cells from MRL/lpr mice treated with 5AzC was accelerated to the same levels as Foxp3 positive cells from MRL/lpr mice without treatment. Furthermore, we will examine the mechanism of preventing symptoms of MRL/lpr mice after 5AzC treatment including phenotypes of Foxp3 positive cells.

T.7. Impaired Recognition of *Porphyromonas Gingivalis* in Rheumatoid Arthritis Patients

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The prevalence of periodontitis is increased in patients with rheumatoid arthritis (RA) and the severity of periodontitis can affect the level of arthritis. *Porphyromonas gingivalis* is one of the main bacteria causing periodontitis and our aim was to determine if there are differences in the innate immune response against *P. gingivalis* between healthy controls and RA patients. Monocyte-derived dendritic cells (DCs) from healthy controls, RA and psoriatic arthritis (PsA) patients were stimulated with *P. gingivalis*, a range of other bacteria and TLR agonists. Cytokine production was determined and blocking studies were performed to determine which receptors were involved in differential recognition of *P. gingivalis*. Cytokines were also determined in peripheral blood mononuclear cell (PBMC) cultures. RA DCs produced markedly less TNFα, IL-12p70 and IL-8 as compared to healthy controls upon stimulation with *P. gingivalis*, but not with the other bacteria tested. In sharp contrast, PsA patients did not differ from healthy controls, suggesting a RA specific deregulated response to *P. gingivalis*. Cytokine production upon *P. gingivalis* stimulation was not correlated with clinical disease characteristics. The difference between RA patients and controls was abolished when complement receptor 3 was blocked. In PBMC cultures interferon gamma induction by *P. gingivalis* was also reduced in RA patients. In conclusion, immune cells from RA patients display a marked diminished response to *P. gingivalis*. This could result in prolonged survival of *P. gingivalis* and an increased oral bacterial burden in RA patients possibly driving autoantibody formation and the characteristic self-perpetuating loop of chronic inflammation.

T.8. A Functional KO of Estrogen Receptor alpha Results in Decreased Inflammatory DCs Correlating with Increased Survival in Lupus-prone Mice

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Lupus is a disease that disproportionately affects females. The etiology of this sex bias is unclear. We previously showed that a functional knockout of estrogen receptor alpha (ERα) resulted in significantly reduced renal disease and increased survival in murine lupus. Dendritic cell development, which requires both estrogen and ERα is impacted, as is activation status and cytokine production. Due to altered hormonal feedback loops, ERαKO mice have hypergonadism and partial endocrine sex reversal. Elevated estrogen (E2) and testosterone levels may have immunomodulating effects. Thus, we studied the phenotype of the lupus-prone ERαKO mouse following ovariectomy (OVX) +/- E2 replacement to preserve a physiologic hormonal state. We found that NZM2410 ERαKO mice were protected from lupus disease expression (no early deaths; no proteinuria at 32 weeks) if they were either unmanipulated or if they were both ovariectomized *and* E2-repleted. These mice also had fewer inflammatory and activated cDCs (CD11c+/CD11b+/MHCII+) cells from Flt3L-cultured bone marrow, which correlated with increased survival. Interestingly, protection was lost after ovariectomy if no E2 pellet was administered, suggesting that the protective effect requires E2 in the system (despite the lack of a functional ERα). A protective effect was not observed in lupus-prone Ex3a mice (ERα-/-) regardless of hormonal state suggesting that the functional mutant in ERKO mice, in the presence of estrogen, potentially modulates disease.

T.9. Serum Circulating Angiogenesis Inhibitor Angiostatin Levels in Patients with Systemic Sclerosis

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Systemic sclerosis (SSc) is an chronic multysystem autoimmune disease, characterized by a widespread microangiopathy. The primary pathological findings are those of raynaud phenomenon, reduced blood flow to skin, finger ulceration and defective wound healing. Angiostatin is a physiologic angiostatic factor derived from the proteolytic cleavage of plasminogen. Angiostatin is also known as a potent antiangiogenic mediator that can be found in increased levels in the patients during various states of inflammation. The purpose of this study was to determine serum levels of angiostatin in patients with systemic sclerosis and comparison with healthy subjects. In this study, 47 patients with SSc (40 female, 7 male, mean age 32,7 ± 4,9 years, mean disease duration 11,4 ± 2,1 months) and 21 healthy controls (18 female, 3 male, mean age 29.2 ± 4,6 years) were included. Serum angiostatin levels were measured by ELISA. The mean serum angiostatin levels were 209,3 ± 27,4 ng/ml in patients with SSc and 69,1 ± 9,2 ng/ml in the healthy controls. According to these results; serum angiostatin levels were significantly higher in patients with SSc compared with healthy controls ($p<0.001$). In addition, serum

angiostatin levels were significantly higher in the patients with raynaud phenomenon and pulmonary involvement ($p<0.001$ and $p<0.01$ respectively). In conclusion, serum levels of angiostatin may play an important role in SSc pathogenesis.

T.10. Investigating the Role of IL-20 in the Pathogenesis of Behçet's Disease

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Background: Behçet's disease (BD) is a multisystem inflammatory disease, characterized by oral aphthous lesions, recurrent uveitis, skin lesions, genital ulceration, and joints involvement. In BD, increased release of proinflammatory cytokines may play a role in inflammatory stages of the disease. IL-20 is a proinflammatory cytokine and it has been reported to be involved in the development of autoimmune diseases. We aimed to search the relation among levels of serum IL-20 with activity of BD. Methods: 64 patients with BD (32 female, 32 male) and 16 healthy controls (7 female, 9 male; mean age 32.5 ± 6.1 years) were enrolled in this study. Thirty-eight patients were in active stage (mean age; 34.9 ± 5.4 years, median disease duration 7 years) and 26 patients were in inactive stage (mean age; 29.5 ± 6.9 years, median disease duration; 8 years). The median serum IL-20 levels were 26,7 pg/ml in healthy controls, 232,6 pg/ml in active BD patients and 47,4 pg/ml in inactive BD patients. Serum IL-20 levels in patients with BD were significantly higher than in healthy controls ($p<0.001$). In active patients with BD, there are statistically significant correlation between serum IL-20 and serum CRP and ESR ($r=0.674$, $p <0.001$, $r=0.602$, $p <0.001$ and respectively). In active BD patients, the mean serum IL-20 level was correlated with uveitis ($r=0.557$ $p=0.01$), and arthritis ($r=0.562$, $p=0.01$ Conclusion: IL-20 was associated with disease activity in BD. So, it may be involved in its pathogenesis.

T.11. A positive feedback mechanism of interleukin 6 through signal transducer and activator of transcription 3 in inflammatory arthritis

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Objective: The molecular mechanisms that link pro-inflammatory cytokines with disease activity in inflammatory arthritis remain incompletely understood. Signal transducer and activator of transcription (STAT) 3 is a signalling protein downstream of IL-6, IL-10, IL-21 and IL-23 receptors. Phosphorylated (P-) STAT3 translocates into the nucleus, where it regulates transcription of its target genes. IL-6 induces P-STAT3, and P-STAT3 promotes un-phosphorylated (U)-STAT3 transcription. We determined peripheral blood (PB) constitutive and induced P-STAT3 and total (T)-STAT3 in early stage inflammatory arthritis. Methods: We used flow cytometry to determine basal and IL-6 or IL-10-stimulated induction of P-STAT3 in PB CD3+ T-cells of 22 untreated RA patients (median symptom duration 16 weeks), 18 treated RA patients (median symptom duration 88 weeks), and controls (23 healthy, 22 non-RA inflammatory arthritis and 35 non-inflammatory arthritis patients). Serum cytokine levels were measured by chemiluminescence. Results: Constitutive P-STAT3 and T-STAT3 levels were significantly higher in T cells of RA patients compared to healthy controls, and P-STAT3 and T-STAT3 were correlated. Constitutive T cell P-STAT3 was positively associated with clinical and laboratory measures of inflammatory activity and with serum IL-6. In longitudinal analyses of RA patients, constitutive P-STAT3 correlated with response to treatment. Conclusions: Our observations are consistent with a positive feedback mechanism for IL-6 through STAT3 in inflammatory arthritis. This mechanism may underlie the potent therapeutic effect of IL-6 inhibitors.

T.12. Dexamethasone-Conditioned Monophosphoryl Lipid A-Activated Dendritic Cells Derived from Rheumatoid Arthritis Patients Exhibit Tolerogenic Features and Induce Poor T Cell Responses to Synovial Antigens

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Objectives: Recently we have developed a shortened protocol for the generation of Tolerogenic dendritic cells (TolDCs) from monocytes of healthy volunteers using dexamethasone and monophosphoryl lipid A (MPLA). Here we characterize TolDCs derived from rheumatoid arthritis (RA) patients concerning phenotype, cytokine profile, migratory properties and T cell-stimulatory capacity to prove their suitability for autologous cellular therapy. Methods: TolDCs were generated from monocytes of RA patients within 5 days, using dexamethasone for tolerization and MPLA for activation (MPLA-tDCs). The phenotype of MPLA-tDCs and their migratory behavior towards lymphoid chemokines were analyzed by flow cytometry in comparison to immature and MPLA-matured DCs. Cytokine secretion of MPLA-tDCs was determined by ELISA and their ability to activate autologous antigen-specific T cells was assessed by flow cytometry via CFSE-dilution and intracellular IFN-gamma and by determination of IL-10 and IL-17 levels in supernatants by ELISA. Results: MPLA-tDCs from RA patients exhibited stable semi-mature characteristics, such as reduced expression of costimulatory and coactivation molecules and the capacity to migrate in response to ligands of lymph node homing receptors CCR7 and CXCR4. These MPLA-tDCs displayed an anti-inflammatory cytokine profile and failed to induce proliferation, IFN-gamma and IL-17 production while inducing IL-10 secretion of autologous CD4+ T cells specific to synovial antigens. Conclusions: Monocyte-derived DCs of RA patients have the potential to develop stable tolerogenic features. Their impaired capacity to induce T cell responses to synovial antigens validates their applicability for treatment of RA. Support by Fondecyt 1140553 and Millennium Institute on Immunology and Immunotherapy P09/016-F.

T.13. The Molecules Involved in IL-6 Signaling an be Used as the Prediction Markers for Clinical Outcome in Rheumatoid Arthritis Patients to IL-6 Blocking Therapy, Tocilizumab

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Interleukin-6 (IL-6) blocking therapy with an anti-IL-6 receptor antibody (Tocilizumab) has been known as a most effective therapy for chronic inflammatory diseases. However, the precise mechanism of this effect has not been realized. Therefore, we have analyzed the improving mechanism to tocilizumab therapy in vitro and in vivo. Furthermore, based on those analytical evidences, we try to find the biological molecules which may respond and predict the clinical response before therapy. IL-6 signal is transduced into cells by two pathways, one is a classical pathway via membrane bound IL-6 receptor (mIL-6R), the other is a trans-signaling pathway via soluble IL-6R (sIL-6R). SIL-6R is an agonist of IL-6 signal but sgp130 is an antagonist of IL-6 signal. In induction of VEGF, CRP, SAA and hepcidin, IL-6 was a pivotal cytokine, and TNF-α or IL-1 complementally acted with IL-6 (Arthritis Rheum 2003, J.Immunol 2008, Gene Cells 2005, Blood 2010). VEGF was also induced by IL-6 with TNF-a or IL-1. Detection of the molecules, cytokine/chemokine/soluble receptor, in serum may be able to predict the clinical outcome before starting tocilizumab therapy. Analysis based on 16 weeks DAS-28 score revealed that sgp130, logIL-6 with some of molecules were markers to predict clinical outcome (complete-remission or non-remission) post tocilizumab therapy. Especially a high level of sgp130 is a highly reliable marker to predict outcome. The detection of pretreatment serum levels of prediction molecules can aware the outcome to tocilizumab therapy and can induce increase the candidate patients who want to treat with IL-6 blockades.

T.14. Secondary Hemophagocytic Syndrome (HLS) or Macrophage Activation Syndrome (MAS) Complicating Presentation of Systemic Lupus in an Elderly Woman

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Secondary hemophagocytic syndrome or macrophage activation syndrome is a well-recognized complication of systemic onset juvenile arthritis (SJIA) and juvenile systemic lupus and has been termed “cytokine storm syndrome”. Less frequently it may be seen in adults. Primary forms (hemophagocytic lymphohistiocytosis) are characterized by familial predominance related to genetic mutations and usually present in childhood. We observed an 72 year old Filipino woman present with fever, waxing waning mental status, pleuropericardial effusions, arthritis, cytopenias, high ferritin (7202 mg/dl (nl< 29)) and rising ANA titer. Work-up demonstrated serial low C3s, trace cryoglobulins, elevated AST/ALT and EEG evidence for encephalopathy. Lumber puncture revealed increase in cells and total protein including IgG. Electrophoresis of the CSF fluid did not demonstrate oligoclonal bands suggesting an impairment of the blood-brain barrier and/or increased IgG synthesis. Treatment with high dose steroids transiently improved her hematologic picture, which then deteriorated (WBC 1,600/ml, hgb

7.6 gm/dl, platelets 38,000/ml). One-day premortem bone marrow evaluation demonstrated a hypercellular marrow with decreased granulopoiesis and increase in histiocytic cells including engulfment of erythrocytes and neutrophils. This patient fulfilled the preliminary diagnostic criteria for MAS complicating SJIA. We believe early institution of definitive therapy was delayed by 3 factors: 1. Cytokine storm syndromes mimic systemic inflammatory response syndromes (i.e. systemic infection), 2. this patient's age and 3. the absence of a prior rheumatic disease diagnosis. The dropping complement levels and trace cryoglobulins suggest immune complex formation may have been etiologic. Definitive diagnosis requires a high index of suspicion and early bone marrow evaluation.

T.15. The Abscence of Estrogen Receptor Alpha Reduces the Activation and Maturation of Plasmacytoid Dendritic Cells in Lupus-Prone Mice

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Systemic Lupus Erythematosus (SLE) is a chronic autoimmune disease that affects women at a 9 to 1 ratio compared to men. To further understand mechanisms underlying the female predominance our laboratory is investigating the role of estrogen receptor alpha (ERα) in SLE disease development. In lupus-prone mice, absence of hormone responsive ERα increased survival and decreased glomerulonephritis despite no effect on autoantibody production or renal immune complex deposition. To explain this protective effect, we hypothesized ERα deficiency impacts innate immune responses and in particular plasmacytoid dendritic cell (pDC) development and function. pDCs are the body's major producer of type I interferons (IFN), a critical cytokine in disease pathogenesis. We found that ERα deficiency reduced the activation state of pDCs *in vivo*. The percentage of spleen pDCs expressing MHC class II was reduced by ERα deficiency. Additionally, pDCs from ERα deficient lupus-prone mice produced less inflammatory cytokines in response to TLR ligands *in vitro*. To explain these findings, we measured the expression of the pDC maturation marker Ly49Q. Ly49Q significantly enhances pDC IFNα production. ERα deficiency reduced both the percentage of pDCs expressing Ly49Q and the median fluorescent intensity of this marker on pDCs *in vivo*. In conclusion, ERα deficiency significantly decreases the activation and function of pDCs in lupus-prone mice. The impact of ERα deficiency on pDC activation and function may be explained by the reduction of Ly49Q expression. These findings may explain the protective role of ERα deficiency in SLE.

T.16. Low Gene Copy Number of Complement C4 is a Risk Factor but High Gene Copy Number of C4 Contributes to Higher Protein Consumption and Possibly More Severe Disease in African American Systemic Lupus Erythematosus

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African American women have higher rates of systemic lupus erythematosus (SLE) and often more severe symptoms, yet the genetic basis and immunologic parameters for Black SLE are understudied. Low and fluctuating levels of blood complement C4 protein levels are well-established phenomenon in human SLE, but the underlying causes have not been clarified. Thus, we investigated gene copy number variations of complement C4A and C4B in African American healthy subjects (n=336; age=37.1±13.4; 70% female) and SLE patients (n=91; age=36.4±13.4; 96% female). All subjects were recruited in central Ohio. Gene copy number (GCN) of C4A and C4B were determined by genomic Southern blots and TaqMan-based realtime qPCR. Plasma complement protein levels were assayed using radial immunodiffusion. C4 genes ranged from 2-6 copies in the study population, but SLE patients displayed higher frequencies in the lower copy number groups (χ^2=6.4, p=0.0113; OR=1.88(1.16-3.04)). There were significant decreases in SLE for C4 protein [mg/dL] (SLE:31.0±15.4, controls:39.3±13.2; $p=4\cdot10^{-7}$). Stratified by C4 GCN groups, we observed more substantial decreases in C4 protein in SLE with higher C4 GCN. Compared with healthy subjects, there was a 34% decrease (SLE:34.4±15.5; controls:52.4±14.9) in C4 protein levels among subjects with five C4 genes, compared to just a 9% decrease among subjects with three C4 genes (SLE:26.5±12.7; controls:29.0±7.9). Our findings support that low C4 GCN is a genetic risk for SLE in African Americans. However, higher C4 GCN leads to higher protein levels and elevated consumption rates, which may contribute to immune-mediated injuries and more severe disease among established SLE patients.

T.17. In Psoriatic Arthritis Synovial Tissue Harbors Expanded T-Cell Clones Which are not Fully Represented in Synovial Fluid or Blood Samples

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Background: T-cells play a key role in psoriatic arthritis (PsA). Nevertheless, the distribution and exact role of these cells remains unclear. Here, we study whether expanded T-cell clones are present in synovial tissue (ST), and whether such clones are also detectable in peripheral blood (PB) and synovial fluid (SF). Methods: ST and SF from inflamed knees was sampled in 2 PsA patients together with paired PB samples. Using next generation sequencing T-cell clones were identified by their unique T-cell receptor β-chain sequence. Clones with a frequency of ≥0.5% were arbitrarily considered to be expanded. Results: ST and SF samples from the same joint shared many of the expanded clones. Ninety percent of the expanded clones in ST were retrieved in SF, 33% of these being expanded in both samples. ST clones accounted for 14% of the SF T-cell repertoire. In PB 83% of the expanded ST clones were retrieved, 17% being expanded in both samples. Analysis of both inflamed knees in patient 1 showed that 83% of the expanded ST clones in the left knee were retrieved in the right knee, 80% of these being expanded in both. Conclusion: In PsA synovial tissue harbors expanded T-cell clones, which are not fully represented in synovial fluid or blood samples. Preliminary analysis indicates that different joints contain identical expanded T cell clones. Our results suggest that in PsA comprehensive analysis of inflammation-related expanded clones best focuses on ST, rather than PB and SF.

T.18. Protoarray Analysis Reveals Novel Autoantigens Targeted by Autoantibodies Associated with DNA-repair Pathway in Systemic Erythematosus Lupus

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Systemic erythematosus lupus (SLE) is an autoimmune disease characterized by presence of autoantibodies (autoAbs) against a broad spectrum of self-antigens. In order to identify novel autoAbs associated with SLE, we utilized a Protoarray bearing 9,500 antigens to screen IgG and IgM autoAbs in sera of SLE patients. 446 IgG and 1218 IgM autoAbs were identified to be significantly elevated in SLE patients compared with healthy controls ($p<0.05$). Protoarray revealed not only the previously described autoAbs such as antibodies against dsDNA, SSA/SSB, Histone and Sm/RNP, but also uncovered autoAbs against a broad range of novel antigens including 151 nuclear-associated antigens and 150 cytoplasma or membrane-associated antigens. The Protoarray results were further validated by ELISA in a larger cohort of SLE patients and controls. Besides, 65 of the IgG autoAb-targeted proteins were also disregulated in SLE on mRNA level by microarray analysis. Pathway analysis recognized significant enrichment of antigens involved in cell proliferation and DNA repair pathways which were targeted by IgG autoAbs including APEX1, AURKA, CSNK1G1, EIF2C1, HMGB1, IFIT5, MAPKAPK3, PADI4, PRKRA, RALGPS1, UBE2S and VRK1. The elevated IgG autoAbs to APEX1 and other proteins in the pathway may reflect the compromised DNA-repair activity during DNA replication in SLE. In conclusion, identification of novel autoAbs by protoarray may shed light on some of the pathogenic pathways leading to disease in SLE.

T.19. Analysis of T cell receptor diversity in peripheral blood of systemic lupus erythematosus subjects by next-generation sequencing

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T cells play an important role in the pathogenesis of systemic lupus erythematosus (SLE). Here, we used next generation sequencing (NGS) to address the hypothesis that T cell receptor (TCR) diversity is restricted in peripheral blood (PB) during increases in SLE disease activity. NGS profiling of complementarity determining region 3 (CDR3) of rearranged TCR beta loci was performed from total RNA extracted from PB of eleven SLE subjects, each with three longitudinal time points (baseline, pre-flare, and flare), and 12 age matched healthy controls (HC). Compared to HC, SLE subjects showed a significant reduction in repertoire diversity in fixed blood volume (p=0.0002) and a more uneven distribution of the repertoire (Gini coefficient, p=0.015). However, the percent of clones that were expanded (clone size >0.1% of total reads) was not different between HC and SLE (p=0.406). In both HC and SLE, the overwhelming majority of these expanded clones showed remarkable stability (over average follow up time in HC, 5.5 months, SLE, 7.2 months). Only two of eleven SLE subjects showed a decreasing trend in overall diversity (using clonality scores), corresponding to increased disease activity. Furthermore, we did not find any overlap of CDR3 amino acids sequences amongst the top 100 clones from any SLE subjects. In conclusion, a significant decrease in TCR repertoire diversity can be noted in PB of SLE compared to HC, however, within a subject, the presence of expanded clones or the dynamics of the clonality score by itself does not always correlate with changes in disease activity.

T.20. Novel Assays Based on Immune Responses to Citrullinated Sm Peptides in the Diagnosis of Systemic Lupus Erythematosus

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Background: Antibodies to Sm proteins are highly specific but insensitive for the diagnosis of systemic lupus erythematosus (SLE), an autoimmune disease that is often difficult to diagnose. Citrullination of autoantigens has been demonstrated to increase the detection of immune responses in several autoimmune conditions but has not yet been investigated in SLE. Methods: Sera from 160 SLE, 164 disease controls and 40 healthy individuals were assayed by ELISA for autoantibodies to a combination of citrullinated and native Sm synthetic peptides. Cytokine production to Sm peptide cocktails was measured in whole blood from 17 patients with SLE, 16 disease and 12 healthy controls. Results: The novel anti-Sm ELISA has a sensitivity of 80.6% and specificity of 92.7% for diagnosis of SLE and is superior to currently used commercial anti-Sm assays which yielded values of 29.4% and 93.1% respectively. The whole blood-Sm peptides stimulation assay using IL-1β as a detection marker shows a sensitivity of 76.5% and specificity of 93.1% for distinguishing patients with SLE from controls. Conclusions: The two assays developed using modified peptides of Sm proteins demonstrate better performance characteristics than other assays currently available for the diagnosis of SLE. Their use concurrently may provide an ideal approach for distinguishing B cell or T cell dominant causation of the disease. The enhanced immunogenicity of citrullinated Sm peptides allows probing of additional repertoires of B and T cell responses to Sm related antigens, and implies that exposure to the postranslationally modified autoantigens may contribute to the pathogenesis of self-reactivity in SLE.

T.21. Severe lupus-like chronic GVH disease develops in STAT deficient mice

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Systemic lupus erythematosus (SLE) is a complex multisystem autoimmune disease driven by genetic and environmental factors. The etiopathogenesis of SLE is still poorly understood. The bm12 to B6 chronic graft-versus-host disease (cGVHD) is a well-studied inducible mouse model of SLE. STAT1 (signal transducers and activators of transcription-1) is dynamically regulated during the course of immune and inflammatory responses. However, little is known about the role of STAT1 in autoimmune diseases. We induced cGVHD in B6.STAT1-KO and B6.WT (H-2^b, Igh^b) mice by intraperitoneally injection of $1´10^8$ splenocytes from bm12 (B6.C-$H2^{bm12}$, Igh^b) mice. Mice were followed for signs of autoimmune manifestation, such as mortality rate, skin rash, and renal involvement. Upon injection, WT mice developed anti-dsDNA autoantibodies starting at week 2 as expected, with decline noted after week 4. In contrast, STAT1-deficient mice exhibited a prolonged and significant increase of anti-dsDNA autoantibody responses compared to WT mice (week 4 to week 8). Severe splenomegaly also developed in cGVHD STAT1-KO mice. Increased autoantibody titers were accompanied by increased proteinuria in mice lacking STAT1 due to immune complex deposition. Three months after injection, 40% STAT1-KO mice died. Further analysis of anti-dsDNA isotypes in cGVH diseased STAT1-KO mice revealed an enhanced IgG1 response but abolished IgG3 response. STAT1 seems to be a suppressor of systemic autoimmunity by altering autoantibody isotype switching. Results from these studies may provide insights into the pathogenesis of STAT1 in SLE.

T.22. Triggering Receptor Expressed on Myeloid cells-1 (TREM-1) in Lupus Nephritis

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Triggering Receptor Expressed by Myeloid Cells -1 (TREM-1) is a potent amplifier of myeloid Toll-like receptor (TLR) and nucleotide-binding oligomerization domain like receptors (NLR) driven innate immune responses. Under conditions of normal homeostasis, TREM-1 is expressed by monocytes and neutrophils in the periphery. Our goal is to determine the expression and function of TREM-1 in lupus nephritis. Previously, we used an anti-glomerular basement membrane antibody-induced nephritis (anti-GBM) model, which shares many features with lupus nephritis to demonstrate that soluble TREM-1 (sTREM-1) levels were elevated in urine of susceptible129/SvJ mice compared to relatively resistant B6 mice. We found that TREM -1 blockade with an inhibitory peptide improved renal inflammation as judged by a number of disease parameters. The anti-GBM studies indicated that elevated TREM-1 expression in 129/SvJ mice had important pathologic consequences. Recently, elevated sTREM-1 levels were detected in serum samples from spontaneous lupus nephritis murine models and in their renal biopsies. Moreover, elevated TREM-1 levels were detected in serum of lupus nephritis patients and in SLE renal biopsies. Preliminary immunohistochemical studies of SLE renal biopsies suggested that TREM-1 was neither expressed on inflammatory infiltrates nor co-localized with profibrotic myofibroblasts-associated α-smooth muscle actin, but was highly upregulated in the kidney in chronic lupus nephritis. These studies indicate that TREM-1 might be expressed by resident cells in the inflamed kidney. Current studies suggest that TREM-1 is a potential therapeutic target for lupus nephritis. These studies could be important in understanding the complex mechanisms driving lupus renal disease.

T.23. Genome-wide Transcriptional Profiling of Isolated Immune Cell Populations from SLE Patients with Different Ancestral Backgrounds

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Background/Purpose: Systemic lupus erythematosus (SLE) is a complex multi-system autoimmune disease of uncertain etiology. Patients from different ancestral backgrounds demonstrate differences in clinical manifestations and autoantibody profiles. In this study we examined genome-wide transcriptional patterns in major immune cell subsets across different ancestral backgrounds.Methods: Peripheral blood was collected from 21 African-American (AA) and 21 European-American (EA) SLE patients, 5 AA and 5 EA controls. $CD4^+$ T-cells, $CD8^+$ T-cells, monocytes and B cells were purified by flow sorting. Each cell subset from each subject was run on an Illumina HumanHT-12 V4 expression BeadChip array (n=208 arrays). Differentially expressed genes (DEGs) were determined by comparing cases and controls of the same ancestral background. Results: The overlap in DEG lists between different cell types from the same ancestral background was very modest (<1%). Typically between 5-10% of DEGs were shared when comparing the same cell type between different ancestral backgrounds. Global IFN-stimulated gene (ISG) expression revealed that AA subjects demonstrated more concordance across all studied cell types. Two subgroups of patients were identified based on the ISG expression profiles. One subgroup showed higher ISGs expression in all cell types, and the other subgroup had higher ISG expression only in T and B lymphocytes but not in monocytes. Conclusion: We find striking differences in gene expression between different immune cell subsets and between ancestral backgrounds in SLE patients. The IFN signature is diverse, with different transcripts represented in different cell populations, and signature-positive cell subsets differed in EA vs. AA patients.

T.24. Circulating HLA-DR^+CD4^+ T Cells Mirror the Functional T Cell Signature of the Inflamed Synovial Microenvironment and Correlate with Resistance to Therapy in Juvenile Idiopathic Arthritis

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Our understanding of the pathogenesis of autoimmune diseases is hampered by the inability to properly identify and investigate pathogenic T cells, which hinders the development of targeted therapeutics. The yet incomplete characterization of inciting antigens frustrates efforts aimed at antigen-based identification of self-reactive T cells. In addition, the sites of autoreactivity are highly enriched in pathogenic T cells, but they are out of reach for most autoimmune diseases. Conversely, blood is easily accessible, but, in spite of massive investigation, results have been disappointing. This is likely due to the strict compartmentalization of tissue-specific immune responses, which are highly diluted in the bloodstream. The goal of our work was to identify the circulating subset of pathogenic T cells in juvenile idiopathic arthritis. We selected a population of circulating HLA-DR$^+$ CD4$^+$ T cells as candidate pathogenic population based on its phenotypical and functional similarity with the CD4$^+$ T cell signature in the synovial microenvironment. Then, we compared their TCR repertoire using next-generation sequencing. Similarly to synovial T cells, blood HLA-DR$^+$ T cells were highly activated, pro-inflammatory antigen-experienced cells, recirculating from the inflamed tissue. More importantly, circulating HLA-DR$^+$ CD4$^+$ T cells were highly enriched in clonotypes found in the synovial microenvironment. Strikingly, HLA-DR$^+$ T cells were significantly expanded in the circulation of therapeutic failures compared to responders. Therefore, HLA-DR$^+$ T cells provide a biological correlate for resistance to therapy and constitute an easily accessible reservoir of pathogenic cells in the bloodstream, which could be exploited for diagnostic and research purposes.

T.25. Systematic comparison of Treg cell therapy protocols for application in autoimmune rheumatological diseases

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Aims: Regulatory T (Treg) cell therapy is a promising approach for severe autoimmunity. Unfortunately, sufficient Treg cell numbers can be obtained only upon *in vitro* culture. Functional stability of human expanded (e)Treg and induced (i)Treg cells has not been thoroughly addressed for all proposed protocols, hindering clinical translation. We undertook a systematic comparison of eTreg and iTreg cells to recommend the most suitable protocol for clinical implementation, and then tested its effectiveness and feasibility with cells from rheumatoid arthritis (RA) patients. Methods: eTreg cells were expanded with rapamycin (rapa), while iTreg cells were induced from naive T cells in the presence of TGF-β with either all-trans retinoic acid (ATRA) or rapa. *FOXP3* expression and demethylation, regulatory molecular signature and suppressive function were evaluated after a first round of differentiation and a secondary restimulation deprived of differentiation factors. Results: Regardless of the protocol, iTreg cells acquired suppressive functions and FOXP3 expression, but lost them upon withdrawal of differentiation factors. In contrast, rapa eTreg cells maintained their regulatory properties and retained *FOXP3* upon restimulation. Demethylation, but not expression, of FOXP3 predicted Treg cell functional stability upon secondary TCR engagement in the absence of stabilizing factors. Importantly, Treg cell expansion with rapa from RA patients produced functionally stable and suppressive Treg cells with yields comparable to healthy donors. Conclusion: Our data indicate *ex vivo* Treg cell expansion with rapa as the protocol of choice for clinical application in rheumatological settings, with assessment of *FOXP3* demethylation as a necessary quality control step.

T.28. Differences in the Auto-Reactive T-Cell Pool when Restricted by the Highly Related Rheumatoid Arthritis Associated HLA-DRB1 Alleles *04:01, *04:04 and *01:01

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The shared epitope (SE) alleles HLA-DRB1*04:01, *01:01 and *04:04 are associated with risk for rheumatoid arthritis (RA) positive for auto-antibodies against e.g. citrullinated α-enolase. Recent studies identified specific citrullinated epitopes presented by *0401 that are associated with disease and provided molecular evidence for how citrullination could break tolerance. So far, the risk conferred by *01:01 and *04:04 is less well characterized. Here, we aimed at the identification and functional characterization of citrullinated α-enolase T-cell epitopes presented by these alleles. Overlapping peptides were screened and several novel α-enolase epitope candidates identified. A large, but not complete, overlap was observed between *0401, *0404 and *0101. Surprisingly, the binding of native and citrullinated epitopes indicated that the bias for citrullinated epitopes of *04:01 appears less pronounced in *0404 or *0101. *In vitro* stimulation of peripheral blood mononuclear cells (PBMCs) demonstrated that a number of the epitope candidates are indeed immunogenic in RA patients

and elicit a pro-inflammatory cytokine response. However, and in agreement with the peptide-binding, a significant preference for citrullinated epitopes was only observed for *04:01. While this study confirms the compelling bias of *04:01 for citrullinated epitopes, it demonstrates its absence in *0404 and *0101. Our data suggests that, despite their name and shared amino acids, significant differences exist in how these HLA-DR alleles confer risk to RA. Altogether this demonstrates how even subtle changes in the MHC peptide-binding groove can have significant immunological impacts and has prompted ongoing structural characterization of the different properties of *04:01 versus *01:01 and *04:04.

T.43. Clinical Relevance of Antibodies against SS-A/Ro specificities in a cohort of Spanish Patients with Connective Tissue Diseases

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Autoantibodies against SS-A/Ro include two specificities (anti-Ro52 and anti-Ro60). Currently the idea that anti-Ro52 and anti-Ro60 should be considered as independent markers in connective tissue diseases (CTD) is gaining ground. Design retrospective (1999-2012). Multicenter study: 21 centers in Spain. Sera from patients with systemic lupus erythematosus (SLE), Sjögren syndrome (pSS), systemic sclerosis (SSc) or poly-dermatomyositis (PM-DM) where tested for SS-A/Ro specificities by ELISA and/or immunoblot. Medical records of positive patients (n=518) were analyzed. 163 presented reactivity only against Ro52 (Ro52+), 66 against Ro60 (Ro60+) and 289 against both specificities (Ro52+Ro60+). The associated diagnoses were: SLE (n=318), SS (n=133), PM-DM (n=29), SSc (n=38). Ro 52+ patients included SLE (45.4%, n=74), SS (23.3%, n=38), SSc (16.0% n=26) and PM-DM (15.3%, n=25). Ro52+Ro60+ included mainly SLE (65.1%, n=188) and pSS (30.8%, n=89) but also SSc (3.8%, n=11) and PM-DM (0.3%, n=1). Finally Ro60+ included only SLE (84.8%, n=56), pSS (9.1%, n=6), SSc (1.5%, n=1) and PM-DM (4.5%, n=3). Ro60+ patients presented more cutaneous involvement (p=0.019) and an increase of Subacute Cutaneous Lupus Erythematosus (p=0.002). Sicca syndrome was observed more commonly in SLE (p=0.018) Ro60+ patients. Furthermore Ro60+ SLE and SSc patients presented more articular involvement (p=0.007, p=0.006). Ro52+ patients presented more Raynaud´s phenomenon (p=0.001) especially in LES subgroup (p=0.003). Interestingly, lung manifestations were increased in Ro52+ patients in all subgroups although no statistical significance was reached. Conclusions: Ro60+ patients presented the classical manifestations associated to SS-A/Ro antibodies, while Ro52 positive patients are a more heterogeneous group with increased Raynaud´s phenomena and lung involvement.

T.103. A Single-Site, Investigator-Initiated, Open-Label Trial of the Adrenocorticotropic Hormone H.P. Acthar® Gel (Repository Corticotropin Injection) for Improving Clinical Disease Activity Among Subjects With Active Systemic Lupus Erythematosus

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Background: Systemic lupus erythematosus (SLE) impacts kidneys, brain, heart, dermatologic manifestations, and blood. A recently completed study by Fiechtner and Montroy demonstrated that treatment with H.P. Acthar® Gel, a long-acting preparation that contains full-length adrenocorticotropic hormone ($ACTH_{1-39}$), resulted in improvement in all 6 outcome measures, and those results are included in a paper currently under review by the journal *Lupus*. This abstract contains additional results that are not presented in that manuscript. Objectives: To assess changes of clinical manifestations among patients with SLE who are treated with $ACTH_{1-39}$.Methods: The British Isles Lupus Assessment Group (BILAG) Index was used to determine changing severity of clinical manifestations of SLE, enabling comparisons between the immediate past 4 weeks and the 4 preceding weeks. Results: After 28 days of treatment with $ACTH_{1-39}$, patients (n=10) with SLE had improvements in all 9 pertinent BILAG Index scores, which included #5 (severe skin eruption), #6 (mild skin eruption), #10 (mild mucosal ulceration), #15 (alopecia-severe), #16 (alopecia-mild), #32 (cerebrovascular disease not due to vasculitis), #37 (lupus headache severe and unremitting), #42 (moderate arthritis), and #43 (mild arthritis). These improvements reveal benefits for skin tissue, cerebrovascular disease, headache, and arthritis. Conclusions: Active lupus disease usually requires treatment with varying doses of prednisone. This trial revealed that, when treated with Acthar® Gel, patients in need of therapeutic alternatives can have improvements in the BILAG Index scores that indicate improvements in quality of life.

F.18. TNF induces the maintenance of the TH17 profile through TNFR1 and TNFR2 receptors

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Introduction: Rheumatoid arthritis (RA) has been described as an autoimmune disease associated with a pathogenic Th17 cells response. Although RA patients have been shown to have increased levels of circulating TNF, there is little information about the direct effect of this cytokine on Th1 and Th17 populations. Aim: This study evaluated the expression of TNF receptors (TNFR1/TNFR2) on Th1 and Th17 cells, and also determined the effect of TNF on Th1 and Th17 populations, in order to contribute in clarifying the role of this cytokine in the pathophysiology of RA. Methodology: Th1 and Th17 cells, purified by cell sorting, were evaluated in their TNFR1 and TNFR2 expression by flow cytometry, while the effect of TNF on these lineages was analyzed in terms of their IL-17 and IFN-gamma-production capabilities after TNF stimulation and TNFRs blocking. Results: Our results showed that TNF promotes the maintenance of the Th17 profile, inducing IL-17 production at the expense of IFN-gamma production in Th1 and Th17 cells. Moreover, the TNF –induced increase of IL-17 production by Th17 cells is mediated by both, TNFR1 and TNFR2, while the decrease of IFN-gamma production by this subset is dependent on TNFR2. Finally, we could not restore the IFN-gamma production, by blocking TNFRs in Th1 cells, suggesting that TNF effect on Th1 population is rather indirect. Conclusion: These results suggest a direct effect of TNF in the maintenance of the Th17 profile, mediated through TNFR1 and TNFR2 in the Th17 population. Support by Millennium Institute on Immunology and Immunotherapy P09/016-F.

Bone Marrow or Stem Cell Transplantation

1102B. FAVORABLE IMPACT OF NATURAL KILLER CELL RECONSTITUTION ON CHRONIC GRAFT-VERSUS-HOST DISEASE AND CMV REACTIVATION AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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NK cells are the first lymphocytes to recover after allo-HSCT. This recovery follows the NK differentiation pattern with an early expansion of the NK $CD56^{bright}$ subset followed by NK $CD56^{dim}CD16^{+}$. In addition, NK-cells that express the activating receptor NKG2C seem crucial in the resolution of CMV episodes. We investigated prospectively NK cell reconstitution in a large cohort of allo-HSCT adult patients (n=439) treated (2005-2011) with non T-cell depleted stem cells from bone marrow (61%) or peripheral blood (24%) and reduced intensity (61%) or myeloablative conditioning (39%). NK-cell subsets were analyzed at months M3, M6, M12 and M24 post-transplantation on freshly collected blood samples. Data were analyzed with respect to conditioning regimen, source of stem cells, underlying disease, occurrence of Graft- versus-Host Disease (GvHD), and profiles of CMV reactivation. In multivariate analysis, acute GvHD impaired reconstitution of total and CD56dim NK cells at M3 (p=0,006 and 0,002). An efficient NK cell reconstitution at M3 was associated with a lower incidence of chronic GvHD, independently of a previous episode of acute GvHD and stem cell source. With regard to CMV, CD56dim and total NK counts were lower at M3 in the group of patients reactivating CMV between M0 and M3 (p=0,03 and 0,01). NKG2C expressing total NK cells and the CD56dim NK subset were significantly increased at M3 in patients who did not experience further reactivation (p=0,011 and 0,007). These data favor a direct role of NKG2C expressing NK cells in the early control of CMV reactivation during allo-HSCT.

OR.38. Potency and stability of LAP+FOXP3+ Tregs in control GVHD development

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Intro: We have developed previously a method to isolated bona fide Tregs from expansion cultures based on their selective surface expression of latency-associated peptide (LAP). In this preclinical study, we investigated their in vivo potency and stability using a humanized GVHD murine model. Methods: CD25+ cells isolated from healthy donor PBMC via AutoMACS were stimulated with OKT3 loaded K562 artificial APC expressing CD64 and CD86 in media +IL2 and rapamycin. 36h post 2nd restimulation at wk2, the Tregs were re-isolated with anti-LAP via AutoMACS. The LAP+ Tregs and LAP- nonTregs were expanded for 2 more wks with 3rd restimulation at wk3. In vitro suppression, phenotype and TSDR demethylation were performed after 4wk expansion. CD25-depleted human PBMC were injected into NSG mice to induce xGVHD and compared with coinjection of LAP+ Tregs or LAP- nonTregs. Results: LAP+ repurification results in >85% LAP+FOXP3+ Tregs, leaving behind FOXP3- and FOXP3+ nonTregs within the LAP- population. After a total of 4wk expansion, LAP+ Tregs were >1 billion cells, >85% FOXP3 purity, diverse in TCR repertoire, highly suppressive and anergic in vitro, and >95% demethylated TSDR ($p<0.05$). In GVHD model, PBMC alone resulted in a median survival of 29 days. The LAP+ Tregs increased median survival to 43 days ($p<0.01$), while the LAP- non-Tregs had a median survival of 28.5 days. Conclusions: LAP+ Tregs are highly purified, stable, and potent based on these preclinical studies. LAP+ Tregs are an ideal immunotherapeutic population for clinical trials to prevent or treat GVHD and other autoimmune diseases

W.44. Adoptive T-cell therapy to Prevent and Treat Human Metapneumovirus (hMPV) Infections Post Hematopoietic Stem Cell Transplant (HSCT)

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Viral infections are a significant cause of morbidity and mortality in allogeneic HSCT recipients. Previous studies by our group and others have demonstrated that CMV, EBV and Adv infections can be treated by adoptively transferring in vitro expanded virus-specific T-cells (VSTs). However, the list of pathogens linked with high morbidity/mortality continues to grow, while VSTs therapy has been limited by the paucity of information regarding immunogenic/protective T-cell target antigens within many viruses. Thus the goal of this study was to characterize the cellular immune response to human metapneumovirus (hMPV), a paramyxoviridae detected in up to 10% of HSCT recipients. To determine which of the 9 expressed antigens (N, M, F, SH, G, M2-1, M2-2, P, L) are most frequently recognized, we generated overlapping peptide libraries (15mers overlapping by 11aa) spanning each of the antigens to activate and expand antigen-specific T-cells. To assess the specificity and define a hierarchy of immunodominance we used an IFNg ELIspot assay. F was recognized most frequently (n=14) and induced the highest frequency of specific cells (mean 504±105 SFC/$2x10^5$), followed, in descending order, by M (n=13, mean 197±40), N (n=13, mean 293±67), M2-1 (n=12, mean 188±42), G (n=10, mean 94±22), P (n=9, mean 196±58) and L (n=6, mean 152±83). Reactivity against SH and M2-2 was detected in 3 and 1 donors, respectively. We next plan to assess the cytolytic capacity of our hMPV-VSTs and identify immunodominant epitopes. Ultimately, we would like to incorporate hMPV-directed T-cells into our ongoing clinical trials.

W.77. A Graft-vs-Host-Disease Impairs a CXCR4-Related Increase of Circulating Human Lymphoid Progenitors after Allogeneic Hematopoietic Stem Cell Transplantation

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Immune recovery after profound lymphopenia is a major challenge in many clinical situations, such as allogeneic hematopoietic stem cell transplantation (allo-HSCT). Recovery depends, in a first step, on hematopoietic lymphoid progenitors production in the bone marrow (BM). In this study, we characterized $CD34^+Lin^-CD10^+$ lymphoid progenitors in the peripheral

blood of allo-HSCT patients. Our data demonstrate a strong recovery of this population 3 months after transplantation. This rebound was abolished in patients who developed acute graft-versus-host disease (aGVHD). A similar recovery profile was found for both $CD24^+$ and $CD24^-$ progenitor subpopulations. $CD34^+lin^-CD10^+CD24^-$ lymphoid progenitors sorted from allo-HSCT patients preserved their T cell potential according to *in vitro* T-cell differentiation assay (OP9-DL1) and the expression profile of 22 genes involved in T-cell differentiation and homing. $CD34^+lin^-CD10^+CD24^-$ cells from patients without aGVHD had reduced CXCR4 gene expression, consistent with an enhanced egress from the BM. CCR7 gene expression was reduced in patients after allo-HSCT, as were its ligands CCL21 and CCL19. This reduction was particularly marked in patients with aGVHD, suggesting a possible impact on thymic homing. Thus, the data presented here identify $CD34^+lin^-CD10^+CD24^-$ lymphoid progenitors bone marrow production and exit as an important early step in T-cell reconstitution after allo-HSCT in humans.

W.79. High Expressing IL-1Receptor Genotype is associated with Robust anti-Cytomegalovirus (CMV) Immune Response and Confers Strong Protection against CMV Complications after Allogeneic Hematopoietic Cell Transplantation

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Background: Reactivation of Cytomegalovirus (CMV) is one of the most frequent viral complications that affect hematopoietic cell transplant (HCT) transplant recipients, primarily due to the deficient anti-CMV immune response during the post HCT immunocompromised state. Cytokines and their receptors act as chief mediators of anti-viral immune response. Genetic control of cytokine production is evidenced by polymorphisms in cytokine gene regulatory regions resulting in low, moderate, or high cytokine production profiles. In the present investigation, we assessed whether gene variants of cytokine or their receptors influences post HCT CMV complications and anti-CMV immune response. Methods: A total of 240 allogeneic HCT donors and 50 healthy individuals were genotyped for 22 single nucleotide variants located in the regulatory and/or exonic regions of 13 cytokine or cytokine receptor genes. PBMNCs from healthy individuals were stimulated with CMV lysate to enumerate CMV specific immune response by measuring IFN-g and TNF-α production using multicolor flow cytometry. Results: Allogeneic HCT recipients receiving grafts form donors carrying low producing genotype of *IL-1R* (-1970 CC genotype) had high incidence of CMV reactivation (p=0.03; HR=2.1) and recurrent CMV infection (p=0.01; HR=2.2). Healthy individuals carrying *IL-1R* 'TT' genotype showed significantly stronger anti-CMV immune response (high production of CMV induced cytokine production) in comparison to individuals carrying IL-1R 'CC' genotype. Conclusions: Our results strongly support the role of high *IL-1R* producing gene variants in mediating anti-CMV immune response and in conferring protection against CMV complications after allogeneic HCT.

W.80. High Incidence of Graft Versus Host Disease after Allogeneic Hematopoietic Cell Transplantation in Recipients receiving Grafts from Donors Carrying Interleukin-10 Low Producing Genotypes

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B: Graft-versus-host disease (GVHD) remains a major complication of allogeneic hematopoietic cell transplantation (HCT) that greatly influences morbidity and mortality associated with HCT. Cytokines act as chief mediators/regulators of allo-immune responses and their expression levels are influenced by gene variants located in their gene regulatory regions. Here, we evaluated the prognostic significance of cytokine gene variants known to impact cytokine production on the occurrence of significant GVHD (defined as grade 2-4 acute GVHD and/or chronic GVHD requiring systemic therapy). Methods: A total of 240 allogeneic HCT donors (discovery, n=146 and validation, n=94 cohort) were genotyped for 22 single nucleotide variants located in the regulatory regions of 13 cytokine/cytokine receptor genes. The panel of genes includes *TNF-a*, *IFN-g*, *IL-1* gene cluster (*IL-1a*, *IL-1b*, *IL-1R*, *IL-1Ra*), *IL-2*, *IL-4*, *IL-4Ra*, *IL-6*, *IL-12*, *IL-10*, and *TGF-b*. Results: HCT recipients receiving grafts from donors carrying low producing genotype of *IL-10* (-1082G/A) were strongly associated with significant GVHD (p=0.01, HR=2.1), as well as with grades II to IV acute GVHD (p=0.001, HR=2.3), and chronic GVHD (p=0.01, HR=1.75). Conclusions: Genetic predisposition to low IL-10 production is a strong predictor of GVHD. Low IL-10 production may contribute to a

deficient regulatory milieu culminating into stronger allo-immune responses involved in the pathogenesis of GVHD. These results implicates the importance of assessment of *IL-10* gene variants for improved allogeneic HCT donor selection - where selecting HLA matched donors that carry high *IL-10* producing genotypes will result in a low likelihood of GVHD.

W.81. 2-Methoxyestradiol (2ME2, Panzem) treatment can modulate Th1/17 polarization and reduce GVHD in mouse BMT model

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Allogeneic stem cell transplantation is an effective therapy for hematological malignancies. But the limiting factor is graft-versus-host-disease (GVHD), a result of alloimmune responses elicited by donor T lymphocytes to major and minor antigens. The molecular and cellular basis of GVHD are not well understood. Our goal is to elucidate the regulatory mechanisms of alloimmune responses and develop novel therapies for tolerance induction and GVHD prevention. 2-Methoxyestradiol (2ME2, Panzem) is an endogenous metabolite that is well-tolerated in phase I/II clinical trials for variety of tumors. The increase of 2ME2 level has also been shown to modify disease activity in autoimmune mouse models, and correlate with the remission of MS and RA symptoms in pregnant women. The anti-inflammatory properties of 2ME2 was established in the mouse model of MS and the mechanism of action is the ability of 2ME2 to inhibit IL-17 production in Th17-polarized cells. Our studies demonstrate that 2ME2 treatment can reduce the mortality and morbidity associated with mouse GVHD. There is a significantly lower number of donor-derived $CD4^+$ and $CD8^+$ T cells in the peripheral lymph node and Peyer's patches of 2ME2-treated mice compared to control recipients. Moreover, 2ME2 exposure can reduce the production of IFN-γ (Th1), IL-17 (Th17) and IL-2 in donor-derived $CD4^+$ T cells in the spleen. However, 2ME2 treatment has no effect on the differentiation of $CD8^+$ effector T cells in vivo and their cytolytic activity remains intact. Therefore, we conclude that 2ME2 treatment can modulates donor T cell activation and Th1/17 polarization in GVHD.

W.82. Early Post-Transplant Lymphoid Expansion after Cy/Flu Minimal Intensity Allotransplant in Humans: Homeostatic Proliferation versus Alloreactivity

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Cytokines/Chemokines

W.3. The functional role of chemokine secretion by T regulatory cells

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Type 1 diabetes (T1D) is an autoimmune disease caused by a breakdown in immune tolerance leading to immune cell-mediated destruction of the insulin-producing beta cells in the pancreas. This can occur by many different mechanisms, but substantial data support a major role for insufficient control of autoimmunity by regulatory T cells (Tregs). However, the mechanistic basis for why Tregs fail to control diabetogenic T cells remains unclear. We recently found that activated Tregs secrete significant amounts of chemokines including CCL3 and CCL4, which are ligands for CCR5. To investigate the function of chemokine production by Tregs, we used transwell migration assays and found that supernatants from Tregs stimulated the migration of wild type, but not CCR5-deficient, CD4+ and CD8+ T cells. We also identified putative FOXP3-binding sites in the promoters for CCL3 and CCL4 and used reporter construct assays to demonstrate that FOXP3 transactivates the promoters for both of these genes. Since expression of FOXP3 is thought to be unstable in T1D we hypothesized that chemokine production from Tregs in T1D patients may be reduced. Indeed, Tregs from children with established T1D produced significantly lower amount of chemokine compared to age-matched controls. These results reveal an unexpected role for chemokine production in the function of Tregs and suggest that reduced production of these proteins may be associated with autoimmunity.

W.21. *P. gingivalis* promotes Th17/IL17 responses in chronic periodontitis

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Chronic Periodontitis and rheumatoid arthritis may have similar pathogenic mechanisms. The Th17/IL-17 pathway has been implicated in the pathogenesis of rheumatoid arthritis, but its role in periodontitis is not fully understood. The aims of our study were to: (1) determine the presence of IL-17+ CD4+ T-cells in inflamed gingival tissue; (2) investigate if and how the periodontal pathogen *Porphyromonas gingivalis* (Pg) promotes a Th17/IL-17 response *in vitro*; and (3) compare the Th17/IL-17 response between healthy controls and patients with periodontitis. We detected IL-17+ CD4+ T-cells in gingival tissue cells from periodontitis lesions (10±2.5 % of CD3+CD4+ cells). In co-cultures of monocytes and CD4+ T-cells from healthy donors, addition of heat-killed Pg (hk-Pg) significantly induced IL-17 production in a dose- and time-dependent manner. Hk-Pg-stimulation of monocytes resulted in a significant increase in the expression of CD40, CD54 and HLA–DR, and enhanced production of TNF-α, IL-1β, IL-6 and IL-23 when compared to controls. Hk-Pg-activated monocytes drove Th17 responses in an IL-1β and IL-23, but not IL-6/TNF-α-dependent manner. Moreover, blockade of TLR-2 and TLR-4 or the CD80/86 pathway decreased IL-17 production in hk-Pg-stimulated co-cultures. Preliminary data further suggest that hk-Pg induced higher IL-17 production in anti-CD3 mAb-stimulated co-cultures from patients with periodontitis (1787±853 pg/ml, n=5) compared to healthy controls (739±384 pg/ml, n=7)**.** Our data show that IL-17-producing CD4+ T-cells are present in gingival tissue from periodontitis lesions, and suggest that *P. gingivalis* can activate monocytes resulting in subsequent induction of IL-17 responses in human CD4+ T-cells, which may be enhanced in patients with periodontitis.

W.26. Interleukin-1 is required for cancer immunosurveillance mediated by tumor-specific Th1 cells

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Pro-inflammatory cytokines like interleukin-1 (IL-1) and tumor necrosis factor a (TNFa) may either promote or suppress cancer. The cellular and molecular basis underlying these opposing outcomes remains unclear. Using the MOPC315 mouse model for myeloma, we have recently reported that inflammation driven by tumor-specific Th1 cells conferred protection against B-cell cancer. Here, we have investigated the contribution of several inflammatory mediators. Myeloma eradication by Th1 cells was not affected by inhibition of TNFa, inducible nitric oxide synthase (iNOS), TNF-related weak inducer of apoptosis (TWEAK), or TNF-related apoptosis-inducing ligand (TRAIL). In contrast, cancer elimination by Th1 cells was severely reduced by *in vivo* blockade of both IL-1a and IL-1b with IL-1 receptor antagonist. Inhibition of IL-1 did not affect the activation of naïve tumor-specific $CD4^+$ T cells or Th1 polarization. In contrast, IL-1 had a central role in promoting a cancer-suppressive pro-inflammatory cytokine milieu at the incipient tumor site. Moreover, IL-1 acted in synergy with interferon-g (IFN-g) for induction of tumoricidal activity in tumor-infiltrating M1 macrophages. Thus, in the presence of Th1-derived IFN-g, IL-1 enhances the adaptive immunity against cancer. This synergy between IL-1 and IFN-g may explain how inflammation, when driven by tumor-specific Th1 cells, represses rather than promotes cancer.

F.1. The Disintegrin and Metalloproteinase ADAM10 Triggers Inflammation and Endothelial Dysfunction in Donor-Specific Antibody-Mediated Rejection

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A Disintegrin and Metalloproteinases (ADAMs) regulate key proteolytic events involved in inflammatory cytokine and chemokine release. ADAM10 is constitutively and preferentially expressed in endothelial cells (EC) whereas ADAM17 is usually induced by inflammation. This study investigated the contribution of ADAMs and Notch signaling to vascular injury and inflammation associated with anti-HLA donor-specific antibody (DSA)-mediated rejection (AMR) in cardiac allografts. Regulation of ADAM10, -15, -17, CD31 and VCAM-1 was analyzed by quantitative PCR and immunohistochemistry in cardiac biopsies from patients with stable graft or non failing heart (n=13) or with AMR (n=8). Crosstalk between inflammation and

ADAM10 proteolytic activity was further investigated in cultured EC from donor transplants using gain and loss of function models. Here, we found that AMR induced by donor-specific anti-HLA is characterized by a significant upregulation of both ADAM10 and ADAM17 mRNAs (respectively 4.3- and 3.4-fold increase versus controls, $p<0.01$) without change in ADAM15 mRNA. ADAM10 is located in graft EC and in infiltrating CD68+ macrophages and some CD3+ T cells. AMR is also associated with an increase in Notch activity reflected by an enhanced Hey1 expression. In cultured EC, the blockade of ADAM10 dysregulates Notch signaling pathway and also efficiently decreases the production of the pro-inflammatory cytokines and chemokines IL-6, IL-8 and MCP-1. We finally show that ADAM10 mediates a Notch-dependant regulation of IL-6 release in human EC. Overall, our findings suggest that ADAM10 is a major metalloproteinase driving proteolytic events involved in inflammatory responses and immune cell recruitment during AMR through the Notch signaling pathway.

F.2. Atomic basis of the decoy trap: how DcR3 neutralizes its ligands and hints for the immunotherapy

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Decoy receptor 3 (DcR3) is a secreted TNF receptor discovered in human beings but not in mice. DcR3 is able to neutralize three TNF ligands, LIGHT, FasL and TL1A and further modulate the immune response. For example, neutralization of LIGHT by DcR3 can manipulate the co-stimulatory signal triggered by LIGHT:HVEM interaction or the cell death signal resulted from LIGHT:LTbR interaction. Upon ligation with FasL, DcR3 can block the cell apoptosis pathway commenced by FasL:Fas interaction. Besides, DcR3 binds to TL1A, which can interrupt its interaction with DR3 to boost T cell immunity or induce cell apoptosis in different cell types. DcR3 is thought to play an important role in regulating the immune response, especially in cancer immunology as suggested by elevated expression levels and the associated poor prognosis in cancer patients. Although the remarkable ability of DcR3 to neutralize three different ligands has already been realized, the mechanisms which support the broad specificity of DcR3 remain to be fully defined. Here I present the novel crystal structures of the FasL:DcR3 and LIGHT:DcR3 complexes. Along with the TL1A:DcR3 structure solved in our lab previously, the whole set of the structures of TNF:TNFR complexes engaged by DcR3 was solved. Our results show that the loops of the ligands are the major determinants for the recognition of DcR3. Based on these structures, we designed a novel LIGHT mutant that specifically recognizes DcR3 and exhibits no interaction with HVEM; this reagent dissects the multiple biochemical functions of LIGHT and offers unique clinical opportunities.

F.3. CXCL17 is a Novel and Important Chemotactic Factor for Monocytes and Macrophages

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Chemokines are a superfamily of chemotactic cytokines that direct the movement of cells throughout the body under homeostatic and inflammatory conditions. CXCL17, a chemokine that exhibits highly specific and restricted expression in mucosal tissues, was the last ligand to be characterized, and therefore its biology has not been well studied. We sought to better characterize CXCL17's functions. To this end, we analyzed its chemoattractive properties *in vivo* and also obtained a Cxcl17 $^{-/-}$ mouse. Injection of Cxcl17 into the peritoneal cavity results in strong recruitment of macrophages into the cavity. Furthermore, Cxcl17 $^{-/-}$ mice exhibit macrophage abnormalities (compared to control mice), including significant defects in monocyte recruitment. We conclude that CXCL17 is an important chemotactic factor for both monocytes and macrophages in mucosal tissues. Taken together, these observations strongly suggest that CXCL17 is a major regulator of macrophage recruitment to mucosal sites. Given the importance of macrophages in inflammatory responses, these observations strongly suggest that CXCL17 is a critical regulatory factor of mucosal inflammatory responses.

F.4. 5-methyl-1-phenyl-2-(1H)-pyridone down regulates inflammatory mediators and modulates endocannabinoids receptors in non-alcoholic steatohepatitis induced by high fat/carbohydrate diet

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Nonalcoholic fatty liver disease begins with liver accumulation of triglycerides, which produce inflammation that eventually lead to unbalance between anti-inflammatory and pro-inflammatory cytokines eading to non-alcoholic steatohepatitis (NASH) and finally liver fibrosis. 5-methyl-1-phenyl-2-(1H)-pyridone (PFD) is indicated for chronic inflammation and fibrogenesis. Furthermore pharmacological modulation of cannabinoid type 1 receptor (Cnr1) and TGFb1 is associated with inflammatory liver damage reduction. By contrast cannabinoid type 2 receptor (Cnr2) activation in chronic inflammatory diseases diminish inflammation by reducing IL17 synthesis. We aimed to determine modulatory effect of PFD in inflammatory liver damage induced by experimental steatosis. Male C57/BL6 mice were fed with isocaloric normal diet (ND) or high-fat/carbohidrate diet (HFHC) (60% fat, 18% protein, 22% carbohydrate, and 55% fructose/45% sucrose in drink water) for 8 weeks. For both diets, PFD 100 mg/kg/d or vehicle was administered intragastrically.

Compared to HFHC mice, HFHC+PFD mice had significantly less weight gain, lower blood glucose, triglycerides and hepatic steatosis. ALT and AST level caused by HFHC diet was significantly reduced by PFD regimen. Also PFD down-regulates IL17 and mRNA expression of Cnr1 and TGFb1 in HFHC mice, which is associated to lower liver inflammation. Also PFD displayed a slight Cnr2 increase, which may explain IL17 reduction and improvement of liver markers. These results suggest that protection from HFHC diet-induced steatohepatitis by PFD is likely associated with the capacity of reduce inflammatory mediators, where IL-17 expression correlates with NASH-related liver diseases and fibrosis.

F.5. N-acetylcysteine Attenuates Tumor Necrosis Factor-α Levels in Autoimmune Inner Ear Disease Patients

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AIED (Autoimmune Inner Ear Disease) is a poorly understood disease marked by bilateral, rapidly progressive hearing loss triggered by unknown stimuli. The mechanism of the disease is not precisely understood, however our previous studies have demonstrated the role of TNF-α in steroid-sensitive immune mediated hearing loss. N-acetylcysteine (NAC), a broad spectrum antioxidant, has been effective in certain autoimmune disorders. NAC has been identified by others as an adjunct treatment for hearing restoration in idiopathic sudden hearing loss, where NAC augmented corticosteroid efficacy. We previously observed that PBMCs from AIED patients exhibited greater TNF-α secretion compared to normal healthy controls. PBMC treatment with NAC effectively abrogates LPS-mediated TNF-α release from PBMC of both AIED patients and controls. Preliminary results by western blot showed that inducible nitric oxide synthase (iNOS) is constitutively activated in PBMCs of AIED patients when compared to controls, where LPS was necessary to induce iNOS expression. These results suggest that in AIED patients, the TNF-α down-stream signaling pathway appears to be constitutively activated. Therefore, given that NAC effectively abrogated LPS-mediated TNF release, NAC has the potential to regulate the down-stream targets of TNF. In support of this hypothesis, AIED patients have almost 3 fold greater myeloperoxidase (MPO) release compared to normal healthy controls and this release is inhibited by NAC. We emphasize that use of antioxidants may be an important adjunct therapy for the treatment of this poorly understood disease, and further understanding of the molecular mechanism of action may elucidate further biologic targets for intervention.

F.6. Flow Cytometric Determination of Vitamin D Receptor Expression in Human Immune Cells

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Vitamin D (VitD) plays roles not only in skeletal homeostasis, but also in immunomodulation, through binding to vitamin D receptor (VDR), a member of the nuclear receptor superfamily, in cells of the immune system. Currently, VDR expression in these cells can only be shown semi quantitatively by PCR analysis and western blotting. In this study, we describe a new multiparametric flow cytometric method for direct quantitation of VDR in monocytes and lymphocytes. A rat monoclonal antibody recognizing both monomeric and dimeric forms of the receptor was optimized using human peripheral blood mononuclear cells. Percentages of VDR in peripheral monocytes, T and B cells in healthy humans were shown as 42.3 ± 12.5%, 25.4 ± 12.4% and 2.5 ± 2.0%, respectively (n = 10). In addition, VDR expression of T cell subgroups and NK cells were also determined. In conclusion, herein we describe the first multi parametric flow cytometric protocol in literature to

quantitatively determine VDR expression in immune cells of humans, which should facilitate future studies on VitD and its receptor in immune system.

F.7. Suppressing Excessive Peripheral Cytokine Responses by Electrical Stimulation of a Brain Cholinergic Region

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Neural signals from the brain to peripheral organs regulate immune responses and cytokine release (Nat Rev Endocrinol, 2012, 8:743). We have indicated a role for brain cholinergic signaling, associated with a vagus nerve-mediated anti-inflammatory circuit in the regulation of peripheral inflammation (PNAS, 2006, 103:5219; Brain Behav Immun, 2009, 23:41). Here we studied whether electrical stimulation of the medial septum, an important constituent of the basal forebrain cholinergic system, alters the peripheral inflammatory response during endotoxemia. The medial septum was stimulated in isoflurane anesthetized C57Bl/6 mice using a stereotaxically placed coaxial electrode 1h prior to endotoxin (8mg/kg, i.p.) administration. Serum cytokine levels were determined 1.5h following LPS administration. Three consecutive trains (30sec each) of stimulation (bipolar rectangular pulses, 250ms, 150mA, 30Hz, 75Hz, 120Hz, respectively) followed by 10min of continuous stimulation (250ms, 150mA, 75Hz) significantly reduced serum TNF, IL-6, CXCL1, IFN-g, and IL-12p70 levels as compared to sham-stimulated controls. Medial septum stimulation was also associated with increased regional cerebral blood flow (rCBF, parietal cortex, laser Doppler flowmetry), but no alterations in heart rate or respiratory rate were observed. Decreasing stimulation intensity to 75mA and duration to 3min resulted in lower magnitude of serum cytokine suppression and rCBF increase. These results, demonstrating a previously unrecognized cytokine modulating effect of medial septum stimulation triggered an ongoing study of brain anti-inflammatory circuitry. Our findings are of interest for further unveiling central mechanisms of regulation of inflammation and development of brain stimulation protocols as novel anti-inflammatory approaches. This study was funded in part by NIH/NIGMS.

F.8. Baseline levels of Soluble Mediators and Connective Tissue Disease Screening Questionnaire Scores Associate with Subsequent Transition to SLE in Previously Unaffected Family Members

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Healthy blood relatives (BRs) of lupus patients have an increased risk of transitioning to classified SLE. Using a unique resource of family members, this study seeks to identify and understand roles of select inflammatory mediators as early biomarkers in at-risk populations. We re-enrolled 409 unaffected BRs of SLE patients with samples available from a previous genetic study; 45 transitioned to classified SLE (≥ 4 ACR criteria). Participants provided detailed clinical and demographic information, and completed the SLE portion of the Connective Tissue Disease Screening Questionnaire (CSQ). Medical records were reviewed for ACR classification criteria. Baseline (BL) and Follow-up (FU) plasma samples were tested for autoantibody production and 52 soluble inflammatory mediators. BRs who transitioned to SLE had significantly higher BL CSQ scores than age/race/gender matched unaffected BRs ($p<0.0001$). Compared to matched unrelated, unaffected individuals, BRs who transitioned to SLE had significantly altered levels of 39/52 soluble mediators. A number of BL mediators, including SCF, MCP-1, MCP-3, and BLyS, positively correlated with cumulative ACR score at FU ($p\leq0.006$) and were significantly higher in BRs who transitioned to SLE ($p\leq0.05$). BL levels of the regulatory mediators IL-10 and TGF-β (negatively correlated with cumulative FU ACR score in BRs, $p=0.012$) were significantly lower in BRs who transitioned to SLE vs. BRs who remained unaffected ($p\leq0.05$). Significantly altered levels of soluble inflammatory mediators are present prior to the transition to active SLE suggesting that perturbations in immune-mediated processes occur before clinical classification and that high-risk, pre-clinical individuals can be identified.

F.9. Overexpression of IL-17 and TGFb-2 in the liver of rats with experimental fibrosis by cholestasis

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Background IL-17R knockout mice have showed an important reduction of liver and lung fibrosis. However, IL-17, RORg and NK cells have been little explored in experimental cholestasis. Likewise, TGFb-2, an important fibrogenic mediator, remains still poorly understood in this model. Aim. To analyze the expression of IL-17, RORg, NKp46 and TGFb-2 in the liver of rats with experimental fibrosis by bile duct ligation (BDL). Methods Liver fibrosis was induced by BDL. Fibrotic animals were sacrificed at 8 and 30 days. qRT-PCR for IL-17A, TGFb-1 and TGFb-2 was achieved with total RNA. Liver homogenates were used to detect IL-17, TGFb-2, RORg and NKp46 by Western-blotting. Liver sections were analyzed to confirm chronic injury and collagen deposition. Results The gene expression of IL-17A dramatically increased 350 and 10 times more than the control group at 8 and 30 days after BDL. TGFb-1 and 2 increased significantly during all the process of liver damage. IL-17 protein, remained significantly elvated at 8 and 30 days after BDL, paralellel TGFb-2 showed a tendency to increase at 8 days, and was significant after 30 days of BDL. Interestingly, RORg increased three times more throughout the whole fibrotic process ($p<0.01$); in contrast, NKp46 was a reduction at 30 days after BDL. Histological analyses showed characteristic alterations of the cholestatic process. Conclusions. Our results suggest that profibrogenic cytokine IL-17, TGFb-1 and TGFb-2 are criticals during early fibrosis and established cirrhosis in the experimental cholestasis. Additionally, Th17 cells might represent an important source of IL-17.

F.10. Differential Expression of CXCR4 and CXCR7 in Breast Cancer Cell Lines Drives Migration in a CXCL12 Dependent Manner

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The CXCL12/CXCR4 axis was proposed to regulate the trafficking of breast cancer cells to sites of metastasis. CXCR4 plays a central role in breast cancer progression by enhancing tumor growth, angiogenesis, and triggering cancer cell invasion in vitro. However, CXCR7 also binds to CXCL12 and it has been recently found to enhance breast primary tumor growth, as well as metastasis. In order to address this issue, we analyzed the CXCR4 and CXCR7 differential co-expression by flow cytometry in three breast cancer cell lines with different molecular classification, MDA-MB-231, MCF-7 and T-47D. Also, we performed a chemotaxis assay with CXCL12 to measure their chemotaxis indexes by the Boyden chamber assay. Our results showed that MDA-MB-231 cell line expressed the highest level of CXCR4 compared to other studied cell lines ($p<0.001$) and it´s expression was correlated with a strong chemotactic ability to migrate in a CXCL12 dependent manner ($p<0.001$). We found that T47D cells expressed higher levels of CXCR7 than others cell lines ($p<0.0001$) and this expression was correlated with a less chemotactic index ($p<0.05$). In conclusion, we found that the up-regulated expression of CXCR4 enhances chemotaxis correlating with the most aggressive phenotype (MDA-MB-231). On the other hand, the up-regulated expression of CXCR7 correlated with a less invasive phenotype (T47D). Taken together, these data suggest that both chemokine receptors can drive cell migration and possibly tumor metastasis in a differential manner; also these findings demonstrate the therapeutic potential for targeting this pathway in cancer management.

F.11. Helper T cell polarization promotes temporal inflammatory lymphangiogenesis

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Lymph node lymphangiogenesis (LNL) is a critical regulator of inflammation, and tumor metastasis. Although it is clear that lymphangiogenic factors VEGF-A and C play a central role in the regulation of this process, recent studies showed that these

effects are balanced with anti-lymphangiogenic forces like IFNg. However, although it is clear that T cells negatively regulate LNL, the effect of their differentiation remains unknown. We analyzed the effects of T cell differentiation on LNL in a mouse model of inflammation and analyzed draining lymph nodes at various time points by flow cytometry, histology, and RT-PCR. We found that CFA/OVA induced inflammation, resulted in a robust lymphangiogenic response in the draining lymph nodes. This process was associated with generation of a mixed Th1,Th2 response, decreased expression of IFNg and IL-17A, and increased expression of VEGF-A/C. Treatment of mice with IFNg, IL13, or IL17A neutralizing antibodies resulted in augmented LNL and increased expression of VEGF-A/C and IFNg. Loss of either CD4 or CD8 T cells results in augmentation of LNL as a result of increased VEGF-A/C expression. In conclusion, we showed that T cell differentiation temporally modulates inflammatory LNL, suggesting that targeting lymphangiogenesis can be a new therapeutic approach for various pathologies.

F.91. Development of a Robust ELISA Kit to Detect Human APRIL (TNFSF13) Homotrimers and APRIL-BAFF Heterotrimers in Human Serum, Plasma, and Other Biological Samples

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APRIL (TNFSF13) is an important secreted protein that stimulates cell proliferation. As with most other TNF family members, APRIL exists as a functional homotrimer. It can bind to two cell-surface receptors: BCMA and TACI, which it shares with BAFF (BLyS or TNFSF13B), to exert downstream T- and B-cell regulatory effects. APRIL is most well known for its tumor proliferation effects. It is a potential biomarker, with serum levels elevated in certain cancers and autoimmune diseases. In fact, recombinant TACI is currently in clinical trials as a neutralization drug against APRIL and BAFF for the treatment of SLE. Besides forming homotrimers, APRIL can also form functional heterotrimers with BAFF. The stoichiometric relationship of the protomeric units is still unclear, however it appears that these heterotrimers are significant since they are elevated in serum of certain autoimmune patients. Both the APRIL and BAFF pathways are currently hot targets for therapeutic intervention. We developed a sandwich ELISA kit to detect human APRIL, including its various multimeric forms, in biological samples. The assay uses two mouse monoclonal antibodies specific against human APRIL, which together recognize APRIL homotrimers, as well as APRIL-BAFF-BAFF and BAFF-APRIL-APRIL heterotrimers, with no cross-reactivity with BAFF itself. Other validation data include recovery, linearity, specificity, sensitivity, interference, and precision. This assay was validated using human serum and plasma samples, as well as stimulated cell culture supernatant. It provides a unique and sensitive tool for measuring the various multimeric forms of human APRIL, which have useful clinical applications in oncology and autoimmunity.

F.92. New Multiplex Assay Panels for Simultaneous Quantification of 13 Th Cytokines or Proinflammatory Chemokines

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Cytokines secreted by T helper cells (Th cytokines) play pivotal roles in regulating the immune responses by stimulating effector cells, such as cytotoxic T cells, B cells and macrophages. Proinflammatory chemokines are chemokines induced by inflammatory stimulations and play important roles in the development of immune responses by attracting inflammatory cells to sites of inflammation. Expression profiling of important cytokines and chemokines, such as Th cytokines and proinflammatory chemokines, is critical in achieving a deeper understanding of the immune responses and states of various inflammatory diseases. We have developed four multiplexed assay panels, i.e., the human and mouse Th cytokine panels and proinflammatory chemokine panels, using fluorescence–encoded beads that are suitable for use on various flow cytometers. The two Th panels allow simultaneous quantification of 13 human or mouse cytokines including interleukins (IL-2, 4, 5, 6, 9, 10, 13, 17A, 17F, 21, 22), IFN-g and TNF-α, which are collectively secreted by Th1, Th2, Th9, Th17, Th22 and T follicular cells. The two proinflammatory chemokine panels detect 13 human or mouse chemokines including MCP-1, RANTES, IP-10, Eotaxin, TARC, MIP-1α, MIP-1β, MIG and MIP-3α. Human panel also includes ENA-78, GROα, I-TAC and IL-8, while mouse panel contains LIX, KC, BLC and MDC. Each antibody pair was carefully selected for assay specificity, sensitivity, accuracy and reproducibility. The panels have been validated by detecting expected changes in biological samples. These panels can be used for serum, plasma, cell culture supernatant and other sample types, offering useful tools for biomedical research and drug discovery.

Diabetes and Other Autoimmune Endocrine Diseases

OR.6. Selective Resistance of Effector Memory Th1 cells to apoptosis in Type 1 Diabetic Patients and Healthy Subjects who carry the PTPN22 Autoimmune Risk Allele

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Individuals who carry the C1858T SNP of PTPN22 have an increased frequency of circulating memory Th1 cells and an increased risk for developing autoimmune disorders, such as rheumatoid arthritis and type 1 diabetes. Here, we examined peripheral T cell populations from genotyped healthy controls and diabetic patients to determine whether this is due to an ability of PTPN22 to modulate the activity of AKT and its downstream effects on T cell survival. We found that in healthy non-risk controls, Akt activity was lower in memory T cells compared to naïve T cells, and even lower in the $CD45RO^{+}CD27^{Neg}Tbet^{Hi}$ effector memory (EM) Th1 subset, which most readily underwent activation-induced cell death. However, in subjects carrying the PTPN22 1858T allele, Akt activity and apoptosis were dysregulated compared to 1858C (non-risk controls). Increased phosphorylation of Akt and resistance to apoptosis were associated with the 1858T allele were most strikingly observed in the $CD45RO^{+}CD27^{Neg}Tbet^{Hi}IFN\gamma^{+}$effector memory Th1 subset. Importantly, the enhancement of Akt activity and increased survival of EM Th1 cells were observed in all Type 1 diabetic subjects tested, irrespective of PTPN22 genotype. Therefore, selective resistance of EM Th1 cells to apoptosis appears to be a common immune phenotype in type 1 diabetes and represents a potential mechanism underlying their accumulation and loss of T cell tolerance in this patient population. *Funded by NIAID.*5 R01 AI083455-01A2

OR.23. Discovery of novel HLA-DQ binding beta-cell peptides uniquely processed and presented by dendritic cells

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Identifying T-cell islet-epitopes is important for development of therapeutic and diagnostic tools and to provide insight into the etiology of type 1 diabetes (T1D). Studies identifying islet-epitopes in T1D have focused on testing synthetic overlapping peptides of islet-autoantigens with arbitrary lengths for T-cell autoreactivity in T1D patients and eluting natural processed and presented epitopes (NPPEs) from HLA class II expressed on B-cells pulsed with islet-autoantigens. Neither approach addresses the role of the professional APC in the priming of autoreactive T cells. We therefore developed a novel, sensitive technique to identify NPPEs after pulsing professional antigen-presenting cells (monocyt-derivedendritic cells (moDCs)), with whole islet-autoantigens (preproinsulin, PPI; GAD65, IA-2). Using low DC numbers ($<50x10^{6}$), we identified IA-2-derived NPPEs eluted from T1D high-risk HLA-DQ2/8 constituting two nested peptide-sets around distinct core-regions of the N-terminus of IA-2. In addition, we confirmed HLA-DR3/4 eluted NPPEs derived from IA-2 and GAD65. Binding of the NPPEs to HLA-DQ was validated. Importantly, our HLA-DR eluted GAD65 NPPEs had previously been identified as CD4 T-cell epitopes in T1D by others. Both HLA-DQ and HLA-DR eluted IA-2 NPPEs were derived from the N-terminus, whereas NPPEs from the C-terminus have been reported for HLA-DR using B-cells. Our study discovers the first IA-2 NPPEs eluted from highest risk HLA-DQ using professional APCs, providing a new tool to study antigen presentation. Using different APC may improve epitope selection for generation of sensitive and specific reagents to detect and monitor autoreactive CD4 T-cells in T1D patients as well as vaccination therapy.

OR.26. Tolerogenic Properties of Dex/VitD2-treated Human Tolerogenic Dendritic Cells Remain Stable after Mimic *in vivo* DC Activation

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Tolerogenic dendritic cells (TDC) that are maturation-resistant and locked in a semimature state show a great promise as a specific form of cell-based therapy for induction or restoring immune tolerance in the context of transplantation and autoimmune diseases. The aim of this study was to establish human monocyte-derived TDC in the cGMP-compliant medium Cell Gro using paricalcitol (19-nor-1,25-dihydroxyvitamin D2), the active form of vitamine D2, glucocorticoid dexamethasone (Dex), and monophosphoryl lipid A (MPLA) and to assess their stability to exogenous maturation stimuli. TDC generated in the presence of Dex, paricalcitol and MPLA exhibited tolerogenic phenotype with reduced expression of maturation markers and increased expression of CD14, ILT-3, TLR2 and TIM-3 molecules compared to control DC. Moreover, cytokine profile of TDC was skewed toward a tolerogenic type with increased secretion of IL-10, reduced secretion of TNF-a and notably undetectable production of IL-12p70 when compared to control DC. Finally, TDC showed a reduced T cell stimulatory capacity, supressed mature DC -induced T cell proliferation and were capable of inducing CD4+CD25+FoxP3+Helios+ T regs. To examine whether TDC were resistant to an exogenous maturation stimulus, we mimicked *in vivo* DC activation by re-stimulating TDC with LPS, poly IC, CD-40L or cytokine mix. We found that TDC remained phenotypically resistant to secondary stimulation and maintained stable regulatory phenotype with increased expression of ILT-3, PD-L1 and TLR-2 molecules, high IL-10/IL-12p70 ratios and reduced T cell stimulatory capacity. These findings supported collectively a stable non-proinflammatory profile for TDC.

OR.32. Analysis of Beta Cell Death in Individuals at Risk For Type 1 Diabetes

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The timing of pathologic events leading to β cell death in T1D and hyperglycemia in non-diabetic relatives of patients who are at risk for the disease, are not well understood. We developed an assay to measure β cell death *in vivo* by measuring unmethylated INS DNA in the circulation by nested PCR and more recently with droplet digital PCR (ddPCR). We first compared the levels and kinetics of β cell death in 21 autoantibody+ at risk subjects in the TrialNet Natural History study (mean age 11.2±0.52yrs, 16F) in serial samples up to 1226 days prior to dx (progressors, n=10) or over the same study period in non-progressors (n=11). By RPANOVA, the progressors showed higher levels of unmethylated INS DNA vs non-progressors (p=0.038). There were significant elevations at intervals 625 days (p=0.006), 375 (p=0.033) prior to and at diagnosis (p=0.008). We then analyzed a second cohort of subjects with > 75% risk of T1D in 5 yrs (n=30, mean age=18.6±2.1 yrs) by ddPCR. The ratio of unmethylated:methylated INS DNA was significantly greater in these subjects vs healthy controls (0.595±0.1 vs 0.211±0.023, p=0.009). Ten of these subjects developed T1D after 99±34 days. We conclude that β cell death can be detected in subjects at-risk for T1D prior to the development of hyperglycemia. Measurement of β cell death may be useful as a biomarker to select individuals for prevention therapies.

OR.42. β-Cell-Specific CD8 T Cell Phenotype in Type 1 Diabetes Reflects Chronic Autoantigen Exposure

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Autoreactive CD8 T cells play a central role in the destruction of pancreatic islet b-cells that leads to type 1 diabetes, yet the key features of this immune-mediated process remain poorly defined. In this study, we combined high definition polychromatic flow cytometry with ultrasensitive peptide-human leukocyte antigen class I (pHLAI) tetramer staining to quantify and characterize b-cell-specific CD8 T cell populations in the blood of patients with newly-diagnosed type 1 diabetes patients and healthy controls. Remarkably, we found that b-cell-specific CD8 T cell frequencies in peripheral blood were similar between subject groups. In contrast to healthy controls, however, patients with newly diagnosed type 1 diabetes displayed hallmarks of

antigen-driven expansion uniquely within the b-cell-specific CD8 T cell compartment. Molecular analysis of selected b-cell-specific CD8 T cell populations revealed highly skewed oligoclonal T cell receptor (TCR) repertoires comprising exclusively private clonotypes. Collectively, these data suggest that CD8 T cell-mediated autoreactivity is intrinsically programmed within the human TCR repertoire and that clonotype-dependent functional antigen encounter is a component of disease pathogenesis.

OR.45. Proinsulin specific, HLA-DQ8 restricted, CD4+ T cells infiltrate the islets in type 1 diabetes

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Type 1 diabetes (T1D) is caused by the T-cell mediated destruction of the insulin-producing beta cells. The HLA genes, HLA DQ8 and HLA DQ2, confer the highest risk of developing T1D implicating $CD4^+$ T-cell responses in the pathogenesis of type 1 diabetes. We have isolated $CD4^+$ T-cell clones from the residual pancreatic islets from a deceased organ donors who suffered from T1D. We tested 53 clones from this donor for responses to 26 peptides including the entire sequence of proinsulin and selected epitopes from other islet antigens. Fifteen of 53 clones (28%) recognized epitopes derived from the C-peptide of human proinsulin. Six distinct T-cell clones recognized the same core C-peptide epitope (PI40-50), while two clones recognized PI50-60. Analysis of the clone's HLA restriction revealed that all except one pair of clones with identical TCRs, were restricted by HLA DQ8 (A1*0301, B1*0302). The remaining clones were restricted by an HLA DQ8 transdimer (DQA1*0501, DQB1*0302). CFSE-based proliferation assays revealed that two of eight children with recent onset type 1 diabetes had $CD4^+$ T-cell responses to these epitopes, but none of five HLA matched control subjects responded to these peptides. Our work greatly extends our knowledge of HLA DQ8 restricted T-cell responses, confirms that central role that $CD4^+$ T-cell responses against proinsulin play in the pathogenesis of human T1D and provide strong new evidence for the autoimmune basis of human type 1 diabetes.

W.23. Gene Expression Profile of Human Peripheral Blood Cells from Pre-diabetics Compared to Control Non-Diabetic Subjects

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As we enter the era of T1D prevention trials, we need validated biomarkers that not only predict risk of T1D development, but can define the stage of disease and predict the rate of progression in at-risk individuals. This information can be used to stratify at-risk individuals and predict how certain subsets of patients may respond to therapy. While serum autoantibodies (AA) and tests of beta cell function remain the gold standard to identify at-risk patients, it is important to find alternative biomarkers that will identify the stage of disease or rate of progression, and subsequently help in devising strategies for intervention trials in at-risk subjects. To identify such biomarker candidates, we performed preliminary genome-wide gene expression analysis of peripheral blood cells from 4 AA+ first degree relatives of T1D patients and 4 healthy controls. We identified 23 significantly up-regulated genes and 461 significantly down-regulated genes in AA+ patients compared with controls (2 fold or greater with P value <0.05). Pathway analysis highlighted the Spliceosome in up-regulated genes, and the Neuroactive ligand-receptor interaction, natural killer cell mediated cytotoxicity, and IL-1 family receptor signaling pathway (*IL1R1*, *IL1RAP* and *IL1RL1*) in down-regulated genes. We also found that several genes encoding secreted proteins (*LIF*, *CD54*, *CSF3*) were significantly up-regulated in AA+ patients and those genes were also included in the T1D susceptibility loci in the meta-analysis of genome wide association studies (GWAS). Our studies have cataloged multiple gene-expression signatures that may play a role in the progression of T1D and could serve as biomarkers.

W.34. Regulatory T cell activation and destabilization in the inflamed islets of NOD mice

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Regulatory T cells (Tregs) are essential to prevent autoimmunity in mice and man. In non-obese diabetic (NOD) mice, Treg function in the pancreaticLN as well as in inflamed islets is essential for effective control of disease progression. In this study, we analyzed Treg activation in inflamed islets of pre-diabetic NOD mice. We found two distinct subsets of intra-islet Tregs based on their expression of CD103. CD103+ Tregs were present mainly in inflamed islets and expressed higher amounts of Foxp3 and Treg effector molecules such as CD25, ICOS, and CTLA-4 than CD103- Tregs. After adoptive transfer into new hosts, >20% of CD103- intra-islet Tregs lost Foxp3 expression and approximately 50% acquired CD103 expression. When CD103+ Tregs were adoptively transferred, more than 90% remained Foxp3+ and 70% maintained CD103 expression. Additionally, 4% of CD103- Tregs produce IFNγ after ex vivo mitogen stimulation compared to <0.5% in CD103+ Tregs. CD103+ intra-islet Tregs from NOD.Nur77-GFP reporter mice expressed higher amounts of GFP suggesting they react more strongly with endogenous antigen in islets. CD103+ Tregs are also more sensitive to ex vivo IL-2 stimulation. Collectively, these results suggest that intra-islet CD103- Tregs have two distinct fates: destabilize to conventional T cells or acquire CD103 expression to commit to the Treg lineage. Responsiveness to TCR and IL-2 stimulations may underlie CD103+ Treg stability. Our findings highlight the impact of the inflammatory milieu of the tissue on Treg commitment and destabilization and have important implications for understanding the pathogenesis of autoimmune diabetes and for identifying therapeutic opportunities.

W.35. Production of Pro-inflammatory Cytokines after TLR Ligands Stimulation in Type 1 Diabetes Patients

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Type 1. diabetes (T1D) is an autoimmune Th1-mediaded disease. Innate immune reactions are important particularly in early stages of T1D development. We have, therefore, studied cytokine responses induced by TLR ligands in T1D patients and their relatives in risk of development of the disease. A set of TLR ligands for TLR4, 7, 8, and 9 were used to stimulate peripheral blood mononuclear cells. Production of IFNα, IL-1β, IL-6, IL-10 and TNFα was measured in 68 patients with T1D onset, 31 long-term treated T1D patients and 67 their first degree relatives. As a disease control group we investigated 14 patients with Sjögren's syndrome and 55 healthy controls. IL-1β was the most prominent cytokine induced by LPS and by TLR7, 8 and 9 ligands loxoribine, ssRNA and CpG2216 in patients with chronic established T1D. IL-1β production in this group was significantly higher in comparison to the controls. Out of other pro-inflammatory cytokines, patients with T1D produced also high levels of IL-6 and TNFα upon TLR9 stimulation with CpG2216. We therefore observed induced production of these cytokines upon stimulation of multiple TLRs in T1D patients. In contrast, pro-inflammatory cytokines IL-1β, IFNα and TNFα were induced in the Sjögren's syndrome group almost exclusively after loxoribine and ssRNA stimulation. In T1D group we observed increased IFNα only after TLR9 stimulation in relatives of T1D patients. In conclusion, pro-inflammatory cytokines, mainly IL-1β, created a typical signature for T1D patients, while IFNα was a prominent cytokine in Sjögren's syndrome patients. This study was supported by projects IGA NT/11407-5 and by GACR P302/10/1679.

W.38. The *PTPN22* risk variant is sufficient to break peripheral T cell tolerance and trigger Type 1 diabetes (T1D) *in vivo* in mice

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An allelic variant of protein tyrosine phosphatase non-receptor 22 (*PTPN22*) is strongly associated with multiple human autoimmune diseases. The risk variant modulates antigen receptor signaling and aged knock-in mice expressing the analogous mutation, Ptpn22 R619W, in a mixed genetic background develop systemic autoimmunity. However, the role of the risk variant in peripheral T cell tolerance has not been directly investigated. In this study, we used the RIP-mOVA T1D mouse model to address this question. Adoptive transfer of Ptpn22 R619W OT-I cells rapidly triggered diabetes in RIP-mOVA recipient mice, while transfer of similar numbers of wild-type (WT) OT-I cells did not. Consistent with these observations, histological analysis of the pancreas from recipients of Ptpn22 R619W OT-I cells revealed severe lymphoid inflammation.

Ptpn22 R619W OT-I cell recipient mice contained 2-fold greater numbers of OT-I T cells within pancreatic lymph nodes and recovered T cells expressed higher levels of CD44 compared with controls. Strikingly, co-transfer of Ptpn22 R619W OT-II T cells with small numbers of OT-I T cells caused diabetes in RIP-mOVA recipients , whereas WT OT-II cells failed to promote disease. These latter findings indicate that Ptpn22 R619W OT-II CD4 T cells can enhance the pathogenic activity of OT-I CD8 T cells. Taken together, our data demonstrate that the *PTPN22* risk variant plays an important role in controlling the effector differentiation of autoreactive T cells. They suggest that, in the setting of a fixed TCR repertoire, altered fine-tuning of TCR signals is sufficient to break T cell tolerance and mediate tissue-specific autoimmunity.

W.45. Inflammation and Hyperglycemia Mediate *Deaf1* Splicing in the Pancreatic Lymph Nodes Through Distinct Splicing Pathways During the Progression of Type 1 Diabetes

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Peripheral tolerance is partially controlled by the expression of peripheral tissue antigens (PTAs) in lymph node stromal cells. We previously identified a transcriptional regulator deformed epidermal autoregualtory factor 1 (*Deaf1*) that can regulate transcription, processing, and presentation of PTA genes in lymph node stromal cells in the pancreatic lymph nodes (PLN). During the progression of Type 1 diabetes (T1D), *Deaf1* is spliced to form the dominant negative isoform *Deaf1-Var*. Here we show that *Deaf1-Var* expression correlates with the severity of disease, and that inflammation and hyperglycemia can independently drive the splicing of *Deaf1* by upregulation of distinct splicing factors. Microarray and qPCR analysis demonstrated that two splicing factors, *Srsf10* and *Ptbp2*, were highly upregulated in the PLN of hyperglycemic 16 week old NOD mice. Remarkably, significant upregulation of both *PTBP2* and *SRSF10* was also found in the PLN of autoantibody positive patients compared to control subjects. *In vivo* experiments showed that inflammation induced by *i.p.* injection of activated splenocytes significantly increased *Deaf1-Var* and *Srsf10,* but not *Ptbp2* expression in the PLN of NOD.SCID mice. Hyperglycemia induced by insulin receptor agonist treatment significantly increased *Deaf1-Var* and *Ptbp2,* but not *Srsf10* expression in the PLN of 10 week old NOD mice. *In vitro* studies showed that overexpression of *PTBP2* and *SRSF10* alone, or in combination, induced *Deaf1* splicing in HEK293T cells. These data demonstrate that inflammation and hyperglycemia that occur during the progression of T1D can mediate the splicing of *Deaf1* by controlling *SRSF10* and *PTBP2* expression in the PLN.

W.101. Immunological Assessment of hu-ESC Derived Islet Cells

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Beta-cell replacement therapy with pancreas or islet cell transplantation is the only curative treatment for advanced type 1 diabetes. Unlimited sources of beta-cells, such as those derived from embryonic stem-cells, could make beta-cell replacement accessible to more patients. Protective encapsulation may help survival of the grafts. Yet, human embryonic stem-cell derived cells may be immune privileged by their nature. We therefore investigated the sensitivity of hu-ESC derived cells to adaptive immune responses that may occur after transplantation. Human embryonic stem-cell line Cyt49 was differentiated into pancreatic endoderm in vitro and further differentiated in mice as macro-encapsulated implants in the subcutis. At posttransplant month 4, implants were retrieved and dissociated, before analyzing their susceptibility to cytotoxic T-cell killing, ADCC and CDC, while assessing CD46, CD55 and CD59 complement receptor expression. Hu-ESC derived endocrine cells were efficiently killed by alloreactive T-cells and CMV-specific cytotoxic T-cells if pulsed with CMV peptide. Alloreactive antibodies lysed endocrine cells through ADCC. This effect was repressed after HLA-upregulation by IFNγ to mimic an inflammatory microenvironment. Endocrine cells expressed complement-protecting receptors and were protected from complement-mediated cytolysis. Our data show that hu-ESC derived islet cells are susceptible to immune responses, indicating the need for an associated anti-rejection strategy. Since they are well protected from complement but vulnerable to direct cellular attack, a cell-impermeable encapsulation may be sufficient to protect their survival as allograft. Yet, if grafted cells unintentionally escape their capsules, they can be eliminated by immune cells, which protects from potentially undesired spreading.

F.12. Immunosuppressive properties of quercetin on macrophage function in mouse model of type 1 diabetes mellitus

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Introduction: Type 1 diabetes mellitus (T1DM) is a chronic autoimmune disease, caused by loss of pancreatic β cells. Studies have demonstrated the role of macrophages (MΦ's) in development of T1DM. Quercetin (QUE) is a flavonoid with immunosuppressive properties, which have been considered for autoimmune diseases treatment, nevertheless there have been no studies to evaluate QUE effect on MΦ's function in mouse model of T1DM. AIM: Investigate the immunosuppressive effect of QUE on MΦ's function in mouse model of T1DM induced by streptozotocin (STZ). Methods: T1DM was induced in ICR male mice injecting STZ-low doses for 5 days (40 mg/kg), and pre-treated with QUE (50,100,150 mg/kg) 3 days prior induction and during it. We obtained peritoneal MΦ's LPS-stimulated to determine NO concentration, IL-6 and TNF-α production . Phagocytic activity was evaluated with flow cytometry. Serum was obtained to determine autoantibodies production. Results: The highest percentage of NO production inhibition (%INO) and phagocytosis inhibition (%IF) were observed on 150 mg/kg group with 69.7±2.8 and 58.6±5.3 respectively. In this group, a decrease on IL-6 and TNF-α was observed (39.4±6.0 and 256.9±94.5 respectively) compared to group control. Levels of anti-insulin and anti-pancreatic islets diminished 63% and 56% respectively. Conclusions: These results suggest that QUE inhibit phagocytosis and NO production, diminishing production levels of anti-insulin, anti-pancreatic islets, IL-6 and TNF-α in mouse model of T1DM.

F.13. Responsiveness to IL-6 is Enhanced in T Cells from Patients with Type 1 Diabetes

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Abnormal IL-6 production or signaling is associated with changes in effector and regulatory T cell functions and has been implicated in the etiology of multiple autoimmune diseases including rheumatoid arthritis and multiple sclerosis. However, in type 1 diabetes the role of IL-6 is not fully understood. Here we show that IL-6-mediated STAT3 phosphorylation is significantly increased in CD4 and CD8 T cells of type 1 diabetic individuals compared to healthy controls. The effect was most pronounced in the CD4 memory and CD8 naïve T cell compartments and appeared to be IL-6 specific as T cell stimulation with other cytokines utilizing the STAT3 pathway, IL-10 and IL-27, did not result in similar differences. An important determinant of IL-6 responsiveness in peripheral T cells was IL-6 receptor surface expression, which correlated with phospho-STAT3 levels. Additionally, enhanced IL-6 mediated pSTAT3 was highest in type 1 diabetic subjects with short disease duration, decreasing with time from diagnosis. In conclusion, our study demonstrates the presence of enhanced IL-6-mediated STAT3 responses in T cells from patients with type 1 diabetes suggesting a role for IL-6 mediated immune dysregulation and providing an argument for IL-6 targeted therapeutic intervention in type 1 diabetes.

F.14. Diminished B Cell Receptor Signaling is Present in Ab$^+$ First Degree Relatives that Progress to Type 1 Diabetes and is Improved in Subjects Responsive to Rituxan Therapy

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Autoreactive B lymphocytes are strongly implicated in development of Type 1 diabetes (T1D), and B cell depletion therapy with rituximab has been shown to slow disease progression in some new onset T1D patients. Previously, we demonstrated expansion of transitional and anergic B cell subsets and diminished BCR signaling in adults with long-standing T1D,

suggesting that these alterations are factors in disease pathogenesis. Here, we investigated whether these phenotypes are evident prior to disease in the TrialNet Pathway to Prevention Study and if they can be modulated by rituximab therapy. We found that BCR-triggered proximal signaling in naïve B cells is modestly increased in Ab^+ non-progressors as compared to age- and gender-matched Ab^{neg} FDR control subjects; but was significantly reduced in Ab^+ progressors when compared to Ab^+ non-progressors. Longitudinal analysis of these cohorts revealed that diminished BCR signaling is present in Ab^+ progressors 6-12 months prior to diagnosis, suggesting that this phenotype is most prominent near disease onset. Parallel analyses of these groups for the composition of the B cell compartment revealed significant expansion of transitional B cells in Ab^+ progressors as compared to Ab^{neg} FDR controls. In our preliminary assessment of participants in the TrialNet rituximab trial before and after treatment, we observed significantly increased BCR-mediated P-PLCγ2 in total B cells from active treatment responders, but not non-responders, at one year post-therapy. These combined findings suggest that altered BCR signaling and transitional B cell homeostasis are features of new onset T1D subjects that can be modulated following intervention with rituximab.

F.15. Reduction in Effector Memory T Lymphocytes in Graves´ Disease

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Graves' disease (GD) is an organ-specific autoimmune disorder characterized by the production of autoantibodies against the thyrotropin receptor (TSHRAb) and lymphocyte infiltration of the thyroid gland. Among infiltrating T lymphocytes, there is a predominance of CD4+ CD45R0+ cells. Imbalances in the naïve/memory CD4 and CD8 T-lymphocytes, regulatory T cells and B-lymphocyte subpopulations in peripheral blood of GD patients have been reported, but a comprehensive multiparametric flow cytometric analysis of these subpopulations is still lacking. The aim of this work is to carry out an extensive analysis of the T and B peripheral blood cell compartments in GD patients in order to identify those subpopulations that are relevant to disease pathogenesis. CD4 and CD8 T cells (including naïve, central memory, effector memory and terminally differentiated effector (TEMRA), Th17 and Tregs) and B cells subsets (naïve, unswitched memory, switched memory and transitional B cells) were analyzed in peripheral blood of stable GD patients (n=26) and healthy donors (HD; n=40) using multiparametric flow cytometry.

We found a reduction of effector memory CD4 and CD8 T cells (p=0,036 and p=0,0023, respectively) as well as a decrease in regulatory T (p=0,020) and B cells (p=0,030) in peripheral blood of GD patients. In contrast, an increase of double positive CD4+CD8+ (DP) lymphocytes with memory phenotype was observed (p=0,049) compared with HD. In conclusion, in GD patients -even in stable clinical situation- there is an increase of effector T cells and a decrease of regulatory lymphocyte subpopulations, suggesting their involvement in the pathogenesis of the disease.

F.16. Measurement of Autoantibodies in LADA Patients HLA-ACE- APO Gene in DM Patients and Adiponectin in GDM pts

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Estimation of autoantibodies and C peptide in A) LADA patients to determine treatment profile B) Overt diabetic – to check prognosis C) Siblings of such patients – if lifestyle needs to be changed early D) Pregnant women to check if these parameters -adiponetin and insulin assay to determine the GDM II HLA,ACE-ApoE in diabetic patients , their siblings to determine the protective gene

	Total Sample	Overt		Siblings		LADA		Pregnancy	
	1652	1202	%	136	%	134	%	180	%

GAD positive		252	21	24	18	87	65	18	10
IA2 positive		42	3.5	4	3	69	52	05	2.5
C peptide Positive		264	10	7	5.5	16	12	45	25
GAD +IA2 Positive		12	1	nil		04	3	05	2.5
Insulin assay		216	18	6	8	77	58	14	7.5
Adiponectin		84	7			22	17		
Any two		36	03	02	2.7	60	45	09	5
Any three		06	0.5	nil		08	5.8	nil	nil

At diagnosis, both ICA and GAD antibodies were shown to be predictors of insulin dependency, but GAD antibodies appeared to have higher sensitivity as predictors than ICA. The adult diabetic patients considered to be insulin-deficient on the basis of their C-peptide responses to glucagon, Siblings with lower values were warned. Patients have DRBI*03, DQBI*0201 or the DRBI*04, DQBI*0302 haplotype. DRBI*0403 was in non diabetic ACE-ApoEassociation were found in three patients .The association of Positivity increases the risk of type 1 diabetes 2 years after delivery, Siblings could be detected early. Adiponectin did not have significant correlation.

F.17. Insulin Impairs Regulatory T Cell Function: Implications for Obesity

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Chronic inflammation is known to drive metabolic dysregulation in obesity. Although the precise origin of the unchecked inflammatory responses in obesity is unclear, it is known that over-production of pro-inflammatory cytokines such as TNF-α by innate immune cells has a key role in the development of insulin resistance. One key hallmark of obesity is high levels of the pancreatic hormone insulin, and we hypothesized that there may be an unknown link between hyperinsulinemia and chronic inflammation. Here we show that high levels of insulin impair the ability of regulatory T cells (Tregs) to suppress inflammatory responses via effects on the AKT/mTOR signaling pathway. Insulin strongly activates AKT/mTOR signalling in Tregs, leading to specific inhibition of the production of the anti-inflammatory cytokine IL-10. As a result, insulin hinders the ability of Tregs to suppress the production of TNF-α by macrophages. Tregs from the visceral adipose tissue of hyperinsulinemic, obese mice also have a decrease in IL-10 production and a parallel increase in production of IFN-γ. Although obese mice are resistant to insulin in several tissues, Tregs from these mice remain insulin sensitive. These data suggest that the hyperinsulinemia associated with obesity may contribute to the development of obesity-associated inflammation via a previously unknown effect on Treg function.

F.19. IL-10 Receptor and Endoplasmic Reticulum Stress Impairs STAT3 Activation in Type 1 Diabetes Patients and Their Healthy First Degree Relatives

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Autoimmune diseases including Type 1 diabetes (T1D) result from disordered immune tolerance. We showed that an endoplasmic reticulum (ER) stress signature predicted poor outcome in recent-onset T1D patients, and is observed in a proportion of at-risk first degree relatives (FDR). To determine the relationship of ER stress to immune cell signalling, we compared monocyte and T cell STAT3 phosphorylation in response to IL-6 and IL-10 in 30 T1D, 35 FDR, 11 healthy controls, and 22 Rheumatoid Arthritis (RA) disease controls. Cytokine-stimulated signalling was detected in peripheral blood monocytes and T cells by flow cytometry. Expression of ER stress genes *GRP78* and *DDIT3* was quantified from PBMCs by RT-PCR.

Induction of phospho (P)-STAT3 by IL-10 but not IL-6 was significantly reduced in T1D and FDR monocytes and T cells relative to healthy or RA controls. Basal levels of total STAT3 were comparable in all groups. This reduction in P-STAT3 response in T1D patients and FDR was associated with reduced IL-10-, but not IL-6-receptor expression by monocytes and T cells. IL-10 receptor expression and P-STAT3 induction in response to IL-6 and IL-10 were negatively associated with *GRP78* and *DDIT3* ER stress gene expression. IL-10 was shown to block ER stress in gut epithelium. Thus, our data implicate a disease-specific IL-10 receptor defect and its consequences on excess ER stress and disordered peripheral tolerance in T1D pathogenesis.

F.20. Enhanced Recognition of Post-translationally Modified Beta Cell Antigens in Subjects with Type 1 Diabetes

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Modification of proteins by citrullination and transglutamination is known to elicit T cell responses in rheumatoid arthritis and celiac disease. Recent studies suggest that responses to self-proteins with similar modifications are relevant in type 1 diabetes. We demonstrate that post-translational modification of beta cell antigens enhances their recognition by CD4 T cells in subjects with type 1 diabetes. In particular, citrullination of amino acids at anchor positions significantly enhanced their binding to diabetes susceptible HLA-DR proteins (such as DRB1*04:01). Corresponding modifications to amino acids at T cell contact positions modulated recognition by self-reactive DRB1*04:01-restricted T cells. Transglutamination of amino acids at anchor positions significantly enhanced their binding to diabetes susceptible HLA-DQ proteins (such as DQB1*03:02). Corresponding modifications to amino acids at T cell contact positions modulated recognition by self-reactive DRB1*03:02-restricted T cells. Using class II tetramers, we verified that T cells specific for modified epitopes were detectable directly *ex vivo* in the peripheral blood of subjects with Type 1 diabetes at significantly higher frequencies than healthy controls. T cells that recognize modified epitopes were either less responsive or non-responsive to their unmodified counterparts. Using these same tetramers, self-reactive T cells that recognize post-translationally modified epitopes could be directly characterized by multiparameter flow cytometry. Our findings demonstrate that post-translational modification contributes to the loss of tolerance to beta cell antigens by activating T cells specific for neo-self epitopes. Since these modifying enzymes are induced under conditions of inflammation, T cell responses to modified epitopes may be less subject to tolerance mechanisms.

F.21. Accentuated Dichotomy between Activation and Resting State Results in Lower Basal IL-2R Signaling Potential in CD4 Memory T Cells of T1D Subjects

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The IL-2/IL-2R pathway is strongly implicated in type 1 diabetes (T1D). However, not all underlying mechanisms are understood. Known genetic risk alleles in humans account for some, but not all, of the reduction in pSTAT5 IL-2R signaling observed in T1D. To determine additional factors, we interrogated signaling in healthy controls and T1D subjects held constant for IL-2R associated risk alleles. Glucose and HbA1c did not correlate with IL-2 response in CD4 memory T cells of established T1D subjects. Reduced IL-2R signaling was not observed in T2D subjects. Nor was reduced signaling restored by resting isolated memory T cells overnight in fresh media. Instead, IL-2 signaling in CD4 memory T cells of T1D subjects remained significantly lower than controls even after in vitro activation and proliferation. Together, these data suggest that intrinsic factors contribute to reduced IL-2R signaling in T1D subjects. We next asked whether these intrinsic factors were caused by divergence to alternative signaling pathways, differentiation or activation state. Akt and Erk phosphorylation were not augmented in T1D with IL-2 stimulation. IL-2R signaling was comparable regardless of differentiation marker expression. However, there was an accentuated dichotomy between IL-2 response in activated versus quiescent cells of T1D subjects. This resulted in a more pronounced reduction in resting cell IL-2/pSTAT5 signaling in T1D. Thus, in addition to known T1D-associated risk alleles, intrinsic properties related to activation may modulate levels of basal IL-2R pSTAT5 signaling. Therapeutically targeting multiple components of the IL-2R pathway may increase treatment options for T1D.

F.22. TCR-Vβ13 CDR1 CDR2 CAA.1WR1 R

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Autoimmune type-1 diabetes (T1D) is common among inbred rat strains with high-risk MHC-II (RT1.B/D^{u}). *Iddm14* on rat chromosome 4 is a strong determinant of diabetes susceptibility. We identified an allele of a T-cell receptor beta chain variable region, TCR-Vβ13a, as the *Iddm14* gene. Depletion of Vβ13+ T-cells prevents T1D and halts early progression of disease, suggesting that Vβ13+ T-cells recognize critical islet auto-antigens early in diabetes progression. TCR-Vβ13+ transcripts accumulate in pre-diabetic LEW.1WR1 rat islets and their frequency increases during diabetes progression. Furthermore, Vβ13-CDR3s display islet-specific pauciclonal expansion early in diabetes progression. In NOD islets , TCR-Vα5D4+ T-cells are abundant and recognize insulin B:9-23 in combination with multiple TCRβ chains. Rat Vα5-CDR3 sequences also display pauciclonal expansion in LEW.1WR1 islets suggesting an autoimmune response to insulin early in rat T1D. Together, this indicates that TCR germline elements (CDR1 and CDR2) are critical for recognition of islet auto-antigens.

To examine how TCR-Vβ13 CDR1 and CDR2 influence anti-islet autoimmunity we employed *in silico* modeling and *in vitro* testing of Vβ13+ and Vα5+ TCRs that displayed pauciclonal expansion. *In silico* modeling of one Vβ13-Vα5 TCR on RT1.B/D^{u} bound to Ins B:11-23 indicates a significant interaction between Vβ13 CDR2 and the pMHC interface. Eight Vβ13 and three Vα5 TCR vectors were constructed for retroviral transduction of hybridoma cells to test multiple Vβ13-Vα5 combinations for reactivity to islet antigens. In addition, Vβ13a sequences were mutagenized to encode Vβ13b CDR1 or CDR2 to test the individual contribution of allelic polymorphisms in those regions toward islet-antigen reactivity.

F.23. Th17-Like Tregs in Patients with Type 1 Diabetes and Response to Anti-CD3 mAb Treatment

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CD4^{+}CD25^{+}CD127^{lo} Tregs exist in distinct subsets similar to helper CD4 T cells. Differences in the number and function of Th1-like Tregs have been implicated in autoimmune disease including type 1 diabetes (T1D). This study aimed to further characterize Th1 and Th17-like Treg subsets in patients with recent-onset T1D and to investigate the effects of teplizumab, a FcR non-binding anti-CD3 mAb. Methods: PBMCs were obtained from 10 healthy controls (HC) and 11 T1D patients before and after teplizumab treatment or placebo. Treg frequencies and subsets were analyzed by FACS (Tregs:CD4^{+}CD25^{+}CD127^{lo}, Th17-like:CXCR3^{-}CCR6^{+}, and Th1-like:CXCR3^{+}CCR6^{-}). CD4^{+}CD25^{+}CD127^{lo} Tregs from patients and controls were sorted and stimulated with PMA/Ionomycin and analyzed by Luminex and qPCR. Baseline comparisons were made to controls, and changes following teplizumab treatment were assessed. Results: Pretreatment, CD4^{+}CD25^{+}CD127^{lo} Tregs were elevated in patients with T1D (p= 0.03) compared to HC. Patients had a decreased frequency of Th17-like Tregs by FACS (p=0.01). CD4^{+}CD25^{+}CD127^{lo} Tregs in patients had decreased IL17 expression (p=0.008) and secretion (p=0.02), and RORγc expression (p=0.07). There were not differences in pretreatment frequency of Th1-like Tregs, expression or secretion of IFNγ or Tbet expression compared to HC. Th17-like Tregs decreased with disease progression (p=0.004), but were not altered by teplizumab treatment. The total circulating Tregs were decreased 6 mo after treatment (p =0.04) compared to placebo. Conclusion: There are differences in frequency of Treg-subsets that change with the course of T1D. They may be related to the evolution of disease, but the circulating Treg-subsets were not affected by teplizumab treatment.

F.24. InsB Gene Transfer to Hepatocytes Induces FoxP3 T Regulatory Cells and Upregulates TGFβ and IL-10 Production Resulting in Protection from Type 1 Diabetes

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Type 1 diabetes (T1D) is a T cell-mediated autoimmune disease with significant clinical and socioeconomic burden. Several preclinical studies indicate that treatment with immunomodulatory drugs and/or FOXP3+ T regulatory cells (Tregs) can lead to immunological tolerance with prevention and cure of T1D. However, induction of antigen (Ag)-specific tolerance to treat T1D has not been achieved yet. The insulin B (InsB) chain carries immunodominant epitopes for T1D in humans and non-obese diabetic (NOD) mice. We previously showed that targeted expression of the Ag to hepatocytes achieved by *in vivo* lentiviral (LV) gene transfer is an effective approach to induce active tolerance towards the encoded Ag. To test whether this approach can induce tolerance in T1D, we generated LV vectors that selectively target InsB9-23 to hepatocytes. LV.InsB9-23 treatment in 10-week-old prediabetic NOD protected 90% of the mice from T1D, induced Ag-specific FoxP3+ Tregs in the liver and increased expression of the Tregs associated markers PDL-1 and CTLA4. FoxP3+Tregs homed to the pancreas and reduced autoreactive lymphocyte infiltration. These cells also blocked transfer of diabetes to NOD/Scid mice. Ex vivo analyses of lymphocytes isolated from spleen and pancreatic lymph nodes of treated NOD mice showed increased production of TGFβ and IL-10, suggesting that these cytokines play a role in mediating protection from T1D. Our findings demonstrate the feasibility of using LV gene therapy as a novel platform for Ag-specific therapy of T1D. We are currently investigating the utilization of this platform alone or in combination with other drugs to reverse overt disease.

F.25. Reduced RNA Virus Sensor Signaling Protects NOD Mice from Type 1 Diabetes

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Type 1 diabetes (T1D) is a devastating autoimmune disease that demands the design of new treatments that consider both the strong genetic influence and environmental stressors linked to the disease. Protective polymorphisms identified in the intracellular virus receptor melanoma differentiation-associated protein 5 (MDA5) gene urges further investigation of RNA virus sensing in T1D pathogenesis. Patients carrying MDA5 protective polymorphisms are heterozygous and functional studies have demonstrated that the protective alleles result in less MDA5 transcripts. This implicates the level of MDA5 signaling in T1D susceptibility. Our lab has previously demonstrated that the enterovirus highly associated with human T1D, Coxsackievirus B4 (CB4), accelerates T1D in the non-obese diabetic (NOD) mouse through the activation of autoreactive T cells. Importantly, CB4 is an RNA virus whose replicative products are recognized by MDA5 and activate subsequent type 1 interferon (IFN) anti-viral responses. We hypothesized that a reduction in MDA5 expression, as with patients carrying IFIH1 protective variants, would alter anti-viral IFN and adaptive responses to still allow for viral clearance but skew T cell responses in favor of T1D protection. We observed in MDA5 heterozygous mice on the NOD background that a 48% reduction in MDA5 protects from spontaneous and CB4-induced disease and results in an increase in regulatory T cells and fewer activated effector T cells at the site of disease after CB4 infection. MDA5hets have a distinct IFN signature that suggests a unique protective signaling mechanism and supports altering MDA5 signaling as a new avenue in T1D therapy.

F.26. Genetic Regulation of TLR7/8 by SNP rs5979785 and Its Impact on T1D

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T1D is an autoimmune disorder that results in loss of insulin secretion and destruction of pancreatic beta cells. There is a strong genetic association with the MHC class II gene region, implicating adaptive immunity in the disease process. Less well appreciated is the genetic association with the innate immune response, which could be key to understanding disease initiation. Toll Like Receptors (TLR) upon attachment to evolutionarily conserved Pathogen Associated Molecular Patterns (PAMP) are involved in the cascade of pro-inflammatory cytokines. Polymorphisms in TLR7/8 locus have been putatively linked to T1D by Genome Wide Association (GWA) studies, however, the functional impact of this SNP remains unknown. We sought to further elucidate a functional role for the SNP (rs5979785) located in the linkage disequilibrium block next to TLR7/8, for which the protective (C) allele has also been associated with another autoimmune disorder, celiac disease with an odds ratio of 0.8. Studies from our lab demonsrated reduced production of IL-6 and IL-1b upon R848 (TLR7/8 agonist) stimulation of whole blood in vitro for the protective allele of rs5979785. We next focused on isolated monocytes to better understand the effect of this SNP, as the highest expression of TLR8 is observed in monocytes.

F.27. Possible Therapeutic Potential of Filarial Proteins in Mice Model with Induced Type 1 Diabetes

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There has been increasing evidence available from several epidemiological and experimental studies in favour of an inverse relationship between parasite infection and T helper type 1/17 (Th1/17)-based inflammatory diseases such as type 1 diabetes (T1D) suggesting the therapeutic potential of the helminth molecules in these conditions. Hence we explored the curative effect of filarial native and recombinant proteins on the development of T1D in BALB/c mice model. As many as 63% diabetic mice treated with *B. malayi* recombinant abundant larval transcript-2 (rBmALT-2) showed recovery from diabetes reflected from significantly reduced fasting blood glucose levels ($p<0.005$), almost normal pancreatic islet cell architecture with minor inflammatory changes and restored normal insulin in circulation. There was also marked reduction in IFN-γ & TNF-α levels and increase in IL-10 & IL-4 levels in the culture supernatants of splenocytes of rBmALT-2 treated group of mice followed by stimulation with the rBmALT-2. In another set of experiments, diabetic mice treated with *B. malayi* microfilarial excretory-secretory antigen (Bm-mf ES) ($p<0.005$) and *B. malayi* microfilarial soluble antigen (Bm-mf S) ($p<0.05$) also showed significantly decreased blood glucose levels associated with reduction in inflammatory cytokines level and increase in anti-inflammatory cytokines level. As many as 70% of mice treated with Bm-mf ES showed recovery from diabetes and marked bias against inflammation associated with reduced pancreatic damage. These findings provided valid proof for exploiting filarial proteins to target immune modulation and thereby to devise effective anti-diabetic therapeutics.

F.93. The Cytokine Milieu of Pancreatic Beta-Cells in Early Type 1 Diabetes Pathogenesis

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In Type 1 Diabetes (T1D), the complex interplay of effector and regulatory T-cells shifts the balance in favor of the development of autoreactive effector T cells, resulting in inflammatory pathology. We believe, that a network of diverse cytokines constituting the "cytokine milieu" of the beta-cell, determines the direction of the immune response towards inflammation, and, as recent findings suggest, this inflammatory milieu may precede autoimmunity. However, it remains elusive which cytokines are produced and when, and how they interact in such a complex network to sustain inflammation. Here we used immunohistochemical and bioinformatics tools to detect important inflammatory cytokines (IL1beta, IL2, IL6, IL10, IL17, IL21, TNFa, IFNg) level on both mRNA and protein levels in specimens from the nPod collection. This allows us to dissect the multiple layers of cytokine interactions during the very early diabetes pathogenesis at the single cell level and to create an “Atlas of Diabetes” as a first step towards the development of immunotherapeutic strategies for the treatment of autoimmune diseases.

Epidemiology

W.76. Celiac Disease in Southern Saudi Arabia: A Retrospective Study

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Introduction: Celiac disease is a gluten-induced autoimmune inflammation of small bowel villi, leading to atrophy and malabsoprtion. The disease prevalence is not well studied in the southern region of Saudi Arabia. The aim of this study is to check the incidence of celiac disease among high risk subjects. Methods: A retrospective case-finding study for a period of three years (2009 - 2012) were retrieved from medical record department of the main tertiary care hospital (A.C.H), Southwest of Saudi Arabia. Laboratory requests for serum antigliadin IgA antibodies (AGA), anti-tissue tranglutaminase (atTG), and anti-endomysin antibodies (EmA) were analyzed along with small intestinal histopathology. Results: From total of 419 (193 male

and 227 female) records, the median age was 9 years (SD ± 10.2), 17.9% (75/419) were positive for at least one antibody marker. We could find results of histopathology for only 40 patients, 22 cases (55%) were confirmed to be positive for duodenal atrophy. The individual IgA antibody positivity for AGA, atTG and EmA were 18%, 23.5% and 18% respectively. The most common clinical condition (47%) associated with requested markers was type 1 diabetes mellitus (T1DM). Failure to thrive (9.3%) and short stature (5.5%) were the second most common reasons for requests. Gastrointestinal symptoms like abdominal pain and chronic diarrhea were only ordered in (9.3%) of the whole records. Anemia, neurological symptoms, down syndrome and ricket's were other conditions screened. Conclusion: The current study showed high frequency of the disease among T1DM. However, more screening studies are required to determine the prevalence of celiac disease among other conditions.

General Autoimmunity

1108A. A Chimeric Human-Mouse Model of Sjögren's Syndrome

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Sjögren's Syndrome (SjS) is a chronic autoimmune disorder that mainly affects the salivary and lacrimal glands. Despite recent advances in the understanding of this disease, the pathogenic mechanisms remain elusive and an ideal model for early drug discovery is not yet available. To establish a suitable animal model, peripheral blood mononuclear cells (PBMCs) from healthy volunteers or patients with SjS were isolated from whole blood and adoptively transferred via intraperitoneal injections into immunodeficient NOD-scid IL-2rγ (null) mouse recipients to produce chimeric mice. Four weeks after adoptive transfer of healthy or SjS PBMCs, flow cytometry detected only T-cells from donors in the blood of chimeric mice. Interestingly, while no difference was observed in the distribution of $CD3^+$, $CD4^+$, or $CD8^+$ T-cells, chimeric mice transferred with PBMCs from SjS patients produced considerably higher cytokine levels, most significantly IFN-γ and IL-10. Histological examination of the target organs in SjS revealed enhanced inflammatory responses in the lacrimal and salivary glands of SjS chimeras, as measured by digital image analysis and blinded histopathological scoring when compared to adoptive transfers from healthy controls. Furthermore, immunohistochemical analyses of these lacrimal and salivary gland infiltrates were primarily $CD4^+$, with minimal detection of $CD8^+$ T-cells and B-cells. Collectively, these results demonstrate a novel chimeric mouse model of human SjS that provides a unique *in vivo* environment to test experimental therapeutics and investigate disease pathology.

OR.16. Carbon Nanomaterials Treat Autoimmune Diseases by Scavenging Superoxide in Autoreactive T Lymphocytes

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Rheumatoid arthritis (RA) and multiple sclerosis (MS) are chronic, idiopathic autoimmune diseases that affect synovial joints and the central nervous system, respectively. Both diseases are mediated by autoreactive $CCR7^-$ effector memory T (T_{EM}) lymphocytes that promote inflammation and the production of deleterious reactive oxygen species. Superoxide (SO), the precursor to other reactive oxygen species, is released from mitochondria upon T cell receptor stimulation and is crucial for signaling pathways in T_{EM} cell proliferation: NFAT activation and IL-2 production. Therefore, a selective inhibitor of SO could serve as an innovative therapy for RA and MS compared to current treatments, which are mainly broad-spectrum immunosuppressants with life-threatening side-effects. Here we report that the nontoxic carbon nanomaterials, PEGylated hydrophilic carbon clusters (PEG-HCCs), which selectively scavenge SO, ameliorate the T_{EM} cell-mediated pathology of RA and MS. We have found that PEG-HCCs rapidly enter primary rat T cells, reduce intracellular SO produced during stimulation, preferentially reduce the *ex vivo* proliferation of primary rat T_{EM} cells without inducing cell death, and decrease their cytokine production. We have also demonstrated that PEG-HCCs have no effect on antigen-presentation or phagocytosis in primary rat macrophages, indicating that they will likely not behave as generalized immunosuppressants. Furthermore, PEG-HCCs decreased inflammation mediated by T_{EM} cells in a delayed-type hypersensitivity model and ameliorated clinical signs of RA

and MS in rat models. This work suggests that PEG-HCCs are a promising therapy for RA and MS and potentially other T_{EM} cell-mediated autoimmune diseases.

OR.29. A Novel Pathway that Controls Autoimmunity: Reciprocal Interaction Between NK Cells and DCs Regulates the Th17 Response by Eliciting an Innate IFN-γ/IL-27 axis

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IFN-γ is a pathogenic cytokine involved in inflammation. Paradoxically, its deficiency exacerbates experimental autoimmune encephalomyelitis, uveitis and arthritis. We previously showed that it is innate IFN-γ that is protective, whereas adaptive IFN-γ is pathogenic. Here we uncover the mechanism of the protective effect by using IFN-γ-deficient mice repleted with IFN-γ-sufficient NK cells in the experimental autoimmune uveitis model. We demonstrate by immunological and imaging methods that innate production of IFN-γ from NK cells is necessary and sufficient to limit autoimmunity and to reduce the number of IL-17A and GM-CSF producing effector T cells in the target tissue. Mechanistic studies demonstrated that, following immunization for uveitis, DC producing CXCR3 ligand chemokines recruited $CXCR3^{+}$IFN-γ-producing NK cells to the draining lymph node (DLN). Two-photon intravital microscopy revealed that DC and recruited NK cells interact in the DLN in a CXCR3-dependent fashion. During the interaction, IFN-γ from NK cells induced DCs to produce IL-27, which in turn enhanced IFN-γ production by NK cells, forming a positive feedback loop. The NK-DC-dependent IL-27 inhibited development of the adaptive pathogenic IL-17 response and induced Tr1-like cells, which upon adoptive transfer ameliorated disease in an IL-10-dependent manner. Our data reveal that an early NK-DC interaction controls the adaptive Th17 response and limits tissue-specific autoimmunity through an innate IFN-γ/IL-27 axis.

OR.40. Autoantigen Microarray Analysis of Sera from New-Onset Pediatric Systemic Lupus Erythematosus Patients Reveal a Signature Associated with Class III/IV Lupus Nephritis

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Objective: Currently, pediatric Systemic Lupus Erythematosus (pSLE) patients suspected to have proliferative nephritis typically undergo a confirmatory kidney biopsy prior to initiation of cytotoxic therapy. Unfortunately, kidney biopsy is unavailable to patients with bleeding risk, represents a significant medical expense, and carries a small risk of complications. With this in mind, we aimed to identify a serum autoantibody signature to confirm the diagnosis of proliferative nephritis in patients with pSLE, obviating the need for a kidney biopsy.

Methods: We used microarrays featuring 140 recombinant or purified autoantigens to compare the serum autoantibody profiles of pSLE patients with (n=24) and without (n=23) biopsy-confirmed class III or IV proliferative nephritis. We performed ELISA with selected autoantigens to validate the microarray findings. We created a multiple logistic regression model, based on the autoantibody ELISA and clinical information, to predict whether a patient had proliferative nephritis, and used a second cohort (n=20) to test its accuracy.

Results: We identified 17 serum autoantibodies at significantly higher levels in pSLE patients with proliferative nephritis than those without. We confirmed five of seven of the autoantigens (dsDNA, C1q, collagen IV, aggrecan, and histone H1) using ELISA. Our regression model correctly identified patients with proliferative nephritis with 90% accuracy.

Conclusion: We have identified a serum autoantibody signature that, when combined with clinical information, is capable of identifying patients with proliferative nephritis with high accuracy, and could be used to safely and inexpensively confirm diagnosis of proliferative nephritis in pSLE patients, perhaps eliminating the need for a kidney biopsy.

W.16. Selective Targeting of the Gut Microbiota with Single Antibiotics Prevents Mortality in the Autoimmune-prone (NZWxBXSB)F_1 Model

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Introduction: The cause of persistent anti-β_2-glycoprotein I (β_2GPI) antibody production in the lupus-associated antiphospholipid syndrome (APS) is unknown. Infectious triggers have been implicated in transient antibody production in both mice and humans. We tested if persistent anti-β_2GPI antibodies that induce microthrombotic myocardial infarctions in the spontaneous (NZWxBXSB)F_1 model of APS are sustained by specific members of the gut microbiota. Methods: (NZWxBXSB)F_1 hybrid males were treated orally with antibiotics or control water starting at 6 weeks of age. Sera, urine and fecal pellets were collected longitudinally and analysed for anti-β_2GPI titers, proteinuria from lupus nephritis and eubacterial 16S rDNA load by real-time PCR. H&E slides were prepared from kidneys and hearts for histologic analysis. Results: Eubacterial load was suppressed after combined treatment with vancomycin, metronidazole, neomycin and ampicillin. Among those, only vancomycin and ampicillin selectively lowered anti-β_2GPI antibodies at 4 months of age (p=0.014) and protected mice from deaths due to coronary microthrombi (p=0.005). Lupus-related proteinuria, known to be mediated by endogenous retroviral protein gp70, was unexpectedly also suppressed in microbiota-depleted mice (p=0.026). Conclusions: Depletion of selective members of the gut microbiota with vancomycin or ampicillin protects from anti-β_2GPI-antibody-induced myocardial infarctions and deaths. These results suggest that gram-positive members of the gut microbiota are fundamentally involved in the pathogenesis of APS. The gut microbiota appear to modulate also endogenous retroviral-driven lupus nephritis in this model, pointing towards interactions between the microbiota and retroviruses. Phylogenetic analysis of the disrupted microbial communities and transfer experiments should reveal the key pathobionts driving systemic autoimmunity.

W.30. Altered CD8$^+$ T Cell Function In Human Non-Infectious Uveitis

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Purpose: Although CD8$^+$ T cells are primarily implicated in the clearance of viral infections, they have also recently been shown to be a key determinant of clinical prognosis in patients with non-ocular autoimmune diseases. This challenges the conventional paradigm of CD4$^+$ T cell driven autoimmunity, and raises new questions about the role of CD8$^+$ cells in non-infectious inflammation. We therefore interrogated the phenotype and function of CD8$^+$ cells in patients with autoimmune uveitis. Methods: Fresh whole blood from uveitis patients (n=90) and healthy controls (HCs, n=65) was analyzed using flow cytometry. Four subsets of CD8$^+$ cells were distinguished based naïve (CCR7+CD45RA+), central memory (CCR7+CD45RA-), effector memory (CCR7-CD45RA-), and effector memory RA+ (CCR7-CD45RA+) cells. Flow cytometry was used to assess intracellular expression of cytotoxic markers, CD107a, granzyme B and the transcription factors T-bet and Eomes , which are master regulators of CD8$^+$ effector differentiation. Results: There was significantly higher percentage of effector memory CD8+ cells in uveitis patients compared to HCs. Effector memory CD8+ cells from uveitis patients contained higher percentage of CD107a+, and higher expression of granzyme B. Furthermore, CD8+ cells from uveitis patients had a significantly higher T-bet:Eomes ratio in comparison with HCs. Conclusions: The increased expression of CD107a and granzyme B in effector memory CD8$^+$ T cells from uveitis patients indicates a greater capacity for cytotoxicity. This is the first report of altered CD8$^+$ cell function in patients with non-infectious uveitis, and further investigations are now needed to determine whether this contributes to the pathogenesis of intraocular inflammation.

W.31. HMGB1 and Soluble RAGE are Correlated to Disease Activity in Sjögren's Syndrome

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Objective:To assess HMGB1 and soluble RAGE serum concentrations in patients with Sjögren's Syndrome (SS) and explore correlations with clinical parameters. Methods: Patients with SS according to ACR diagnostic criteria and healthy donors were included in this cross-sectional study. The patients were recruited between 2011 and 2013 at the outpatient clinic of the Department of Rheumatology and Clinical Immunology, University Medical Center Freiburg. Clinical data and laboratory values were obtained from all patients. Disease activity was assessed by EULAR SS disease activity index (ESSDAI). Serum

samples were collected and HMGB1, anti-HMGB1 IgG and IgM antibody levels were measured by enzyme-linked immunosorbentassay (ELISA), furthermore HMGB1 serum amounts were detected by Western blot (WB). Results: Forty-one patients with SS and 21 healthy volunteers were included in this study. HMGB1 levels were highly increased in patients with SS compared to HD (1926.8 ng/ml [IQR 1372.7-3167.2] versus 1202.4 ng/ml [IQR 927.8-2058.2], p=0.009) and were significantly higher in patients with extraglandular manifestations compared to patients without extraglandular involvement (3298.0 ng/ml [IQR 5970.8-2316.5] versus 1593.2 ng/ml [IQR 2404.8-1327.2], p=0.006). Moreover HMGB1 and soluble RAGE serum concentrations correlated with disease activity as determined by the ESSDAI. Conclusion: Serum concentrations of HMGB1 and RAGE were elevated in SS and correlated to disease activity as measured by the ESSDAI.

W.37. The Balance of T Cell Costimulation and Exhaustion Determines Contrasting Clinical Outcomes in Autoimmunity and Infection

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The clinical course of autoimmune disease varies greatly even between individuals with the same condition. An understanding of the molecular basis for this heterogeneity could lead to improvements in monitoring and treatment. During chronic infection the process of T cell exhaustion inhibits the immune response, facilitating viral persistence. Since persistent antigen is targeted in both autoimmunity and chronic infection, similar pathways could dictate the success of that response. However, whereas a successful response to pathogen results in clearance, a robust response to self-antigen might drive relapsing autoimmunity. We show that a common transcriptional signature reflecting high levels of T cell exhaustion predicts poor viral clearance (HIV), but conversely predicts better prognosis in multiple autoimmune diseases. In autoimmunity, we found that where evidence of CD4 costimulation was pronounced, that of CD8 exhaustion was reduced. We used this costimulation signature to identify signals preventing the development of T cell exhaustion in vitro, and showed that inhibitory signalling through PD-1 can partially reverse that effect. Robust detection of a costimulation signature in independent datasets comprising 1145 samples from 504 individuals confirmed an association with outcome or response to therapy in infection (dengue virus, hepatitis C virus (HCV)), vaccination (yellow fever, malaria, influenza) and autoimmunity (type 1 diabetes (T1D), anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV), systemic lupus erythematosus (SLE) and idiopathic pulmonary fibrosis (IPF)). We have shown that T cell exhaustion plays a central role in determining outcome in autoimmune disease and that targeted manipulation of this process could lead to new therapeutic opportunities.

W.40. C1q Suppresses the Production of Cytokines through LAIR-1 Engagement in TLR9-triggered Human Monocytes

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Systemic lupus erythematosus (SLE) is a chronic autoimmune disease that leads to progressive loss of tolerance to self antigens. Monocytes are a key component of the innate immune system involved in the regulation of the adaptive immune response, are efficient producers of multiple inflammatory cytokines by pathogen-associated molecule patterns but they are inappropriately activated in SLE. We have previously reported that Leukocyte-associated Ig-like receptor-1 (LAIR-1), when engaged by C1q, acts to block the differentiation of DCs from monocytes and to inhibit the production of IFNα in CpG-stimulated pDCs. In this study, we demonstrate that CpG-challenged monocytes also can be suppressed by C1q acting through LAIR-1. We observed that the stimulation of LAIR-1 by C1q or anti-LAIR-1 monoclonal antibody resulted in less induction of type I IFN, interleukin-6 and TNFα and less nuclear translocation of Interferon Regulatory Factors (IRF-5 and IRF-3) compared to CpG stimulation alone. C1q-mediated suppression was decreased by LAIR-1 siRNA. C1q collagen-like tail which contains the binding region for LAIR-1, suppressed cytokine production as well. Interestingly, LAIR-1 was associated with the inactive form of Src tyrosine kinase Hck following CpG stimulation. When LAIR-1 was engaged by C1q or anti-LAIR-1 antibody, the interaction between Hck and LAIR-1 was not observed but an interaction between Src homology 2 domain-containing protein tyrosine phosphatase 1 (SHP-1) and LAIR-1 was observed. These studies provide a better understanding of the molecular mechanisms by which LAIR-1 and C1q maintain immune quiescence.

F.28. Identification of Candidate *cis*-Regulatory Elements Governing *Aire* Expression

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The autoimmune regulator (AIRE) protein is critical to immune tolerance; loss-of-function mutations in *AIRE* lead to autoimmune polyendocrine syndrome type 1 (APS1). *Aire* is expressed by two rare cell populations: medullary thymic epithelial cells (mTECs) and extrathymic *Aire*-expressing cells (eTACs), which are located in the spleen and lymph nodes. In these cells, Aire promotes expression of tissue-specific proteins, such as insulin. Display of peptides from these proteins permits interaction with autoreactive T cells specific for these antigens, leading to their death, inactivation, or conversion to regulatory T cells. The regulation of *Aire* expression remains poorly understood and the critical *cis*-regulatory elements (CREs) remain wholly undefined. We previously generated an *Aire*-recapitulating GFP-reporter BAC transgenic mouse, thereby defining a 180 kb region containing all essential CREs. Using sequence conservation analysis and chromatin immunoprecipitation and high-throughput sequencing (ChIP-seq) we have identified several candidate *Aire* CREs in this region. We have begun to functionally assay these candidates. We have profiled a CNS several kb away from *Aire*. We identified putative NF-κB binding sites in this CNS and show p50 and p52 binding using EMSA. Using *in vivo* transgenesis, we find this CNS is necessary but not sufficient for *Aire* reporter expression in both mTECs and eTACs.

F.29. Sodium Chloride Induces Human Regulatory T Cell Dysfunction

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In understanding that the pathogenic niche of autoimmune disease is the result of both a hyper-inflammatory and dysregulated immune response, we were curious as to the impact of high-salt on the regulatory T cell (Treg) subset. We have recently reported that increased sodium chloride (high-salt) concentrations significantly enhance Th17 induction. Such changes are mediated by the serum/glucocorticoid-regulated kinase (SGK1), which causes downstream activation of both p38/MAPK pathway and nuclear factor of activated T cells 5 (NFAT5) during cytokine-induced Th17 polarization. Recent data have demonstrated that similarly increasing sodium chloride concentrations by 40mM renders Tregs functionally dysregulated in standard *in vitro* assays; high-salt Tregs lack their suppressive capacity. Such functional changes are likely secondary to activation of similar pathways causing Tregs to shift towards a pro-inflammatory and effector-cell-like phenotype. In line with these data, human Tregs are unable to control xenogeneic graft versus host disease in vivo, induced by the adoptive transfer of PBMC into immune deficient mice placed on a high-salt diet. These *in vitro* and *in vivo* results demonstrate a putative role for sodium chloride in mediating a dysfunctional and pro-inflammatory environment. Given that Western society is on a high-salt diet unless specific and severe dietary restrictions are implemented, we suggest that high dietary salt may function as an environmental trigger for autoimmunity in a genetically susceptible host. Thus, these data could in part provide a mechanistic explanation for the increasing rates of autoimmune diseases in the Western world.

F.30. *Nitric oxide synthase 2* is Down-regulated by *Retinoic Acid* During Behçet's disease: A Study in Algerian Patients

Zineb Djeraba[1], *Djemouai Boumedine*[2], *Zahira Chikh-Salah*[2], *Amina Arroul-Lammali*[1], *Oussama Medjeber*[1], *Fatmazohra Djaballah-Ider*[1], *Houda Belguendouz*[1], *Fifi Otmani,*[2] ***Chafia Touil-Boukoffa***[1]. [1]*University of Sciences and Technology (USTHB), Laboratory of Cellular and Molecular Biology (LBCM), Biological Sciences Faculty, Algiers, Algeria;* [2]*Mustapha Bacha Hospital, Algiers, Algeria* One of the chronic inflammatory disorder highly observed in the Mediterranean basin, including Algeria, is known as Behçet's disease (BD). This dreaded disease is characterized by recurrent oral and genital ulcerations, skin lesions, uveitis and which frequently affects joints, gastrointestinal tract, vascular and central nervous systems. Even if many hypotheses have been advanced in pathogenesis of BD, its specific etiology remains uncertain. The

involvement of nitric oxide (NO) during chronic inflammation of BD has been suggested by higher levels of its metabolites observed in patients' sera. The aim of our study was focused on the effect of the most potent active metabolite of vitamin A: retinoic acid (RA), on NO pathway in Algerian BD patients. For that purpose, PBMCs isolated from active (*n*=19) and inactive (*n*=22) BD patients and healthy controls (*n*=15) were cultured with different concentrations of RA. After 20 h of incubation, cells were harvested for immunostaining analysis of nitric oxide synthase 2 (NOS2) and NF-kB activity. Culture supernatants were used for NO estimation with the Griess method. Our results showed a significant production of NO in active BD compared with the inactive stage and healthy controls (all $p<0.0001$). Treatment of PBMCs cultures with RA exerts inhibitory and dose-dependent effect on NO production in BD both in active and inactive stages ($p<0.0001$). We observed that NOS2 expression and NF-kB translocation were also inhibited. In conclusion, RA down-regulates NO production in BD patients, probably through NF-kB pathway, suggesting that RA could be considered as a promising therapy for BD.

F.31. Vasculitis Treatment Intervention through Intravenous Immunoglobulins

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IVIG contains pooled IgG immunoglobulins extracted from the plasma of blood donors, and was initially used to treat immunodeficiencies. The exact mechanism of IVIG's immunomodulatory effects for the vasculitic syndromes is unclear. Proposed hypotheses include the clearance of anti-idiotype antibodies, blockade of Fc receptors on phagocytic cells, downregulation of T- and B-cell function, and anticytokine effects.There were suggestions that IVIG acquires its anti-inflammatory activity from sialylation of the Fc core polysaccharide.IVIG has been used for the treatment of polyarteritis nodosa and Henoch-Schönlein purpura. However, the role of IVIG for the treatment of other forms of systemic vasculitis has not been clearly defined. Wegener's granulomatosis (WG), small-vessel vasculitides are frequently associated with antineutrophil cytoplasmic autoantibodies (ANCAs) and for these diseases Corticosteroids in combination with immunosuppressants are given.However, when therapy is discontinued, relapses are common. But in small prospective studies on persistent ANCA-associated vasculitides, complete or partial responses were obtained in 45–75% of the patients given IVIG alone or in combination with other drugs . Moreover, IVIGs have an excellent therapeutic/side-effects index. Comparatively IVIG is a very good therapeutic option for treaing vasculitis.So in this article we are presenting a review about the safety and efficacy of intravenous immunoglobulins and its usefulness in treating various vasculitis patients and in future we are going to prove the mechanism of action of IVIG in vasculitis intervention through our research.

F.32. Antigen Specific T-cells: Tipping the Balance from Effector to Regulatory

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Antigen specific T-cell responses directed against self-peptides are central to the development of many autoimmune disorders. In the non-obese diabetic (NOD) mouse model of spontaneous autoimmune diabetes, the insulin B-chain peptide consisting of amino acids 9-23, is a primary autoantigen. Previously, we employed a structure-based small molecule strategy to identify molecules capable of altering antigen presentation and resultant T-cell reactivity. One compound, glyphosine, enhanced IL10 production *in vitro* and *in vivo* by B:9-23 peptide stimulated splenocytes and prevented NOD diabetes. We assessed the tolerogenic properties of glyphosine on T-cell homeostasis using the insulin B:9-23 CD4 T-cell transgenic mouse, BDC12-4.1 $Rag^{-/-}$. Treatment with glyphosine or vehicle (n=8) for three weeks resulted in significant increases in regulatory $CD4^{+}CD25^{+}Foxp3^{+}$ T-cells in the spleen (5.5+1.0% vs 0.25+0.1%, p=0.0001) and pancreatic lymph nodes (14.5% vs. 1.5%, p=0.002) of treated mice. There was a decrease in effector ($CD44^{hi}CD62L^{lo}$) cells in the treated mice, suggesting a change in insulin specific CD4 T-cell phenotype with glyphosine therapy. Furthermore, cytokine secretion measured by enzyme-linked immunospot assay revealed more IL10 and less IFN-ɣ secreting T-cells in treated mice. Similar results were observed in the NOD mouse model. Analyzing pancreatic islet infiltrating cells from NOD mice (n=5), the number of insulin specific CD4 T-cells stained by tetramer decreased fourfold in treated mice compared to controls. In conclusion, glyphosine therapy converts insulin specific effector T-cells into Tregs with ability to inhibit T-cell targeting of pancreatic islets. Our findings have broad relevance to modulating antigen specific T-cell responses for the treatment of autoimmunity.

F.33. Corticosteroid Resistant Th17 Cells are Sensitive to Calcineurin Inhibition

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The first-line therapy for uveitis, which is a leading cause of blindness, is corticosteroids. However, up to a third of patients fail to achieve disease remission, and these so-called steroid refractory (SR) patients are commonly clinically rescued with calcineurin inhibitors such as cyclosporine A (CsA). Recent reports have suggested that $CD4^+$ cells expressing IL-17A are SR, and we therefore hypothesised that these Th17 cells are susceptible to calcineurin inhibition. To test this, Th17 cells were derived from FACS sorted $CD4^+CCR6^+$ cells from SR and steroid sensitive (SS) patients, and healthy volunteers (HV) (23 donors in total). Unpolarised CD4+CCR6- Th0 cells were used as controls. *Ex vivo* Th17 cultures from SR, SS and HV donors all exhibited a restricted response to corticosteroids in terms of intracellular cytokine (IL-17 and IFN-γ) and gene expression (RORC, Tbet, IL-17 and IFN-γ), whereas CsA potently inhibited Th17 cells compared with Th0 controls. This corticosteroid-resistant and CsA-sensitive Th17 phenotype was further confirmed at a genome-wide level (PCA of 24 Affymetrix U133 2.0 GeneChips). This was despite equivalent glucocorticoid receptor isoform expression (assessed by PCR) and nuclear translocation (quantified by confocal microscopy). Concordant results were obtained *in vivo* using the murine experimental autoimmune uveitis model, whereby retina infiltrating Th17 cells escaped corticosteroid suppression, but were inhibited by CsA in terms of both intracellular cytokine and gene expression. We therefore propose that Th17 cells are candidate targets for selective CsA therapy in human SR autoimmune diseases.

F.34. The Reciprocity of IL-17 and IL-10 in Corticosteroid Resistant Uveitis

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Th17 cells have been implicated in the pathogenesis of several autoimmune disorders, primarily through production of IL-17A and IL-22. Corticosteroid therapy remains the primary treatment approach for these conditions, but around 30% of the population are unresponsive to this therapy and hence classified as steroid resistant (SR). We have previously identified a subpopulation of steroid refractory $CD4^+$ T cells in SR patients with the autoimmune eye disease uveitis. Following recent reports which suggest that Th17 cells are refractory to corticosteroid treatment, we examined the intra-cellular expression of IL-17A and IFN-gamma from SR and steroid sensitive (SS) patients (N=25). This confirmed that upon activation $CD4^+$ T cells from SR patients show significantly greater expression of IL-17 compared to SS controls, and this IL-17 expression was not inhibited by the synthetic corticosteroid dexamethasone (Dex). However, corticosteroids have wide ranging effects on $CD4^+$ T cells; key among these is enhancement of IL-10 production *in vitro*. We therefore concurrently interrogated the effect of Dex on intracellular IL-10 expression following TCR ligation with α-CD3/α-CD28. This demonstrated that the ratio of IL-10 to IL-17 was significantly reduced in $CD4^+$ T cells from SR patients which exhibited a more pathogenic, IL-17 profile than the IL-10 dominant SS patients. We therefore conclude that $CD4^+$T cells from SR patients are biased to express IL-17 and fail to induce IL-10 in response to corticosteroid treatment.

F.35. Untreated Juvenile Dermatomyositis Muscle: Downregulation of miR-10a is Associated with Increased Pro-inflammatory Cytokines, von Willebrand Factor Antigen, Disease Activity Score, and Earlier Diagnosis

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Objective: To identify the association of miRNA in muscle biopsies (MBx) from untreated children with JDM with inflammatory cytokines and clinical variables. Methods: 18 children with definite JDM (14 White, 4 Hispanic, 10 ♀) and 6 controls enrolled: 8 with short duration untreated disease (DUD) ≤3 months, 10 with long DUD >3 months. miRNA profiles in muscle (Exiquon), and plasma levels of IL- 6, IL-8, TNF-α, MCP-1, VCAM-1, and IL-1β (Mesoscale) were determined. Clinical variables: disease activity scores (DAS, skin, muscle, total score), von Willebrand factor antigen (vWF:Ag), DUD, and the TNF-α-308 A allele. Results: 19 differentially expressed miRNAs were identified. The top miRNAs were: miR-10a and miR-10b with fold changes of -1.96 (p=0.0028), and fold change of -1.62, (p= 0.0027), respectively. miR-10a and miR-10b regulate NFκB pathway, controlling inflammatory cytokines. Plasma levels of IL- 6, IL-8, TNF-α, MCP-1, VCAM-1, but not IL-1β, were increased in untreated JDM vs controls. miR-10a, but not miR-10b, was associated with: a short DUD ≤3 mo. (p=0.014); TNF-α-308 A polymorphism (p=0.006) and increases in both DAS Total (p=0.03) and vWF:Ag level (p=0.004). Conclusion: We conclude that miR-10a downregulation is critical in early JDM pathophysiology fostering inflammatory cytokines production. Downregulation of miR-10a, but not miR-10b, was associated with increased vWF:Ag, indicating damaged endothelial cells and a more severe disease onset with a higher DAS, contributing to earlier diagnosis and a shorter DUD. We speculate that miR10a may be a potential therapeutic target early in JDM disease course.

F.36. Radioiodine Treatment in Graves' Disease Patients Induces Changes in Frequency of Peripferal Regulatory T Cells and Natural Killer (NK) T Cells

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Graves' disease (GD) involves autoimmunity against thyrotropin receptor (TSHR) bearing cells, leading to hyperthyroidism and often orbitopathy. When hyperthyroidism is treated with radioactive iodine (RAI), exacerbation of the orbital disease can occur. We hypothesized that RAI has immune effects affecting the balance between auto-reactive T cells and T cells with regulatory properties. We monitored lymphocyte populations in peripheral blood of GD patients, patients with non-autoimmune goiter (NG), and healthy controls. Circulating T cell interferon gamma production in the presence of TSHR peptides was measured in 10 GD patients and 10 healthy controls. Significant response to at least one peptide was measured in 2/10 and 4/10 GD patients before and after RAI therapy, respectively, and in none of the controls. Regulatory $CD4^+CD25^{high}FOXP3^+$ T cells (Tregs) and $V\alpha24^+V\beta11^+CD3^+$ Natural Killer T cells (NKT) were counted by flow cytometry in 16 GD patients and in 5 NG patients before and one month post-RAI treatment, as well as in 7 untreated healthy subjects over the same time period. Variance of Tregs and NKT cells before and after RAI therapy was greater in GD patients compared to NG (Treg: p=0,0053; NKT : p=0,0117) and to controls (Treg : p <0,0001; NKT: p=0,0217). Post-RAI therapy, frequency of Tregs was positively correlated with NKT cells numbers in GD patients (p=0,0422). Collectively, RAI therapy has a mild effect on auto-reactive T cells specific to thyroid peptides. However, variation in Tregs and NKT cells after thyroid radiation appears to be greater in Graves' disease patients.

F.37. $CD14^{++}CD16^+$ Monocytes are Enriched in Inflammatory Diseases and Promote the Sensitivity of T Cells to Corticosteroids

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The phenotype of $CD4^+$ T cells is in part influenced by interactions with monocytes. However, little is known about the impact of monocyte subsets on T cell differentiation and how this may be skewed under inflammatory conditions or influenced by corticosteroid treatment. We therefore used fluorescent activated cell sorting to obtain $CD14^{++}CD16^-$ and $CD14^{++}CD16^+$ monocytes from healthy volunteers (n = 14) and co-cultured each of these subsets for 5 days with CFSE labelled memory $CD4^+$ T cells from the same donor. This demonstrated that $CD14^{++}CD16^+$ monocytes drove less T cell proliferation (by CFSE dilution, 29% v 38%; p=0.009), less IL-17 expression (9% v 13%; p<0.001) but similar IFNγ production (29% v 26%), compared to $CD14^{++}CD16^-$ monocytes. In separate experiments, each monocyte subset was cultured alone for 24 hr with the synthetic corticosteroid dexamethasone prior to T cell co-culture. T cell proliferation and IFNγ production was abrogated to a greater degree in co-cultures with dexamethasone treated $CD14^{++}CD16^+$ monocytes than with dexamethasone treated

$CD14^{++}CD16^{-}$ monocytes (59% v 37%, p=0.04; 54% v 20%, p=0.04, respectively). The proportions of each monocyte subset were then quantified in the peripheral blood of patients with non-infectious uveitis (n = 98) and alcoholic hepatitis (n = 15). $CD14^{++}CD16^{+}$ monocytes were enriched in both conditions. We therefore conclude that $CD14^{++}CD16^{+}$ monocytes, which are expanded in the peripheral circulation of patients with inflammatory diseases, have a reduced capacity to drive T cell proliferation and IL-17 production. Furthermore, they also promote greater T cell corticosteroid sensitivity than $CD14^{++}CD16^{-}$ cells.

F.38. High Salt (NaCl) Reduces the Activation of Alternatively Activated (M2) Macrophages via Epigenetic Modifications

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High intake of dietary salt (sodium chloride; NaCl) is attributed to the development of cardiovascular disease, and has been proposed to be a cause for the rapid increase in autoimmune diseases in western civilisations. We have recently shown that NaCl has a pro-inflammatory effect and boosts the activation of Th17 cells *in vitro,* with mice fed a high salt diet having an accelerated and more severe experimental autoimmune encephalomyelitis. In this study, we examine how the activation of alternatively activated (M2) macrophages is affected by NaCl. In stark contrast to our study with Th17 cells, we find that high salt dose-dependently decreased M2 activation in IL-4+IL-13 stimulated BM-derived mouse macrophages. Genes important for M2 activation including Mrc1, Arg1, Ym1, Fizz1 and PD-L2, all had a blunted expression in the presence of NaCl; an effect which was not observed in tonicity controls (mannitol or urea), implying a specific action of NaCl. Transcription factors important for modulating M2 function (Irf4 and Klf4) were similarly affected. To explore the mechanism for the effect of NaCl on M2 activation we performed gene expression analysis simultaneously with genome wide epigenetic modification analysis (ChIP-seq for H3K4me3 and H3ac). The results of this revealed that NaCl modulates epigenetic marks at genes important for M2 activation, and additionally identified new genes which were affected. Our study reveals a novel effect of NaCl on M2 activation and gives support to the notion that the modulation of immune cell function by high dietary salt is relevant to autoimmune disease.

F.39. Infiltrating Th1/Th17 Cells Predominate in Hashimoto's Thyroiditis

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Hashimoto's thyroiditis (HT) is an autoimmune thyroid disease characterized by lymphocyte infiltration, fibrosis, and parenchymal atrophy of the thyroid gland. However, little is known about the role of infiltrating T-cells in the etiopathogenesis of HT. The aim of the study was to characterize the phenotype and function of isolated T cells (Th1, Th17 versus regulatory T cells, Tregs) from the thyroid gland. Thyroid tissue and peripheral blood mononuclear cells (PBMCs) were obtained from seven HT patients who underwent thyroidectomy. After in vitro expansion, the cytokine production profiles of isolated infiltrating T-cells and PBMCs were assessed by flow cytometry following unspecific stimulation. Cell surface markers, chemokine-ligand 20 (CCL20) and Treg transcription factor FoxP3 were analyzed by immunohistochemistry. Infiltrating cells were mostly $CD4^{+}$ T-cells with a predominant memory-effector phenotype $CD4^{+}CD45RO^{+}CD27^{+}$. Infiltrating $CD4^{+}$ T-cells up-regulated the expression of chemokine-receptors CCR5 (5.5%), CCR6 (9.6%) and CXCR3 (9.3%), producing high amounts of IFNgamma (41.3%) and IL-17 (4.0%) compared to circulating $CD4^{+}$ T-cells. High numbers of $CD4^{+}CD25^{+}CD127^{-}FoxP3^{+}$ (Tregs) were found in infiltrating cells (2.9%) compared to peripheral blood. Immunohistological analysis of the thyroid gland demonstrated the abundance of $CD4^{+}CD45RO^{+}CD27^{+}$ T-cells, the expression of CCL20 and expression of FoxP3. Our findings suggest that infiltrating T-cells, possibly driven by chemokine-receptors, belong to the Th1/Th17 effector T-cell phenotype producing high amounts of IFNgamma and IL-17. The presence of Tregs within inflammatory tissue may indicate a role of these cells in modulating inflammation, but it has to be proven whether infiltrating Tregs are functionally active on suppression of Th1/Th17 responses.

F.40. Promotion of STAT1 and STAT4 Expression by Estrogen Receptor α Suggests a Novel IFNα Independent Mechanism of Systemic Lupus Erythematosus Pathogenesis

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Systemic lupus erythematosus (SLE) is a devastating autoimmune disease displaying an overwhelming female predilection. Estrogen (E2) influence has been suggested in SLE pathogenesis and E2 has been shown to regulate Signal Transducer and Activator of Transcription (STAT) 1 and STAT4 function. STAT1 is located just upstream of STAT4 and ChIP-seq data revealed an intragenic estrogen response element binding peak within the STAT1 locus with E2 treatment of MCF7 cells, suggesting that estrogen receptor α (ERα) may be promoting the expression of both genes. To demonstrate E2-mediated induction of STAT1 and STAT4, primary human peripheral blood mononuclear cells (PBMCs) from SLE patients and healthy subjects were treated with a physiological dose of E2. Expression of STAT1 and STAT4 was significantly induced relative to untreated controls. Given that we have previously observed that toll-like receptor 8 (TLR8) expression is higher in SLE and induced with E2 treatment, a *bona fide* STAT1 binding region proximal to the TRL8 gene was used in EMSA analysis and showed enhanced DNA-protein complex formation with E2 stimulation. Moreover, siRNA blocking STAT1 significantly reduced E2-mediated TLR8 induction. In primary human cells and hematopoietic cell lines, siRNA blocking ERα inhibited STAT1 and STAT4 induction with E2 stimulation, while targeting IFNα had no effect. Thus, E2 can stimulate the expression of STAT1 and STAT4 in PBMCs through ERα transcriptional enhancement and subsequently could contribute to SLE pathogenesis. These results suggest novel therapeutic opportunities in targeting E2/ERα-induced STAT1 and STAT4 signaling in the treatment of SLE.

F.41. Selectivity of Toll-like Receptor Stimulation in Human B Cells

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B cells activated by nucleic-acid sensing Toll-like receptor (TLR) 7 and TLR9 proliferate and secrete immune globulin (Ig). Memory B cells are more responsive due to higher TLR expression levels, but further selectivity remains largely unknown. In this study, human B cells stimulated by TLR ligands, with or without IFNα, or by coincubation with CD40L and IL-21were examined to identify differentially responsive subsets. These were defined phenotypically (as naïve, IgM memory, or class-switched memory) or functionally (based on secreted Ig isotype and binding profiles of secreted Ig).

Both stimuli induced CD27hiCD38hi plasmablasts, with TLR ligands inducing more IgM+ plasmablasts and IgM+ CD27hiCD38lo pre-plasmablasts than CD40L/IL-21. TLR stimulation also increased the relative proportion of IgM+ memory B cells, while CD40L/IL-21 expanded class-switched memory cells.

TLR stimulation promoted increased IgM secretion relative to CD40L/IL-21, while the latter led to higher levels of secreted IgG. Binding profiles of secreted antibodies were tested using multiplexed protein microarrays for both IgM and IgG isotypes. TLR stimulation induced antibodies recognizing carbohydrates, such as blood group antigens; autoantigens, including insulin and nuclear antigens; and pathogens, including influenza and measles, for both tested isotypes. In contrast, antibodies secreted following CD40L/IL-21 stimulation were largely non-reactive with the 60 autoantigens tested, showing only IgG recognition of pathogen antigens.

Human B cell subsets from healthy donors show differential responsiveness to TLR stimulation, including autoantibody secretion following TLR stimulation. These autoantibodies might serve as protective natural antibodies and help to maintain homeostasis.

F.42. CD146 Expressing T-Cells are Elevated in Numerous Human Auto-Immune Disorders may bt the Major Source of IL-17a Producing Cells at the Sites of Inflammation

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IL-17 has been implicated in the pathogenesis of various autoimmune and inflammatory diseases in humans and has been demonstrated to be secreted by both CD4 T-cells (Th17) and CD8 T-cells (Tc17). CD146 is also expressed on a low percentage (2-4%) of circulating T cells in healthy individuals. CD146, an endothelial adhesion molecule, facilitates binding of lymphocytes to endothelium. It is not known why CD146expression occurs on T cells, however, these cells have been found in elevated numbers in patients with inflammatory autoimmune diseases such as Crohn's disease, ulcerative colitis, Behcet's disease, sarcoidosis, rheumatoid arthritis, multiple sclerosis, and psoriasis.

We found that these cells display multiple features of Th17 or Tc17 phenotype and are capable of secreting signature cytokines without any polarization. CD146+ T cells are pronounced at the sites of inflammation in autoimmune diseases such as in the synovial fluid in RA and in skin lesions in psoriasis and in the latter are the major producers of IL17, suggesting that these cells play a crucial role at the sites of inflammation.

We conclude that CD146+CD4+T-cells are elevated in human autoimmune and inflammatory diseases. CD146 expression appears to facilitate the migration of T cells to the sites of inflammation is a component of Th17 and Tc17 responses. Given that healthy donors maintain small pool of these cells in the peripheral circulation, which become elevated during active disease, it could be speculated that these cells might act as early mediators/responders in IL17-mediated inflammation.

F.43. Protein Kinase C (PKC)-θ-Selective Inhibitor R683 Blocks TH17 Accumulation in the CNS and Suppresses EAE in Mice

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PKC-θ is a key signaling molecule that is critical for T cell activation, proliferation, and cytokine production, making it an attractive target for therapeutic intervention in autoimmune and inflammatory diseases. High-throughput screening and optimization for suppression of TCR-induced IL-2 production in human primary T cells generated a series of small molecule inhibitors resulting in 2 potent and specific compounds: R896 and R683. Both compounds are highly efficacious in acute (SEB-induced IL-2, DTH) and chronic disease (AIA, transplant rejection, EAE) rodent models when administered orally, highlighting the central role of PKC-θ in T cell-dependent pathogenic processes. Here, we explore the effect of R683 on T cells in vivo, during active EAE. At 5 time-points around the peak of clinical disease, mice from R683-treated or vehicle control groups were sacrificed, and CNS (brain and spinal cord), peripheral lymph nodes, and spleen were harvested. Mononuclear cells were isolated and stained for TH17 markers ($CD4^+IL\text{-}17A^+$) or Treg markers ($CD4^+FoxP3^+$). R683 treatment reduced the total influx of $CD4^+$ T cells into the CNS during EAE. We found that R683 greatly reduced the percentage and number of TH17 cells at disease onset, while it had a moderate effect on Treg cells, which increase in percentage and number during peak disease (prior to remission). Thus, the efficacy of R683 in preventing EAE correlates with its ability to reduce TH17 cells in the CNS.

F.44. Aire-Dependent Negative Selection of Insulin-specific T Cells

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Type I diabetes (T1D) is characterized by the destruction of pancreatic islet cells primarily by $CD4^+$ and $CD8^+$ T cells that escape mechanisms of self-tolerance. Autoimmune regulator (Aire) promotes the expression of peripheral self-antigens within the thymus, and is important for the negative selection of autoreactive T cells. In humans with T1D and in the NOD mouse

model of the disease, an insulin peptide (insB:9-23) is a major autoantigen detected by pathogenic T cells, and Aire controls insulin expression in the thymus. Still, NOD.*Aire*$^{-/-}$ mice are resistant to TID, and there is little direct evidence to support our hypothesis that Aire influences the development of diabetogenic T cells. To test this, we measured the frequency of insulin-reactive T cells in the polyclonal T cell population after immunization. We detected an increase in the frequency of peripheral insulin-specific T cells in insulin-deficient mice that express a non-immunogenic insulin transgene, and a modest increase of insulin-specific T cells in Aire-deficient mice. These results suggest that Aire has a partial role in deletion of insulin-reactive T cells. To extend this observation, we analyzed T cell development in the absence of insulin or Aire using a novel transgenic mouse that expresses a T cell receptor that recognizes insB:9-23, and found evidence that Aire contributes to the deletion of a pathogenic T cell clone. These results provide insight into the basic mechanism of insulin-specific T cell deletion.

F.45. Insights into the Specificity of BPI and BPIFB1 Autoantibodies in Lung Disease

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Bactericidal/Permeability Increasing (BPI) Protein is a component of azurophilic granules of neutrophils and is expressed on epithelial surfaces of the respiratory tract in humans. It functions as part of the innate immune system by binding to gram negative bacteria, leading to bacterial death. BPI and related proteins have had their nomenclature adjusted with the broader term 'BPI-fold' containing proteins or BPIF. One of these, BPIFB1 (with two active domains and high level expression along respiratory mucosal surfaces) is of particular interest as patients with Cystic Fibrosis (CF) have upregulated levels[1] and some individuals with idiopathic Interstitial Lung Disease (ILD) develop autoantibodies to it[2]. These findings have been suggested to have aetiological and diagnostic implications. We have assessed sera of 47 patients with ILD, 3 of which were positive and 41 patients with CF, 14 which were positive for IgG BPI autoantibodies. These results suggest that autoantibodies to BPI are neither causative nor diagnostically specific. To ensure that this interpretation applies also to BPIFB1 autoantibodies (BPI and BPIFB1 are structurally homologous) as originally described by *Shum et* al[2], a competitive inhibition ELISA with BPIFB1 as the inhibitor was developed. We propose that development of antibodies to BPIFB1 are epiphenomena to chronic lung inflammation in some individuals, with these autoantibodies able to cross-react with the structurally related BPI found in neutrophils and are not in fact a sero-specific marker of a subset of patients with ILD.

F.46. Model of Autoimmune Liver Disease Using Precision Cut Liver Slices Incubated With Ethanol

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Recent studies have shown that precision cut liver slices (PCLSs) maintain viability and biological function for 72-96 hours *in vitro*. Therefore, PCLSs were prepared and served as both immunogens and targets in order to determine the effects immune responses on the liver following the metabolism of ethanol. Mice were immunized once a week intraperitoneally for 5 weeks in the absence of adjuvant with 100 μg of whole cell homogenates from PCLSs (Immunogens). PCLSs or whole cell homogenates (WCH) of PCLSs from chow-fed mice served as targets in the assays performed. Splenocytes were fractionated to $CD4^+$ and $CD8^+$ T cells. Serum antibodies (100 μl/well), $CD4^+$ or $CD8^+$ T lymphocytes were evaluated for activity by standard ELISA, proliferation and cytotoxicity assays. In these studies it was shown that antibodies to WCH proteins from control PCLSs were detected in the serum from mice immunized with WCH that had been incubated in the presence of EtOH. $CD4^+$ T helper cell responses from these same mice were significantly increased ($p < 0.05$) in response to Control PCLS WCH and EtOH PCLS WCH as the stimulating antigen. Additionally, the CD8+ T cytotoxic cell responses from these mice showed a significant 2-3 fold increase in cytotoxicity (~60%) on intact Control PCLSs. Therefore, this system appears to be capable of generating modified self proteins for use as immunogens, and providing a source of viable tissue as targets for studying the role of the immune system in the development of liver damage.

F.47 Reversal of Autoimmune Hepatitis Induced by Anti-CTLA4 Using Teplizumab in Humanized Mice

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Immunodeficient mice reconstituted with human stem cells (humanized mice) can mount adaptive immune responses that mimic human immune responses. We have found that humanized mice treated with anti-CTLA4 antibody (ipilimumab) develop autoimmune disease characterized by hepatitis, adrenalitis, sialitis, ANAs, and weight loss. This induction of autoimmunity involved activation of T cells and cytokine production and increased T cell – antigen presenting cell interactions. When anti-CTLA-4 mAb treated mice were co-treated with anti-CD3 mAb (teplizumab) hepatitis and ANAs were no longer seen and weight loss did not occur. The anti-CD3 blocked proliferation and activation of T cells that was induced with anti-CTLA-4 mAb. Macrophage infiltration into the liver was reduced as well as the production of IP-10, IFN-γ and TNF in the liver. In addition, there was an increase in the proportion of CD8 central memory T cells in liver and mesenteric lymphnodes reminiscent of finding in clinical responders to the drug among patients with Type 1 diabetes. We also found increased levels of . Tregs (CD25+CD127-) in the spleen and mesenteric lymph nodes in the mice treated with both antibodies and greater constitutive phosphorylation of STAT5 in spleen cells compared to mice treated with anti-CTLA-4 mAb alone. We conclude that humanized mice may be useful for understanding the mechanisms of biologics that are used in patients. Anti-CD3 mAb was able to block the ability of anti-CTLA-4 mAb to induce T cell activation and autoimmune disease but did not inhibit T cell activation.

F.48. Nanoparticles Loaded with IL-2 and TGF-β Enhance the Generation and Stabilize CD4+Foxp3+ Regulatory T Cells from the Inhibitory Effects of Pro-inflammatory Cytokines

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The maintenance of peripheral tolerance and immune homeostasis is in large part mediated through the immunosuppressive properties of CD4+ regulatory T cells (Tregs). These cells turnover rapidly and require IL-2 and TGF-β for fitness and survival. In autoimmune diseases the production or responsiveness to these cytokines is impaired. Since both IL-2 and TGF-β are pleiotropic and have very short half-lives, systemic administration of these cytokines is toxic and inefficient. Therefore, a strategy to deliver sustained amounts of these cytokines in physiologic concentrations specifically to Tregs is a major unmet need. We demonstrate here a novel approach to adoptive therapy whereby regulatory factors are continually delivered to transferred Tregs in a paracrine fashion to reinforce a Treg phenotype. PLGA nanoparticles were loaded with TGF-β and IL-2 and were bound to the Treg membrane through CD4. Nanoparticle-mediated delivery enhanced the bioactivity of released cytokines relative to soluble cytokine. Nanoparticle-bound Tregs retained Foxp3 expression in the presence of inflammatory cytokines. In comparing the effects of retinoic acid, certain ppAR agonists and histone methylase inhibitors on stabilizing human CD4Tregs against the inhibitory effects of IL-1 and IL-6, NP (IL-2,TGF-β) was superior to the other agents. These studies indicate that nanoparticle-mediated paracrine signaling may be a useful approach for maintaining adoptively transferred Treg function as an autoimmune therapy.

F.49. Quick and Efficient Method of Deriving Antigen-Specific, T Cell Hybridomas and Their Phenotypic Characterization Using Major Histocompatibility Complex Class II Dextramers

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We recently created, next-generation major histocompatibility complex (MHC) class II tetramers, designated 'dextramers'. The dextramer reagents were helpful to enumerate the frequencies of autoreactive CD4 T cells *ex vivo* in several murine autoimmune disease models. Importantly, the detection-sensitivity of dextramers was at least five-fold higher than that could be achieved with tetramers. In this report, we describe a method of generating T cell hybridomas specific to two autoantigens namely, IA^s/myelin proteolipid protein 139-151, and IA^k/cardiac myosin heavy chain-α 334-352 using the corresponding dextramers. Using dextramers and a panel of antibodies, hybridoma clones obtained after primary fusions could be directly screened for antigen-specificity, and also for expression of CD3 and CD4 including T cell receptor (TCR) vβs by multicolor flow cytometry. The monospecific T cell hybridoma clones were then obtained by single cell sorting. We have also successfully optimized the conditions to generate T cell hybridomas using as few as $2x10^5$ antigen-specific CD4 T cells sorted by utilizing the dextramers and a cocktail of TCR vβs. Together, the use of dextramers permitted us to derive antigen-specific T cell hybridoma clones in less than 3 weeks, thereby, avoiding the lengthy and laborious/conventional screening procedures.

F.50. The Role of Anti-apolopoprotein A1 Autoantibodies in Cardiovascular Risk Stratification

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Autoantibodies to apolipoprotein AI (anti-ApoAI IgG) have been shown to be both markers and mediators of cardiovascular disease, promoting atherogenesis and unstable atherosclerotic plaque. Previous studies showed that high levels of anti-ApoAI IgGs are independently associated with major adverse cardiovascular events in patients with myocardial infarction. Autoantibody responses can be polyclonal and it is possible that more than one epitope may exist. In order to identify the specific immunoreactive peptides in ApoAI, we generated high purity ApoAI from human plasma, using thiophilic interaction chromatography followed by enzymatic digestion specifically at lysine or arginine residues. Immunoreactivity to the different peptides generated was tested by ELISA using serum obtained from patients with acute myocardial infarction and high titers of autoantibodies to native ApoAI. The immunoreactive peptides were further sequenced by mass spectrometry. Our approach successfully identified two novel immunoreactive peptides, recognized by the autoantibodies. Moreover, a short synthetic ApoA1 peptide with proper tertiary structure, and similar to the endogenous peptide, was shown to compete the endogenous epitope. We determined the diagnostic accuracy of patient immunoreactivity to this peptide for non-ST elevation myocardial infarction (NSTEMI) diagnosis on 132 consecutive patients presenting at the emergency room for acute chest pain. ROC curve analyses demonstrated that synthetic peptide was a significant predictor for NSTEMI diagnosis (AUC:0.64; p=0.01), and risk analyses demonstrated that high levels of immunoreactivity to the peptide was associated with a 7-fold risk of NSTEMI (OR:6.98, p=0.001). These findings may open innovative prognostic and therapeutic avenues potentially suitable to improve current cardiovascular risk stratification.

Genetics

1107. Genetic and Epigenetic Fine-Mapping of Causal Variants in Autoimmune Disease

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Genome wide association studies (GWAS) have identified genetic loci underlying human diseases and traits. However, the precise nucleotide changes and the mechanisms that cause heritable differences among individuals remain largely unknown. To identify and characterize the causal variants driving autoimmune disease risk, we leveraged a new generation of dense genotyping data and a novel algorithm for fine-mapping single nucleotide polymorphisms (SNPs) to explicitly predict for each individual SNP associated with 22 autoimmune diseases, the likelihood that it represents a causal mutation. We then integrated these data with transcription and *cis*-regulatory element annotations, which we derived by mapping RNA and chromatin state in key immune cell types, including $CD4^+$ T-cell subsets in resting and activated states, $FoxP3^+$ regulatory cells, $CD8^+$ T-cells, B-cells, and monocytes. We find that the causal variants in 88% of GWAS loci are noncoding, with a majority mapping to immune-cell specific enhancers, many of which transcribe enhancer-associated 'eRNAs' and increase histone acetylation upon immune activation. Candidate causal variants tend to coincide with nucleosome-depleted regions bound by master regulators of immune differentiation and stimulus-dependent gene activation, including IRF4, PU.1, NFKB and AP-1 family transcription factors. However, our analysis suggests that only a minority of causal variants directly alter cognate transcription factor binding motifs. Rather, most variants that confer disease risk, including those that alter gene expression, instead alter adjacent, non-canonical sequence determinants. This suggests that current models of gene regulation may be insufficient to capture the mechanisms of most causal mutations associated with complex genetic diseases.

1110A. Gene Targets of STAT5A and STAT5B in Human CD4 T cells

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The signal transducers and activators of transcription (STAT) family of proteins mediate a number of biological activities including immune cell regulation and responsiveness to growth factors. STAT5A and STAT5B are highly homologous proteins whose distinctive roles in human immunity remain unclear. In mice, their complete deficiency is lethal. In humans, STAT5B deficiency alone is characterized by chronic lung disease, growth failure, and autoimmunity associated with regulatory T cell (Treg) reduction, while STAT5A deficiency has not been reported. Although STAT5A and STAT5B peptide sequences share >90% similarity, their structural differences suggest non-redundant roles in gene regulation. To identify candidate targets of STAT5A and/or STAT5B, we performed chromatin immunoprecipitation then DNA sequencing on human $CD4^+$ T cells. Then, to validate our findings, we performed quantitative-PCR in *STAT5A* or *STAT5B* knock-down (KD) human $CD4^+$ T cells. Our data indicate that STAT5A and STAT5B are both involved in cell proliferation and apoptosis via *SGK1* interaction. Interestingly, STAT5A appears to bind to genes involved in neural development and function (*NDRG1*, *DNAJC6*, and *SSH2*), while STAT5B plays a distinct role in T cell development and function via binding to *DOCK8*, *SNX9*, *FOXP3* and *IL2RA*. Our results also suggest that co-activators for STAT5A and/or STAT5B may play important roles in establishing different binding abilities and gene regulation behaviors. The new identification of these genes regulated by STAT5A and/or STAT5B has major implications for our understanding of pathophysiology of cancer progression, neural disorders, and immune abnormalities.

OR.3. Molecular Mechanisms of ITGAM and Lupus Susceptibility: Combined Effects of Protein and DNA

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Integrin alpha M (*ITGAM*; CD11b) is a component of the macrophage-1 antigen complex, which mediates leukocyte adhesion, migration and phagocytosis as part of the immune system. We previously identified a missense polymorphism, rs1143679 (R77H), strongly associated with systemic lupus erythematosus (SLE). However, the molecular mechanisms of this variant are incompletely understood. A meta-analysis using published and novel data on 28,439 individuals with European, African, Hispanic, and Asian ancestries reinforces genetic association between rs1143679 and SLE ($P_{meta} = 3.60x10^{-90}$, OR = 1.76). Since rs1143679 is in the most active region of chromatin regulation and transcription factor binding in *ITGAM*, we quantitated *ITGAM* RNA and surface protein levels in monocytes from patients with each rs1143679 genotype. We observed that transcript levels significantly decreased for the risk allele ('A') relative to the non-risk allele ('G'), in a dose-dependent fashion: ('AA'<'AG'<'GG'). CD11b protein levels in patients' monocytes were also directly correlated to RNA levels. Strikingly, heterozygous individuals express much lower (average 10-15 fold reduction) amounts of the 'A' transcript than 'G' transcript. We found that the non-risk sequence surrounding rs1143679 exhibits transcriptional enhancer activity *in vivo* and binds to Ku70/80, NF-κB1 and EBF1 *in vitro*, functions that are significantly reduced in the risk allele. Mutant CD11b protein shows significantly reduced binding to fibrinogen and vitronectin, relative to non-risk, both in purified protein and in cellular models. This two-pronged contribution (nucleic acid- and protein-level) of the rs1143679 risk allele to decreasing *ITGAM* activity provides insight into the molecular mechanisms of its potent association with SLE.

W.11. Expansion of the IgD class switched B cell population in carriers of the autoimmune disease associated risk haplotype in *BLK*

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Polymorphisms in *BLK* are associated with numerous autoimmune diseases including rheumatoid arthritis and lupus. BLK (B lymphocyte kinase) is a tyrosine kinase involved in signaling through the B cell receptor (BCR). The disease risk haplotype is known to be associated with reduced expression of *BLK* transcript and the most significant association of reduced *BLK* transcript is with a candidate causal SNP rs922483. We have previously shown that expression of BLK is reduced in cord blood B cells from carriers of rs922483 risk alleles (HMG 2012). B cell development is perturbed in BLK transgenic and knockout mice, and B cells from these mice are hyper responsive to BCR stimulation. To determine if reduced BLK expression affects the function of primary human B cells we measured CD86 expression in cells stimulated with anti-IgM. As in mice, cord

blood B cells from risk allele carriers are hyper-responsive to BCR stimulation. We also assessed if BLK risk variants convey any variation on the composition of circulating B cell populations of healthy adults. We observed that subjects with BLK risk variants have an expansion in the proportion and number of IgD class-switched B cells, and an increase in the total number of class-switched memory B cells. These results suggest that BLK risk haplotypes confer autoimmune disease susceptibility by reducing a negative regulatory effect of BLK, making B cells more likely to receive help from CD4 T cells and to undergo class switch recombination.

Immune Monitoring

OR.11. Differential expression of TIGIT and FcRL3 surface markers accurately discriminates between Helios+ and Helios- human memory Treg cell subsets

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Foxp3$^+$ regulatory T cells (Treg) are critical mediators of self-tolerance and their absence results in severe multiorgan autoimmunity in humans and mice. Two subsets of Foxp3$^+$ Treg cells have been described based on the expression of Helios, a transcription factor of the Ikaros family. Efforts to understand the origin and respective roles of these distinct Treg populations in regulating immune responses have, however, been hindered by the paucity of reliable surface markers to distinguish and isolate these two subsets in humans. In this study, we show that the co-expression of T cell Ig and ITIM domain (TIGIT), and Fc receptor-like protein 3 (FcRL3) on memory CD4$^+$CD25$^+$CD127^{low} accurately identifies a highly enriched FOXP3$^+$Helios$^+$ population in peripheral blood directly *ex vivo*, in expanded primary FOXP3$^+$ clones of healthy individuals, and in human cohorts of inflammatory disorders. Conversely, the lack of TIGIT/FcRL3 expression on memory CD4$^+$CD25$^+$CD127^{low} identifies a Helios$^-$FOXP3$^+$population in similar settings. Importantly, this novel marker combination relieves the need for stringent CD25 gating whose inconsistency has long complicated analysis of Treg cells in human disorders. While Helios$^+$FOXP3$^+$ cells exhibit stronger suppressive abilities, Helios$^-$FOXP3$^+$ cells are the sole producers of the inflammatory cytokines IFN-gamma, IL-2 and IL-17, suggesting different functional roles for the two subsets. This study provides novel reliable surface markers to distinguish, monitor and isolate Helios$^+$ and Helios$^-$ Treg cells, thus enhancing our ability to isolate Treg cells with superior purity and consistency compared to conventional markers, and paving the way for further elucidation of Helios-based Treg heterogeneity.

OR.33. Large-scale and Comprehensive Immune Profiling and Functional Analysis of Normal Human Aging

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Age-associated immune changes have been reported; however, systematic efforts to study immunological mechanisms are lacking due to the absence of comprehensive and measureable immunological parameters for analysis of the functional status of immunity in varied settings including old age, health and disease. Thus, we embarked on a large-scale study to collect and analyze phenotypic information in multiple scales (epidemiological, clinical, anthropometric, and, at the cell and molecular level, of immune function) and proteomic and genomic information (gene and protein expression) of resting and activated peripheral blood mononuclear cells (PBMCs) and whole blood on healthy individuals between the ages of 40 and 90+ years. Specifically, we developed a 700+ person population-based database of healthy adults throughout 7 decades of life that included subjects' physical function, depression, frailty, medication and co-morbidities, and a CBC, metabolic profile, lipid panel and CRP. For 200+ of these participants, we used Luminex to measure cytokines in serum and supernatants from PBMCs stimulated with a stimulus cocktail as well as genechip analysis of RNA from these PBMCs (resting or stimulated) and whole blood. CyTOF was used to phenotype the different cellular compositions together with phosphoepitope flow cytometry to analyze cytokine-stimulated pathways. These heterogeneous results confirmed some known age-related changes while revealing novel findings regarding the changes in cytokine expression that are modulated upon stimulation of PBMCs, cellular composition changes in the blood, and how these changes associate with CMV infection status. Despite aging, a healthy immune system remains extremely robust on the molecular and cellular level.

W.18. Human T Cell Homeostasis in Humanized Mice

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In mice, T cell homeostasis is primarily governed by access to IL-7 as well as TCR interactions with self-peptide MHC on antigen presenting cells (APCs). In our humanized mouse model (human thymus + autologous human CD34+ cells: Hu/Hu), we achieve robust long-term human chimerism. T cell chimerism may partially reflect significant cross-reactivity with murine IL-7. While the MHC ligands responsible for positive selection of murine T cells have been shown to be the same ligands providing homeostatic TCR signals in the periphery, it is unknown if this model applies to human T cells. We have developed a humanized mouse model in which human T cells develop in a fetal porcine thymus (Sw/Hu). In this model, human T cells undergo positive selection on swine MHC ligands, but interact with human APCs in the periphery of the mice. We have obtained evidence for TCR-APC interactions in the spleen and lymph nodes of our Hu/Hu mice by upregulation of surface CD5 and phosphorylation of the TCRζ chain *in vivo*, indicating tonic TCR signaling. We are assessing whether this tonic TCR signal is intact in Sw/Hu mice. Interestingly, human T cells in the periphery of Sw/Hu mice exhibit reduced proportions of naive T cells, reduced CD8+ T cell percentages, and reduced lymphopenia-driven expansion compared to Hu/Hu mice. The use of this model to determine the source and quality of homeostatic factors maintaining the peripheral human T cell pool will have important implications for understanding human adaptive immunity, disease and immunotherapies.

W.42. Functional Analysis of Immune Responsiveness using Standardized Whole Blood Stimulation Systems

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Standardization of immunophenotyping procedures has become a high priority. We have developed a suite of whole blood, syringe-based assay systems that minimizes pre-analytical errors, to reproducibly assess induced innate or adaptive immune responses. In a specific collection of healthy individuals with homogeneous ethnic background, we have defined the protein signatures induced by complex microbes, MAMPs, cytokines and T cell activators, providing healthy donor reference values for induced cytokines and chemokines. To establish the relationship between induced protein secretion and the dynamics of mRNA expression, we defined the transcriptional signatures at three serial time-points. This pilot study also permitted us to assess different methodologies for RNA extraction and quantification. Using an optimized single-step extraction method we quantitated mRNA expression using a hybridization-based multiplex technology (Nanostring), which showed intra-individual coefficients of variance (CVs) of <5% and a >5-log dynamic range. With this method, we observed differential patterns of expression for the unique immune stimulation systems, correlating gene and protein expression patterns. The observed naturally occurring variation of the immune response may help to explain differential susceptibility to disease or response to therapeutic intervention. The implementation of a general solution for point-of-care assessment of functional immune responses will help support harmonization of clinical studies and data sharing.

T.42. Multiparametric Analysis by Flow Cytometry of Leukocyte Subpopulations in Peripheral Blood of Healthy Donors

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Multiparametric flow cytometry (MFC) is an important tool to characterise leukocyte subpopulations. A standardization of the immunophenotype in peripheral blood is still lacking. In this context, a consortium of laboratories was formed in 2009, led by the NIH and FOCIS. In Europe, The COST-ENTIRE initiative established standardised whole blood panels based on their

recommendations. Six FCE's of ENTIRE agreed to analyze 50 healthy donors (HD). Here the results of FCE-Barcelona are presented. Methods: Peripheral blood of 50 HD (BST,Spain) (28F/22M; aged 21-66) was analyzed by MFC (BDLSR Fortessa®) using panels established in the ENTIRE-HIPC protocol. Results: Regarding T cells, the major CD8+ subset was naïve cells (CCR7+CD45RA+; 39.89±17.43%), followed by TEMRA (CCR7-CD45RA+; 31.29±16.33%). In CD4+, the major subset was naïve (CD45RA+CCR7+; 41.53±13.74%) followed by CM cells (CD45RA-CCR7+; 39.03±10.17%). The percentage of CD4+T_{regs} was 5.21±1.54%, half of them HLA-DR+ (2.02 ± 0.73%). In relation to B cells, 51.78±16.72% were Naïve (CD27-IgD+). The major NK subpopulation was CD56brCD16+ (87.30±8.48%) cytotoxic subpopulation. Monocytes were divided in classic (CD14+CD16-) (91.59±5.48%) and non-classic ($CD14^{low}$CD16+) (6.06±3.15%) subpopulations. Dendritic cell subpopulations were classified as myeloid (CD123lowCD11c+) (38.83±15.34%) or plasmacitoid (CD123+CD11c-) (11.25±15.30%) DCs. Myeloid DCs Slan+ (proinflammatory subset) represented 58.96±14.45% of them. Conclusions: These results, together with those of the collaborative COST-ENTIRE initiative will help to get a range of % of leukocyte subpopulations representative of HD. This will be essential to better utilize MFC in multicentric clinical trials and for diagnosis monitoring and follow up of treatments in immuno-mediated inflammatory diseases in daily clinical practice.

T.63. Predictive Individualized Immune Signatures using Transcriptome Analysis

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Inflammation and immunity are critical pathophysiological factors for many diseases with interplay between immunity and medical interventions dictating clinical outcomes. Platforms capable of monitoring immune status and predicting response potential of different types of patients could transform medical practice by foreshadowing disease course and likely response to therapy at the time of initial diagnosis. Here we develop an approach using RNA expression patterns for determining the immune status and response potential of individuals. Using bioinformatics strategies to define immune response elements of a healthy immune system which are constant within an individual over time, but which vary significantly among individuals in a cohort, we identify individualized molecular immune response signatures (MIRS) which describe individual differences in both immune status and response potential. We demonstrate using in vitro stimulation of CD4 cells with anti-CD3/CD28 coated beads how response prediction gene sets (RPGS) derived from the MIRS from resting cells can serve as biomarkers predictive of induced responses . These findings have far reaching implications for how human genome technologies can be used to characterize the human immune system, providing reproducible detailed information about the immune status and response potential on an individual basis.

T.64. A Mass Cytometry Assay to Interrogate Intracellular Signaling Pathways in Human Whole Blood

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The ability to assess the function of a range of cytokine, antigen receptor, and Toll-like receptor (TLR) signaling pathways in a range of immune cells could provide a kind of fingerprint of the state of the human immune system. The mass cytometry, or CyTOF, platform allows for the parallel application of about 40 labeled antibodies to a single sample, creating the possibility to read out many cell types and signaling pathways in a single small blood sample. We developed such a mass cytometry panel, consisting of 22 antibodies to cell-surface lineage markers and 8 antibodies to phospho-specific epitopes of signaling proteins. These antibodies were chosen to discriminate all major white blood cell lineages, to a level of detail that includes subsets such as naïve, central memory, effector memory, and late effector CD4+ and CD8+ T cells, naïve, transitional, and switched memory B cells, plasmablasts, myeloid and plasmacytoid dendritic cells, CD16+ and CD16+CD56+ NK cells, CD16+ and classical monocytes, etc. 32 such cell subsets are defined in our standard gating scheme. The eight phospho-specific antibodies were chosen to represent major signaling nodes responsive to cytokine, TLR, and antigen receptor signaling. This antibody panel is used with 8 standard stimulation conditions (unstimulated, IFNa, IL-6, IL-7, IL-10, IL-21, LPS, and PMA+ionomycin), although other stimuli can be added. We show that reproducible data can be obtained with this panel in healthy human subjects. Comparison of controls to subjects with immune deficiencies of unknown etiology may help to elucidate the mechanisms of such deficiencies.

T.65. Barcoding of live PBMC for multiplexed mass cytometry analyses

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Mass cytometry allows massively multiparametric and high-resolution immunophenotyping on a single-cell level. We present here a sample barcoding approach for live human PBMC, allowing for identical conditions during immunostaining, fixation, and permeabilization of PBMC, as well as during acquisition on the CyTOF™ instrument. Using unique combinations of 3 out 6 different anti-CD45 conjugates, we barcoded up to 20 different samples. Besides using In-113 and In-115 conjugates, we labeled antibodies with four hitherto unused metal isotopes, i.e. Pd-104, Pd-106, Pd-108 and Pd-110. These reagents were generated by the reaction of palladium-loaded isothiocyanobenzyl-EDTA with primary amines of the anti-CD45 antibody. CD45-barcoding was successfully applied to immunophenotyping of PBMC. There was good correlation of cell population frequencies derived from single, successively acquired samples, and from deconvoluted barcoded sample data. Using three and only three out of the possible six antibodies allowed us to remove any events that did not satisfy that requirement. This facilitated electronic removal of cell aggregates, thus addressing an important limitation of mass cytometry data and its interpretation. The aggregate removal during data deconvolution allowed for less restrictive gating on DNA and cell length parameters, thus allowing more events to be analyzed. As a result, electronic cell recoveries after denconvolution of barcoded samples and separately acquired samples were similar. In summary, CD45-barcoding speeds sample acquisition, allows aggregate removal, minimizes antibody use, and improves data accuracy by harmonizing sample preparation and acquisition conditions.

T.66. Age-related Changes in Stimulated Cytokine Production Assessed by a Simple *in vitro* Assay

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Many of the age-related changes in the immune system are only subtly reflected in the proportions of cell subsets, but may be more clearly reflected in their functional capacities. We sought to design a simple functional assay that could, in a single well, obtain information about the responses of human PBMC to stimulation through cytokine, Toll-like receptor (TLR), and antigen receptor pathways. We designed a cocktail of IFNa, LPS, anti-IgG, anti-IgM, and CD3+CD28 beads, which were added to 0.5 million cryopreserved and thawed PBMC for four hours. The supernatant was then collected for Luminex analysis of 51 cytokines, while the cells were placed in Trizol reagent for RNA extraction and gene expression microarray studies. We initially showed that the genes responsive to individual stimuli were largely independent, resulting in mostly additive results when using the stimulation cocktail. Furthermore, gene expression changes generally tracked well with changes in the corresponding protein by Luminex. Finally, we applied this assay to PBMC from 223 healthy subjects age 40-90+. Luminex results from stimulated and unstimulated control wells were analyzed. A log2 transformation followed by z score transformation was used to correct for batch effects across samples. The fold-changes (stimulated/unstimulated) of 13 of 51 cytokines were found to be significantly correlated with age. However, results were quite heterogeneous within each age group, suggesting that changes over time for a given individual may be more informative than single time point results.

T.67. Cellular Immune Responses to Adeno-Associated Virus (AAV) Vector in a Gene Therapy Trial for Hemophilia B

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Hemophilia B is a bleeding disorder caused by the absence of functional factor IX. Relatively low levels of the protein ameliorate the clinical phenotype significantly and therefore Hemophilia is a good model for gene transfer. We are presenting cellular immune response data to the vector from a clinical study of gene transfer for hemophilia B where an adeno-associated

virus vector serotype 8 (AAV8) expressing the FIX transgene was administered intravenously in ten affected subjects. Cellular immune responses to vector capsid and to Factor IX were monitored by interferon-gamma (IFN-γ) ELISpot and polyfunctional T cell analysis of peripheral blood mononuclear cells (PBMCs). While AAV is non-pathogenic in humans, dose-dependent cellular immune responses to the viral capsid could be detected in both assays concurrent with a slight elevation of liver enzymes. A response to the FIX transgene could not be detected. Liver enzyme elevation could only be detected in 4 out of 6 subjects treated at the highest dose in a time window of 7-10 weeks after gene transfer. Polyfunctional analysis revealed CD4+ and CD8+ T cell involvement and further substantiates the results by coexpression of other cytokines as well. It is important to note that not all subjects treated at the high dose developed an immune response. By continued monitoring and future studies we aim to identify the underlying mechanism and to define predictive parameters for a cellular immune response.

T.68. Dry and Temperature-stable Reagents for Tobust Multi-color Flow Cytometry Applications

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Multicolor flow cytometry is a powerful tool to monitor immune responses. Standardization of sample preparation and analysis methods is crucial for longitudinal and multi-site studies. The use of Flow-Set™ Pro fluorospheres to set instrument settings, a standard operating procedure combined with central data analysis, contributes significantly to the standardization. However, additional parameters such as reagent handling and the degradation of the antibody conjugate cocktail due to light exposure or inappropriate storage conditions may contribute to assay variability. To circumvent these drawbacks, Beckman Coulter has developed the DuraClone technology that allows antibody conjugate stabilization in a dry format and enables long term storage at room temperature. Using a 9-color dry cocktail for the identification of the main white blood circulating cells, it has been demonstrated that the performance of the antibody-fluorochromes is not impacted by the drying process. Moreover, this reagent format is compatible with the most commonly used red blood cell lysis reagents and sample preparation protocols. A preliminary stability study was performed by exposure of the dry cocktail to 60°C for 7 days. The performance of the dry cocktail in the study was similar to its liquid counterpart which was stored at 4°C. This study demonstrates the potential of DuraClone technology to generate a stable and functional reagent format which can be a powerful tool for the standardization of longitudinal and multicentric immune profiling studies.

T.69. Sub-maximal Aerobic Exercise Induces Changes in Peripheral Blood Cell Subpopulation and Increases Serum Concentration of IL-6 and NEFAs

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Introduction: Physical activity is an essential practice for the care and maintenance of the functions in human body. Several studies have shown that regular exercise prevents diseases developing due to stimulation of the immune response components. **Aim:** To determine the effect of moderate aerobic exercise on levels of leukocytes and mediators of the inflammatory response in amateur athletes after a session sub-maximal aerobic exercise. **Material and methods**: Eighteen amateur athletes performed a session sub-maximal aerobic exercise for 120 minutes on a cycle ergometer. Three samples of blood (before, after and 2 h post-test) were taken to determine the cells subpopulations percentage; furthermore, the serum concentrations of TNF-α, IL-6 and NEFAs were determined by ELISA. Statistical analysis was performed with SPSS v20. Friedman test was used to compare. **Results**: A sub-maximal aerobic exercise session induces significant increase in PMNs counts after the test and 2 hours post-test ($p < 0.000$). The percentage of lymphocytes and eosinophils decreased after exercise as 2 h post-test ($p < 0.000$). An increase in serum concentration of IL-6 was shown after the test ($p < 0.01$) and after 2 h of rest ($p < 0.05$). A significant increased was observed in NEFAs serum concentration after test ($p < 0.000$), and after 2 hours of rest ($p < 0.05$). **Conclusions**: These data suggest that aerobic exercise at sub-maximal level induces an increase in the percentage of neutrophils in the blood, which promotes the release of pro-inflammatory mediators and the subsequent establishment of an acute inflammatory response.

T.70. Thymus, Bone and CD34+ Cell Requirements for Functional Follicular Lymphoid Structures and Antibody Responses in Humanized Mice

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Background: The development of human monoclonal antibodies would minimize the side effects of antibody-based immunotherapy with xenogeneic antibodies. Method: To investigate the effect of hematopoietic and lymphoid tissues on functional lymphoid structure and humoral immune responses, we generated 4 types of humanized NOD/SCID IL2Rγc null (NSG) mice with following combinations: the transplantation of (1) human fetal thymus (T), bone (B) and CD34+ cells (TB/CD34+-NSG), (2) T/CD34+-NSG, (3) B/CD34+-NSG and (4) CD34+-NSG mice. Results: The TB/CD34+-NSG mice showed excellent maturation and collaboration among human (h) B cells, T cells and dendritic cells (DCs), thereby providing an enhanced platform for human immune responses and active B cell immunity with abundant plasma cells and well-developed germinal centers. Furthermore, germinal center B cells were highly activated as hCD71 and activation-induced (cytidine) deaminase (AID) expression. These mice also had significantly higher levels of hIgG and hIgM than did other humanized mice examined. Immunization with DNP-KLH led to significantly higher titers of antigen-specific Igs, especially antigen-specific hIgG, in TB/CD34+-NSG mice. We also established hybridoma cell lines, consisting of dual hIg-producing cells secreting both hIgM and hIgG monoclonal antibodies to DNP-KLH, using TB/CD34+-NSG mice. However, only hIgM-producing hybridomas specific to DNP-KLH were generated using control humanized mice, immunized with DNP-KLH. Human IgG levels of our hybridoma cell lines were up-regulated in the culture supplemented with hCD40L, hIL4 and hIL21. Conclusion: Our results demonstrate that humanized TB/CD34+-NSG mice may provide an unprecedented platform for human immunity and the generation of human monoclonal antibodies for immunotherapy

T.71. Tools for Optimization of Adoptive Cellular Therapy

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Cell-based therapies using lymphocytes are promising approaches for immunotherapy. AdoptiveT cell therapy is an effective treatment for viral infections in immune-compromised patients, and has induced regression of cancer in early-stage clinical trials. Adoptive T cell therapy can be optimized in several ways: Patient stratification - only those that benefit from therapy are treated, Measurement of T-cell immunity pre and post treatment - to evaluate if the desired immune response has been induced and additional therapy is needed Quantitative quality control of the cellular product prior to infusion - to ensure consistent cell number and quality Multiple adoptive cell therapy trials are investigating the benefit of transferring CMV-specific T cells to transplant patients to avoid reactivation of CMV. We used the Dextramer CMV assay, to quantitate CMV-specific T cells in whole blood from transplant patients and in processed cell samples.
The reconstitution of CMV immunity was successfully followed in >90 transplant patients and it was shown that induction of CMV-specific immunity upon adoptive transfer of CMV-specific T cells could be reliably measured. Furthermore we showed that CMV Dextramers can be used to characterize cellular products with respect to CMV-specific T-cell composition upon in vitro expansion. Our results demonstrate that the CMV Dextramer assay is a valuable tool that may improve CMV-specific adoptive cellular therapy. The Dextramer assay allows: Stratification – Identification of patients with low CMV-specific immunity, Immune monitoring – Determination of the induced cellular response in patients, Quality control - QC of manufactured cellular products.

T.72. Healthy Preterm Newborns Show Increased Frequency of $CD4^+CD25^{high}CD127^{low}FoxP3^+$ Regulatory T cells in Comparison to Term Ones

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Regulatory T cells (Tregs) have a crucial role in controlling the development of a healthy immune system and, its absence, is responsible for the range of inflammatory and autoimmune manifestations observed in patients with IPEX

(Immunodysregulation Polyendocrinopathy Enteropathy X-linked Syndrome). Preliminary results of our group revealed greater ability of newborns to produce pro-inflammatory response when compared to adults, which was further accentuated by the decreased production of IL-10, which suggests a reduced regulatory function. The assessment of the number and frequency of Treg population in newborns from different gestational ages can be used as a reference and help the diagnosis of IPEX cases. Thus, the objective is phenotypically characterize Treg population by flow cytometry in cord blood of preterm newborns born at 30-33$^{6}/_{7}$ gestation weeks (Group 1), 34-36$^{6}/_{7}$ gestation weeks (Group 2) and term newborns born at 37–41 gestation weeks (Group 3), all healthy and appropriate for gestational age, compared to healthy adults. The results showed that Groups 1 and 2 had significantly higher frequency of $CD4^{+}CD25^{hi}CD127^{lo}FoxP3^{+}$ regulatory T cells when compared to Group 3 and adults. Significantly higher numbers of Treg cells were observed in all neonates compared to adults due to the characteristic leukocytosis found at birth. Moreover, the results showed that the three newborn groups presented significantly increased frequencies of Treg with naïve phenotype ($CD45RA^{+}$) compared to adults. In conclusion, preterm newborns present higher Treg population, however an appropriate number of cells does not indicate proper function. **Financial Support:** FAPESP (Grant number 2012/10928-8).

T.73. Immune parameters in Aging Individuals: Female and Male Differences

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There are differences in the incidence and severity of infections when aging female and male are compared. We compared immune parameters of individuals (female, F n=25 and male, M n=19) from 60 to 65 years old. Blood was collected and leukocytes obtained for flow cytometry analysis (naïve, effector, and memory). Blood was diluted in PBS and added to Ficoll for isolation of PBMCs. Cells were stained with monoclonal antibodies (CD3 APC, CD4 PerCP Cy5.5, CD8 APC Cy7, CD27 FITC, CD45RA PE) and evaluated in cytometer. Clinical baseline parameters were in F and M respectively: Cr 0.84 ± 0.14 mg/dL x 1.02 ± 0.2 mg/dL (p=0.0007), Gl 103.0 ± 22.6 mg/dL x 90.9 ± 11.4 mg/dL, Al 3.7 ± 0.2 g/dL x 4.0 0.3 g/dL (p=0.01). CD4, CD8, CD4/C8 and CD19 were similar when female and male were compared. CD4+CD45RA+CD27+, CD4+CD45RA+CD27- and CD4+CD45RA-CD27+ cells were similar in female and male whereas CD4+CD45RA-CD27- (F 13.9% ± 7.8% x M 18.0% ± 8.5%, p=0.077) were higher in male. Female and male CD8+CD45RA+CD27- and CD8+CD45RA-CD27+ were similar when compared whereas CD8+CD45RA+CD27+ was higher (F 33.9% ± 14% x M 29.2% ± 12.6%, p=0.065) and CD8+CD45-CD27- was lower (F 11.6% ± 9.7% x M 15.6% ± 9.7%, p=0.047) in female. CD19CD27- (F 60.1% ± 16.7% x M 62.7% ± 13.1%) and CD19CD27+ (F 37.7% ± 17.2% x M 34.7% ± 15.5%) were similar. In conclusion, female presented better clinical conditions and higher expression of naïve CD8+ T cells and male had higher expression of memory CD4+ T and CD8+ T cells.

T.74. Evaluation of Renal Function and Immune System Cells in Elderly Individuals

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Renal and immune system functions decline with age. Some studies have shown that the decline in kidney function is associated with the worsening of the immune system. Our aim was to correlate the percentages of CD4+, CD8+, and B cells with renal function in non-institutionalized individuals from 60 to 101 years old. Blood was collected for glucose, albumin, and creatinine measurement besides of T (CD4+, CD8+) and B (CD19+) evaluation by flow cytometry. We calculated the estimated glomerular filtration rate (eGFR) using the Modification of Diet in Renal Disease equation (MDRD: 186 x serum creatinine$^{-1.154}$ x age$^{-0.208}$ x 0.742 if female). Baseline clinical characteristics of the 237 elderly individuals (130 female, 107 male) were: glucose 95.0 ± 34.4 mg/dL (32-373 mg/dL), albumin 3.79 ± 0.36 g/dL (2.8-5.7 g/dL), and creatinine 1.0 ± 0.35 mg/ dL (0.4-3.37 mg/dL). MDRD equation showed that most of the individuals (148 from 237) evaluated presented eGFR > 60mL/min/1.73m^2. The increase in age was negatively correlated with eGFR (r=-0.398, p<0.0005) for both gender. We then compared individuals with eGFR lower and higher than 60mL/min/1.73m^2 for immune parameters. eGFR <60mL/min/1.73m^2 was associated with lower percentages of CD4+ and higher percentages of CD8+ whereas CD19+ was similar. Even though most of our studied population did not present kidney disease stage 3A (45-60 mL/min/1.73m^2) or worst it was possible to show that decreased renal function is associated with changes in percentages of CD4+ and CD8+ T cells and thus could contribute for the impairment in elderly immune response.

T.75. Extensive Profiling of CMV-specific Immune Responses Post Solid Organ Transplantation
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Human cytomegalovirus (HCMV) represents the most important viral infection in solid organ transplant (SOT) recipients. Pediatric patients are at highest risk of developing morbidity and mortality from these viral infections because of the higher incidence of primary infection. The current practice of monitoring just viral load is insufficient for predicting future clinical outcomes and there is broad consensus that T cell immunity is most important in controlling viral replication. However, despite extensive knowledge of epitopes and T cell responses in healthy donors/SOT recipients, there is still no proven consensus on what constitutes a protective T cell response to any of the chronic viruses. Using a new, highly multiparametric, 38-marker, mass cytometry technique (CyTOF), we have simultaneously analyzed T cell phenotypes, functions, and epitope specificity on individual cells, from 20 healthy CMV seropositive donors and 20 kidney and liver transplant patients, in response to eight different CMV antigens from the early and late stages of the viral life cycle. Our results show that polyfunctional T cell responses (producing 3 or more cytokines) may be correlated with lack of viremia at subsequent time points. We did not find any significant trend for protective immune responses focused on specific viral proteins in SOT patients. We further assessed levels of CD57+NKG2Chigh NK cells, which may also be informative. Knowledge about what constitutes a "protective" T-cell response would allow for development of an individually-tailored strategy to control patient-specific viremia and would also be helpful in testing of new vaccine candidates against many chronic viruses.

T.76. Comparison and Determination of the Best Collection Tube for Complement Analysis
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Complement is important not only as the body's first line of defense against infection, but it also has critical roles in autoimmunity and inflammatory regulations. Complement is being connected to a growing number of diseases and disorders which combined with growing number of novel complement therapeutics are increasing the importance of appropriate complement analysis for patients. The first step any laboratory analysis is proper sample collection; this is especially true for complement. In this effort we investigated the effects of different common laboratory collection tube types on the outcome of different complement diagnostic tests. The tube types tested include EDTA (lavender top), EGTA (lab prepared), Citrate (Blue Top), Sodium Oxalate (Gray Top) Heparin (Green Top), serum separator tube (SST, tiger top), and plain serum tube (red top). We also tested Lepirudin, an anticoagulant that is no longer available from the manufacturer. Lepirudin is recombinant Hirudin, which is from leech saliva. Unlike most available anti-coagulants lepirudin specifically inhibits thrombin, but does not inhibit any part of the complement cascade. The assays tested include the measurement of CH50 hemolytic activity. In addition we tested the measurement of activation products which are used to monitor activation in a patient and to investigate possible activation by a drug in development. The final testing was measurements of the ability to activate complement in each tube type ex vivo by using a known complement activator. This analysis helps inform the use of the proper anti-coagulant which will achieve the truest analytical result.

T.77. A Novel Particle-Based Peptide/MHC Multimer to Detect and Influence Antigen-Specific Lymphocytes
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Major histocompatibility complex (MHC) tetramer technology revolutionized the field of immunology by allowing the detection and isolation of antigen-specific T cells and prompted the commercialization of a number of improved MHC multimer platforms. We have further enhanced options to analyze epitope-restricted immune cells by developing a fully customizable biocompatible particle that incorporates the stable conjugation of peptide/MHC molecules (without streptavidin/biotin chemistry) and fluorescent labeling. Our proprietary multimer reagent detects mouse or human lymphocytes with the ability to isolate cells for further experimentation such as expanding immune cell subsets *in vitro* or cloning antigen-specific receptors. Additionally, the MHC-specific particle maintains low immunogenicity *in vivo*, allowing for its extended use in animal models and clinical specimens that involve immune cell trafficking and localization studies. The unique nature of the particle also permits the incorporation of small molecules (regardless of solubility) (e.g., cytotoxic drugs, immune modulators) alongside

MHC attachment and could, therefore, represent a desirable therapeutic agent for conditions such as leukemia where precise destruction of cancerous cells would be ideal or in settings that require the regulation of immune cell function. Altogether, this novel MHC multimer technology can be applied to a variety of immunologically-related areas, particularly, where epitope-restricted lymphocytes are detected/isolated for research and clinical purposes or in cases where the particle delivers an intended payload upon binding its cognate receptor.

T.78. Long Term Stability of Multi-Color Cocktails: One Study Consortium Panels as Examples of Robust Formulation and Tandem Dye Stability

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Multi-color applications for flow cytometry constitute powerful tools to monitoring immune responses. Six 7- to 9- color leukocyte profiling panels have been designed in collaboration with the ONE Study Consortium, which has used these panels to demonstrate high levels of standardization across clinical studies at multiple sites. We have investigated the stability of these panels in two configurations: 1) formulated into 3-vials with at most 3 fluors per vial and where APC/APC tandem and PE/PE tandem conjugates do not co-exist in the same vial to prevent potential dye interaction and 2) the entire panel formulated as a single cocktail. Three-vial configurations of different ages were evaluated versus fresh reference cocktail using two primary criteria: mean fluorescence intensity and population percentage compared to predetermined ranges. Each 1-vial format was evaluated in real time versus a 3-vial reference. Our data show that all panels provided stable performances up to at least a year after formulation, regardless of single-vial or three-vial configuration, and all measures in at least one panel showed satisfactory performance to 18 months. PE and APC tandem dyes in these formulations showed acceptable performance beyond 12 months in cocktail, indicating that tandem dyes are suitable for cocktails that require long-term stability. This study demonstrates that high plex antibody cocktails, when well-formulated, are stable for extended periods, providing powerful tools for multi-center and longitudinal immune profiling studies.

T.79. Standardized Whole Blood Immunophenotyping

***Thomas Giese**. University of Heidelberg, HEIDELBERG, Germany*

Standardization of multicolor immunophenotyping in diagnostic settings is an important and central project of the COST Action ENTIRE (1). Therefore, this initiative established a standardized whole blood assay based on the recommendations of the HIP-consortium (2). The SOP for this procedure is publicly available. Several European FCE's of the ENTIRE group agreed to analyze healthy donors to build a comprehensive reference database. In parallel the panel is successful employed in daily laboratory diagnostics and monitoring of patients with immune deficiency diseases (ID), immune mediated inflammatory diseases (IMID) and after solid organ transplantation (SOT). Importantly, identifying changes in leukocyte subpopulations, not routinely investigated in daily practice accelerated the diagnosis of patients with novel and rare ID's. In addition, monitoring a cohort of patients after SOT provides new insights into alterations of the immune system caused by the transplant and accompanied immunosuppressive therapy. Recently the panel was adapted to study leukocyte populations in solid organs such as liver, colon and pancreas. Results and conclusions from this collaborative project will be relevant to understand which are the real gaps in standardization of immunophenotyping and how to possibly overcome them in order to better utilize flow cytometry in multi-centric clinical trials, as well as in daily clinical immunological practice.

T.80. Serum Procalcitonin Levels in Patients with Intracellular Bacterial Infections

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Procalcitonin (PCT) is a novel marker of the inflammatory response to infection. However, there are very few data on serum PCT levels in intracellular bacterial infections. The aim of the present study was to elucidate PCT levels in sera of patients with active intracellular bacterial infections. For this purpose, 72 patients with intracellular bacterial infections [tuberculosis (14), brucellosis (30), tularemia (28)] and 47 patients with extracellular bacterial infections were enrolled into a prospective study.

We measured serum concentrations of PCT by using enzyme-linked fluorescent assay. Serum levels of PCT were significantly lower in patients with intracellular bacterial infections compared with extracellular bacterial infection group ($p<0.001$). This study showed that PCT levels differed significantly in patients with intracellular and extracellular bacterial infections. Altough there are a great number of evidences showing that PCT increase in extracellular bacterial infections, further large-scale studies are needed to analyze the PCT levels in intracellular bacterial infections.

T.81. Essrimation of Precision of Whole Blood Immune Phenotype Monitoring Using the Example of the One Study

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Flow cytometry has become a powerful tool in immune monitoring for studying the effects of therapeutics aimed at reducing transplant rejection or autoimmune disease flares. However, assay complexity and pre-analytical factors cause high assay variability. Standardization and validation are essential to control variables and improve precision performance, particularly in multi-center trials. Precision performance is estimated by calculating the coefficient of variation (CV%) based on measurements of intra-assay replicates. Due to counting statistics and cytometer performance, precision of enumerating leukocyte subsets depends on the range of measurements. Since leukocyte subsets vary dramatically in size (frequency and cell count) and also sensitivity to variability, one unique CV for all subsets cannot be defined. For the ONE Study, robust panels were developed to allow immune profiling by flow cytometry in a multi-center approach. Comparable results were achieved between centers with a relatively low inter-assay variability(0.05% to 20%). A precision profile approach was used to characterize repeatability performance throughout the range of measurements. Precision profile is a mathematical function based on the relationship between the mean for each sample and CV%. Applying this function, we calculated the CV% for each reported leukocyte subset, which enables estimation of significant differences between patient groups within the ONE Study. In conclusion, the precision profile is efficiently used to validate flow based test systems. It was and will continue to be applied to report changes in precision depending on further developments of the test design, such as minimization of variability by implementation of dry technology of antibody panels.

T.82. Automation of Immune Monitoring and Immune Phenotyping Using Robotics and Multiparametric Flow Cytometry Platform

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One of the biggest challenges in human immune phenotyping/monitoring is the control of many variables such as sample collection, handling and preparation that ultimately affects the quality of data. Automation of assays has been recognized as a natural way to standardize processes and improve precision, accuracy, and throughput with the additional benefits of eliminating errors from manual and repetitive tasks. We have developed a robotic flow platform that allows in depth phenotyping of the immune response. Our platform consisted of two Biomek robots (FXp and NXp) and 2 LSR Fortessa cytometers equipped with high throughput 96 well samplers. We have designed, validated and used automated methods on the Biomeks to thaw batches of 24 or 48 coded clinical samples. Samples were washed, counted (Guava) and the cell suspension adjusted to the appropriate concentration, before staining or stimulation and staining in a 96 well format using the automated platform. Data generated using Diva software consisting of fcs files of 10 phenotypic and functional markers (surface and intracellular) are analyzed using Flowjo or an automated analysis software. Using this automated approach of processing the samples with minimal operator interference, we were able to generate reproducible data with a CV≤5% and a correlation of 0.992 between repeat samples processed on different weeks. This platform has been used in different projects (T1D, west Nile, influenza, multiple sclerosis). We are currently implementing automation to process fresh clinical samples across different projects involving different investigators allowing the interrogation of the same sample on different levels.

T.83. TCRm Arrays Combined with Label-free Screening Technology to Identify Secreted Cancer-associated Peptide HLA Biomarkers

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In this work we present T-cell receptor-mimicking antibodies (TCRm) used in a label-free, multiplexing array format to enable a breakthrough biomarker screening system for cancer diagnostics. Conventional laboratory diagnostic approaches require time- and labor-intensive processing that are not easily integrated in a clinical setting. Additionally, conformational changes occurring to the analyte after binding to an immobilized antibody may result in loss of detection antibody specificity. Label-free bioassay methods monitor antibody-analyte binding directly without requiring a detection antibody for readout. Furthermore, label-free approaches monitor immunoassays in real time, thus minimizing laboratory processing steps and achieving faster results. In this work we apply the ResoSens bioassay system (developed by Resonant Sensors) for high throughput screening of secreted peptide-HLA (sHLA) molecules in plasma. We present detection of four cancer related peptide-HLA complexes (as potential biomarkers) in human plasma samples utilizing TCRm antibody arrays and the ResoSens bioassay system. We monitor sensitivity and specificity in this multi-analyte array. We also generate a signature binding profile (on rate) for each antibody-target biomarker pair that can be used in verification of the specificity of the measurement (as distinguished from the binding characteristics of non-specific proteins and other molecules). Thus, TCRm arrays combined with label-free screening technology can identify cancer-associated peptides presented by sHLA molecules. This provides a nonintrusive method to detect existing and new cancer biomarkers for diagnosis and immunotherapy.

T.84. Assessing Immunogenicity of rhGAA in Adult Pompe Disease Subjects

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Pompe disease (PD) is an inherited metabolic myopathy caused by a deficiency of the lysosomal enzyme acid α-glucosidase (GAA). Enzyme replacement therapy (ERT) has improved the outcome of PD, unfortunately the immune responses against GAA are not uncommon and in some cases prevent therapeutic efficacy. The aim of this study was to investigate the mechanisms of the immune responses against recombinant human GAA (rhGAA) in adult patients undergoing ERT. About one-third of all subjects screened (n=35) showed significant levels of anti-rhGAA binding antibodies, mainly IgG4. After dendritic cell maturation in the presence of rhGAA a positive IFN-gamma ELISpot signal was detectable in some of the subject's peripheral blood mononuclear cells, indicating GAA-specific T cell reactivity. Interestingly we were able to detect a response in PD subject previously untreated with rhGAA, thus naive to the antigen. The *in vitro* re-stimulation with rhGAA resulted in upregulation of proinflammatory cytokines and chemokines (IL-8, IL-12p70, MIP1β, TNF-α and MCP-1) in PD subjects but not in healthy donors who, conversely, secreted higher levels of IL-10. Similarly, markers of immune activation such as IL-1β, IFN-γ, GM-CSF, TNF-α, IL-6, and IL-4 were readily detectable in sera isolated from PD subjects undergoing ERT. While additional studies are needed to better define the subsets of B and T cells involved in immune responses to GAA, these study provide a comprehensive overview of activation of immunity in response to ERT in Pompe patients and may provide tools and strategies to monitor and manage immunogenicity of biotherapeutics.

T.85. Heterogeneity of Id3 in Regulatory T cells

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The basic helix-loop-helix transcriptional regulators Id2 and Id3 (inhibitor of DNA binding) help control cell fate decisions in a variety of biological systems. Id proteins function by binding to and inhibiting the activity of E-protein transcription factors. In T cells, the dynamic regulation of Id2 and Id3 expression helps control T cell development in the thymus and effector/memory T cell differentiation in the periphery. $CD4^+Foxp3^+$ regulatory T cells (T_R) are potent anti-inflammatory cells that dampen immune responses to both self- and foreign-antigens. Recently it has been demonstrated that T_R are phenotypically and functionally heterogeneous, with distinct populations subject to unique homeostatic constraints and behaviors based on localization. However, the molecular basis for the phenotypic and functional properties of different T_R populations is still poorly understood. Our data shows that expression of both Id2 and Id3 is dynamically regulated in T_R in a subset- and tissue-specific

fashion. For instance, although Id2 is expressed by most developing T cells and effector T cells, it is downregulated during T_R development in the thymus. Whereas Id3 is expressed by essentially all T_R exiting the thymus, loss of Id3 expression in the periphery is associated with enhanced T_R proliferation and dissemination into inflamed non-lymphoid sites. Based on the known roles of Id2 and Id3 in directly controlling the functional differentiation of $CD8^+$ effector T cells, we hypothesize that the dynamic regulation of Id2 and Id3 expression underlies the differentiation and functional specialization of T_R, and is essential for their ability to maintain tolerance *in vivo*.

T.104. Selecting Cryopreserved Peripheral Blood Mononuclear Cells for use in High Throughput Screening and Cell Based Assays

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Use of primary cells in high throughput screening (HTS), rather than cell lines, allows for the measurement of endogenous protein target expression in an environment that mimics physiological reactions. Peripheral blood mononuclear cells (PBMCs) are excellent for understanding *in vivo* physiological and metabolic activity. PBMCs have broad ranging applications from basic discovery to preclinical and clinical studies, including direct monitoring of immune responses to therapeutic and vaccine development. The availability of sufficient cells, especially in the case of large screening campaigns, and ensuring lot to lot reproducibility, are limiting constraints for HTS. Precision AccuCell® Cryopreserved PBMCs solve these challenges by providing large quantities of consistent and reproducible cells from a single donation. AccuCell PBMCs are available with diverse donor demographics and superior viability and post thaw recovery, in lots of over 300 vials, ensuring matched samples or standards for hundreds to thousands of assays. Standardized cryopreservation and thawing provides optimal preservation of fundamental PBMC characteristics and functionality. Data has been generated to help select optimal screening lots based on distinct characteristics. Cryopreserved PBMCs functionality is highlighted by the phenotypic characterization of major PBMC subsets from different lots, along with viability and apoptosis data, and cytokine responses to PHA, CMV, and CEF. Available, well-characterized, cryopreserved PBMCs enable *ex vivo* testing of target candidates, while the large lot sizes contribute to consistent and reproducible results; providing controls for whole studies. Cyropreserved PBMCs facilitate progress in all stages of drug development through increased use of HTS.

Immunity and Infection

1100A. Regulatory T cells Represent an Important Fraction of HIV-specific T cells

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Regulatory T-cells (Tregs) play a dual role in HIV infection. Tregs decrease immune activation but they also block anti-HIV-specific immune responses. Their role is thus important in the study of novel vaccines effectiveness. We employed a novel assay in which co-expression of CD25 and CD134 along with FoxP3 and CD39 on antigen-stimulated CD4+ T-cells identifies antigen-specific Tregs cells. Upon antigen stimulation, a large fraction of $CD25^+CD134^+$ cells, co-expressing FoxP3 and CD39 did not secrete cytokines (IFNγ, IL2, TNFα). In HIV-infected patients, 50-60% of HIV-specific cells were $FoxP3^+CD39^+$ which could partially explain the low IFNγ-response observed in these patients. By sorting 3 populations of $CD4^+$ T-cells based on CD25 high, intermediate or low expression, we showed that $CD25^+CD134^+CD39^+FoxP3^+$ antigen-specific Tregs originated from $FoxP3^+CD25^+$ Tregs that upregulated CD134 upon stimulation with the cognate antigen. Next we used this methodology to analyze samples from HIV^+-patients vaccinated with a novel dendritic cell-based vaccine. The results showed a relative increase in the percentage of HIV-specific cells following vaccination and interestingly, we were able to distinguish good responders from the poor ones. In all vaccinees, the percentage of antigen-specific Tregs ($CD25^+CD134^+CD39^+FoxP3^+$) inversely correlated with the percentage of IFNγ-producing cells. When Tregs were depleted prior to stimulation with HIV-derived antigens, we observed an increase in IFNγ, IL2 and TNFα-responses. All together, these data reveal that Tregs

represent an important fraction of HIV-specific T-cells. Thus the ability to measure the inducibility of Tregs prior to vaccination is important for more efficient antigen-incorporation strategies in future vaccines.

1100B. The Adaptor Protein TOLLIP is Associated with Pediatric Tuberculosis Susceptibility in South Africa

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Regulation of Toll-like receptor (TLR)-mediated signaling pathways is a critical mechanism for control of tuberculosis (TB). We previously demonstrated that common variation in human Toll-Interacting Protein (TOLLIP) negatively regulates TLR2 signaling, alters the cytokine response to TB, and is associated with susceptibility to adult TB. Due to the importance of innate immunity in childhood TB, we hypothesized that TOLLIP variation is associated with susceptibility to TB in infants. We performed a candidate gene association study in infants who were vaccinated with BCG at birth and prospectively followed for 2 years to determine if they developed TB disease. We enrolled 232 TB cases and 620 controls and genotyped 18 haplotype-tagging SNPs that spanned the TOLLIP genomic region. Four SNPs were associated with susceptibility to TB in a genotypic model ($P<0.05$). The associations for all four SNPs fit a dominant model with three associated with protection (rs5743890, rs5743854, rs7481967, OR 0.41-0.66, P=0.002-0.037) and one with increased susceptibility (rs5743915, OR 1.54, P=0.01). The associations remained significant after adjusting for gender and ethnicity. Linkage disequilibrium was low - moderate among these 4 SNPs, implying multiple susceptibility loci (pairwise R^2 0.02-0.68). Our data suggests that common variants in the TOLLIP gene are associated with susceptibility to TB across multiple ethnicities. Studies are ongoing to determine the causal SNP and the mechanism underlying TOLLIP's regulation of TB immunity. This study illuminates an important role for TOLLIP in the innate immune response to TB and suggests a new avenue for drug therapy and vaccine adjuvants.

1101A. Negative Regulators of Human B Cell Response: Age, CMV, TNF-a, and MiRs

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Our laboratory previously showed autonomous B cell defects and decreased serum antibody response to the influenza vaccine (by HAI, hemagglutination inhibition) in human subjects 60 years of age and over. Higher initial serum TNF-a and TNF-a in ex vivo unstimulated B cells predicted lower B cell function, measured by activation-induced cytidine deaminase (AID) in stimulated cells in vitro, and we showed that this correlates well with the lower serum response to the vaccine. We hypothesized that the increased pro-inflammatory status of the elderly, including CMV (with sera positive for IgG anti-cytomegalovirus) would negatively impact B cell function. From the 2011/2012 and 2012/2013 vaccine seasons, CMV seropositivity was positively associated with lower in vivo and in vitro vaccine responses in both 36 young and 26 elderly individuals. CMV-specific IgG was also associated with increased serum TNF-a and age. Our next hypothesis was that particular microRNAs (miRs) could contribute to decreased AID in elderly B cells. We show preliminary data that AID mRNA stability is lower in elderly B cells, and we measured miR-155, 16, and 93 as they are implicated in germinal center formation and AID expression. All three miRs were increased in unstimulated B cells from the elderly and negatively associated with AID in activated B cells from the same individuals. Mir-155 was negatively associated with HAI. These results contribute to novel molecular pathways generating reduced effective antibody in the elderly and suggest targets for further vaccine development.

OR.5. Immune Perturbation in patients with TGFbeta pathway defects

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Rationale: Knowledge of TGFβ regulation of the immune system stems predominantly from animal and in vitro studies. Heterozygous mutations in TGFbR1, TGFbR2 and SMAD3 have been associated with familial thoracic aortic aneurysms and aortic dissections (TAAD). These patients offer an opportunity to study their immune development when the TGFβ pathway is

defective. Methods: Flow cytometry was used to analyze PBMC from patients with TAAD (n=9) and age-matched healthy controls (HC, n=8). Th1 and Th17 were determined with intracellular cytokine staining for IFNg and IL17A. Foxp3+ Tregs were detected with anti-Foxp3 (259D). CD19+ were analyzed for naive (IgD+CD27-), unswitched (IgD+CD27+) and switched memory (IgD-CD27+). Plasmacytoid (CD303+pDC) and myeloid (CD1c+mDC) were defined within lineage-1 negative population. Results: %CD3-CD16+NK, CD3+CD16+NKT, CD4+, CD8+ and CD4+CD45RA+ in TAAD were similar to HC. Average %CD19+ (20.8vs7.3, p=0.006) and naive B cells (81.3 vs 66.6, p=0.004) were higher in TAAD. The unswitched were similar but the switched B cells were lower (8.6 vs 15.5, p=0.01). While the %Tregs were similar, there was a remarkable reduction (1/2-3 folds) in Foxp3 concentration based on median fluorescence intensity of Foxp3 in TAAD. There was a significant reduction of %Th17 (0.14 vs 0.61, p=0.01), while the Th1 were similar. %pDC (9.4 vs 24.1, p=0.009) and %mDC (11.4 vs 17.9, p=0.01) were also lower in TAAD. Conclusions: These results demonstrate for the first time in humans of the involvement of TGFβ signaling in B cells, DCs, Th17 and Treg development. Further studies and monitoring of the clinical effects of these immunological perturbations in these TAAD patients are needed to appreciate the impact of their underlying disease.

OR.7. Retonic Acid Regulates T-helper Cell Differntiation an Plasticity

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Upon antigenic stimulation, naïve CD4 T cells differentiate into T-helper cell subsets characterized by distinct cytokine profiles. Expression of master regulator transcription factors control T-cell fate decisions. Classically, Th subsets were considered fixed within their lineage however recent evidence suggests that Th-cells retain a degree of flexibility in their cytokine profiles. The factors that govern Th plasticity are only partially determined. Previously we showed that retinoic acid was critical for effector immunity in a model of allograft rejection. However the cellular and molecular mechanisms underlying this block were unidentified. Here we show that retinoic acid (RA) controls differentiation of Th1 cells. Th1 cells with genetic disruption of RA signaling, exhibit enhanced plasticity. Alterations in levels of *Tbx21* results in enhanced Gata3 expression in vitro and RORgt in vivo, Furthermore, mice with genetic disruption of RA signaling within the CD4+ T-cell compartment exhibit impaired antigen specific Th1 responses to *Listeria monocytogenes*, establishing the functional importance of the RA signaling pathway. This study demonstrates a critical role for retinoic acid in T-helper cell fate decisions through regulation of lineage determining transcription factors in differentiated Th1 cells and confirms the dominance of the master regulator Tbet in suppressing alternative T-helper cell lineages. Th-cell plasticity has been implicated in autoimmune diseases and the RA signaling pathway is an attractive therapeutic target for modulation of Th responses in disease.

OR.14. CREM Promotes Th17 Differentiation and Dependent Autoimmune Pathology

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CREM alpha is a transcription factor induced in human and mouse T cells by cAMP. T cells from patients with the autoimmune disease systemic lupus erythematosus (SLE) express abnormally high levels of CREM which causes abnormal cytokine production. For example, CREM promotes IL-17 production but inhibits IL-2 production through distinct epigenetic remodeling of the respective loci. Nevertheless, little is known about the role of CREM in the activation process of T cells. Here, we addressed the role of CREM in the differentiation of T cells into the Th17 effector subset. In vitro Th17 differentiation was impaired in T cells from CREM deficient animals whereas IL-2 production was significantly increased in T cell cultures of CREM deficient mice when compared to cells from CREM sufficient animals. Therefore, we speculated that increased IL-2 production inhibited Th17 differentiation in cultures from CREM deficient cells. To test the importance of this observation in an *in vivo* setting, we induced experimental anti-glomerular basement membrane antibody glomerulonephritis (AIGN) and experimental autoimmune encephalomyelitis model (EAE) in CREM deficient animals. Clinical scores for nephritis and encephalomyelitis were diminished in CREM deficient mice compared to clinical scores recorded in CREM sufficient animals. Collectively, our results suggest that CREM is a transcription factor that participates in the Th17 differentiation process *in vitro* and mitigates Th17-dependent autoimmune pathology.

W.2. Organ T-cell Profiling in a Mouse Model of Polymicrobial Sepsis
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Sepsis is defined as a systemic inflammatory response that can lead to multiple organ dysfunction and lethality as well as secondary infections due to associated immune-suppression. Among immune cells in responding to sepsis, macrophages and neutrophils have been extensively studied, while the contribution of T lymphocytes and natural killer T (NKT) cells is not well characterized. Here we monitored the change of T and NKT cell populations in various lymphoid and non-lymphoid organs of mice after induction of sepsis. To study this, male C57BL/6 mice were subjected to cecal ligation and puncture (CLP), a polymicrobial sepsis model and 20 h later organs were harvested. Total lymphocyte count showed a significant 73% reduction in thymus and a 41.1% reduction in CLP spleen compared to sham. Thymic $CD4^+CD8^+$ lymphocyte subset showed 81.9% reduction in absolute cell numbers after CLP. Splenic $CD4^+$ T and $CD8^+$ T lymphocyte numbers were also reduced by 45.5% and 36.5%, respectively. By contrast, the frequency of $CD4^+CD25^+$ regulatory T (Treg) and $CD69^+$ activated $CD4^+$ T in CLP spleen was significantly increased by 1.7-fold and 2.5-fold, respectively. In contrast to spleen, after CLP hepatic $CD8^+$ T cell numbers were significantly increased by 2.6-fold, whereas hepatic NKT cell numbers were reduced by 71.1%, but they were more activated with increased CD69 and CD25 expression. In the lungs, the lymphocyte numbers were decreased by 81%. Overall, this study provides important information on the trafficking of lymphocytes during sepsis and identification of potential targeted T-cell populations for a better management of sepsis.

W.10. A Novel *ex vivo* HIV Entry Assay to Identify Early HIV Target Cells in the Female Genital Tract
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The cellular targets of HIV infection in the female genital tract (FGT) are poorly defined, but the correlates of susceptibility may include the early activation marker CD69, HIV co-receptor CCR5, and the mucosal homing integrin alpha4beta7 We developed a novel *ex vivo* assay to identify T cell targets of HIV entry in the FGT. Blood and cervical specimens were incubated *ex vivo* with R5-tropic HIV pseudovirus incorporating beta-lactamase-vpr in its capsid, which cleaves a fluorescent reporter dye upon viral entry. HIV entry in various T cell subsets was analyzed 12h post-infection. Viral entry was specific to cervical CD4 T cells, and blocked by CCR5-inhibitor maraviroc but not by CXCR4-inhibitor AMD3100. Cervical $CD69^+$ CD4 T cells had 1.9-fold higher HIV entry than $CD69^-$ CD4 T cells ($p=0.001$), possibly due to 2.4-fold higher CCR5 expression ($p<0.0001$). Similarly, cervical $alpha4beta7^+$ CD4 T cells had 1.6-fold higher viral entry than $alpha4beta7^-$ CD4 T cells ($p=0.02$), while CCR5 expression was 1.9 fold greater ($p=0.0006$). Cervical CD4 T cells had 2.8-fold greater HIV entry compared to blood ($p=0.0003$) and HIV entry correlated between the two compartments in matched participants ($r^2=0.56$, $p=0.0004$). In summary, we developed a rapid and sensitive assay to quantify HIV entry into cervical CD4 T cells; this assay demonstrates that activated CD4 T cells and $alpha4beta7^+$ CD4 T cells are targets of R5-dependent HIV entry in the FGT.

W.19. Dynamic Changes in Human Regulatory T Cell Populations with Age in Lymphoid and Mucosal Tissue Sites
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The function of regulatory T cells (Treg) in humans and their distribution in tissue sites over time remains unclear. In collaboration with the New York Organ Donor Network, we have established a protocol allowing access to multiple primary and secondary lymphoid as well as mucosal tissue sites from individual organ donors over a broad age range. Using this resource, we can uniquely examine Treg populations in tissue sites over decades of life. Strikingly, the frequency of CD25+CD127-FOXP3+ Tregs decreases significantly over the human lifespan comprising nearly 30% of the CD4 T cell pool in infants and below 10% in individuals over the age of 20, coincident with decreased FOXP3 staining intensity. Monitoring double positive CD4 and CD8 T cell populations in the thymus, we found marked cessation of functional thymopoiesis in donors beginning at age of 40, indicating that Treg populations decrease prior to waning thymic function while naïve T cells

are maintained for decades. The decrease in Treg proportion is coincident with an increasing frequency of memory T cells in tissue sites. Furthermore, Tregs are differentially compartmentalized in children versus adults with high frequencies in the small intestines during infancy and only low levels of Tregs in intestinal sites throughout adulthood. Together these results indicate a tissue intrinsic and age-dependent pattern of T reg compartmentalization in the human suggesting that Tregs may be more functional in maintaining immune homeostasis early in life.

W.39. Human Cytokine Defined B cell Subsets Regulate Pathogen-associated T cell Responses

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Increasing evidence indicates that cytokine-defined B cell subsets play important roles in animal models of several human diseases. We and others have previously identified several cytokine-defined human B cell subsets including IL-10 producing B cells(Breg) and novel subset we describe as $TNF^{high}IL\text{-}6^{+}GM\text{-}CSF^{+}$ B effector(Beff) cells. However the function of these subsets in human is still poorly understood. In this study, we wished to determine how these subsets may regulate antigen specific T cell responses. We first developed a novel antigen-specific human B and T cell co-culture system, using either *Candida albicans* or *Staphylococcus aureus* as the antigen. Interestingly, B cells promoted *C.albicans* associated Th17 response, while in the *S.aureus* system B cells mainly induced Th1 responses. We further found that MHC-II and co-stimulatory molecules expressed by B cells are essential for B cells to promote pathogen-associated T cell responses, as functionally blocking these molecules almost totally abrogated T cell responses. Furthermore, using polarized B cell subsets, we found that Beff strongly enhanced naïve T cell differentiation toward Th1/Th17 as compared to un-polarized-B-cell and Breg, which was dependent on the higher CD80 and CD86 expression on Beff cells. We also show that IL-6 secretion from Beff cells is involved in *C.albicans* induced Th17. Finally, using PBMCs collected from patients with MS who underwent B-cell-depletion treatment, we demonstrated that pathogen-associated T cell responses are strongly decreased after B cell depletion in *vivo.* Here, we show that distinct cytokine-defined human B cell subsets can importantly contribute to, and shape pathogen-associated T cell responses.

T.34. Organ T-cell Profiling in a Mouse Model of Polymicrobial Sepsis

Sepsis is defined as a systemic inflammatory response that can lead to multiple organ dysfunction and lethality as well as secondary infections due to associated immune-suppression. Among immune cells in responding to sepsis, macrophages and neutrophils have been extensively studied, while the contribution of T lymphocytes and natural killer T (NKT) cells is not well characterized. Here we monitored the change of T and NKT cell populations in various lymphoid and non-lymphoid organs of mice after induction of sepsis. To study this, male C57BL/6 mice were subjected to cecal ligation and puncture (CLP), a polymicrobial sepsis model and 20 h later organs were harvested. Total lymphocyte count showed a significant 73% reduction in thymus and a 41.1% reduction in CLP spleen compared to sham. Thymic $CD4^{+}CD8^{+}$ lymphocyte subset showed 81.9% reduction in absolute cell numbers after CLP. Splenic $CD4^{+}$ T and $CD8^{+}$ T lymphocyte numbers were also reduced by 45.5% and 36.5%, respectively. By contrast, the frequency of $CD4^{+}CD25^{+}$ regulatory T (Treg) and $CD69^{+}$ activated $CD4^{+}$ T in CLP spleen was significantly increased by 1.7-fold and 2.5-fold, respectively. In contrast to spleen, after CLP hepatic $CD8^{+}$ T cell numbers were significantly increased by 2.6-fold, whereas hepatic NKT cell numbers were reduced by 71.1%, but they were more activated with increased CD69 and CD25 expression. In the lungs, the lymphocyte numbers were decreased by 81%. Overall, this study provides important information on the trafficking of lymphocytes during sepsis and identification of potential targeted T-cell populations for a better management of sepsis.

F.51. Bacterial DNA Promotes IgA Production in Newborn Infants

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The absence of mucosal immunoglobulin A (IgA) in the intestinal tract renders very young infants highly susceptible to enteric infections. Moreover, most vaccines administered to newborns and young infants induce limited IgA responses and require multiple boosters to be effective. Therefore, the identification of conditions that can enhance the development of protective IgA early in life is essential. We found the novel combination of the bacterial DNA analogue CpG combined with both soluble (IL-21, IL-4) and cognate (anti-CD40) T cell factors (TCF) promoted the production of IgA from newborn infant B cells in a direct and CpG dose-dependent manner. CpG and TCF co-stimulation not only increased the expression of known factors that promote IgA class switching and B cell differentiation (e.g., AID, Blimp-1 [from RNA seq]), but increased CpG binding and expression of toll-like receptor 9 (TLR9), a receptor for CpG, on newborn infant B cells as well. Indeed, IgA production required the engagement of and signaling through TLR9 by CpG. Moreover, TLR9 engagement increased B cell surface expression of both IL-21 receptor and CD40. Thus, exposure to bacterial DNA may enhance responsiveness of newborn infant B cells to T cell help and induce IgA production. We propose that bacterial DNA or its analogues (e.g., CpG) may serve as effective adjuvants to promote mucosal IgA responses to vaccines and to provide early protection against enteric infections in very young infants.

F.52. *T. CRASSICEPS – P. YOELII* CO-infection the Outcome Depends on the Moment of the Second Infection

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In malaria endemic regions people are often exposed to several pathogens at the same time. Currently it has been suggested that immune response already present due to an in progress infection may influence the immune response to second challenge. To test this we developed a murine model of co-infection with *Taenia crassiceps* (Tc) and *Plasmodium yoelii* 17XL (Py) parasites. BALB/c mice are able to develop Th1 or Th2 immune response at different times after Tc infection. Here we explored if the profile of cytokines in the *in vivo* environment of a Tc parasitized host modifies the outcome of a second infection with Py. BALB/c mice were i.p. infected with ten non-budding cysticerci of Tc and after 2 or 8 weeks were coinfected, via i.v., with 10^3 Py parasitised red blood cells. Py infection showed low serum levels of IL-12p40, IL-4 and high levels of IFN-γ which were associated with increased mortality. In contrast, mice were protected when co-infection was performed after Tc infection: Coinfected mice after 2 weeks of Tc infection showed reduced mortality, associated to high levels of IL-1β, IL-12 and TNF-α. However, high pathology was present. Interestingly, co-infection at 8 weeks increased the synthesis of Th1/Th2 cytokines (IL-4, IL-10 e IL-12), this was associated to low inflammatory response, thereby inhibiting the pathology that increased survival. Our data suggest that a mixed Th1/Th2 profile supports the resistance to *Plasmodium*. Thus when a parasitized animal is challenged with a new pathogen the course of the second infection can be modified.

F.53. A unique population of IgG-expressing plasma cells lacking CD19 is enriched in human bone marrow

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Specific serum antibodies mediating humoral immunity and autoimmunity are provided by mature plasma cells residing in the bone marrow (BM). We here characterize a unique subset of plasma cells lacking surface expression of CD19 that show previously described characteristics of murine long-lived plasma cells. Unlike $CD19^+$ plasma cells, $CD19^-$ plasma cells were restricted to bone marrow, expressed predominantly IgG and a pro-survival and distinctly mature phenotype, i.e. HLA-DR^{low}Ki-

67^{-}CD95^{low}CD28^{+}CD56$^{+/-}$*bcl-2*^{high} and resisted their mobilization from the bone marrow after systemic vaccination. Fewer mutations within $V_HDJ_HC_\gamma$ gene rearrangements of CD19^{-} bone marrow plasma cells compared to their CD19-positive counterparts in blood and bone marrow indicate limited re-activation of their precursors. Likewise, the resistance of CD19^{-} bone marrow plasma fells to *in vivo* B cell depletion, i.e. their independency from supply with new plasmablasts is consistent with a long-term stability of this plasma cell subset in the bone marrow. Moreover, CD19^{-} plasma cells were detectable in chronically inflamed tissues and secreted autoantibodies. We propose a multi-layer model of plasma cell memory in which CD19^{+} and CD19^{-} plasma cells represent dynamic and static components, respectively, allowing for both adaptation and stability of humoral immune protection.

F.54. Co-expression of 2B4 and PD1 Delineates a Dysfunctional CD8+ T cell Subset with Increased Frequencies in HTLV-1 Infected Asymptomatic Carriers

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HTLV-1(Human T-lymphotropic virus-1) infection is a worldwide burden endemic in Japan amongst others in which carriers persist without clinical symptoms and are termed asymptomatic carriers. In the response to viral infections, CD8+T cells are important and its function affected by the presence of co-inhibitory receptors including 2B4(Natural killer cell receptor, CD244) and PD1(Programmed death cell receptor, CD279), molecules associated with CD8+Tcell exhaustion. To characterize the modulation of these receptors in HTLV-1 infection and the effect of intercepting the receptor-ligand interaction on effector function, we performed flow cytometric analyses of the co-expression of 2B4 and PD1 on HTLV-1 infected asymptomatic carriers and seronegative controls. We observed primarily high convergent staining for both 2B4 and PD1 in HTLV-1 infected significantly different from uninfected subjects. Similarly, in the prevalent immune-dominant HLA restricted epitopes, HLA-A*02 and A*24, further upregulation of co-expressed 2B4 and PD1 on Tax specific CD8+T cells, which were predominantly 2B4+PD1+; in contrast to co-expression on HLA- restricted CMVpp65 CD8+T cells. On interrupting the receptor-ligand interaction using blocking antibodies to the ligands we observed an improvement of effector CD8+T cell function.These results suggest a distinct dysfunctional CD8+Tcell subset co-expressing both 2B4 and PD1. High co-expression on CD8+Tcells and further upregulation on Tax-specific CD8+T cells is consistent with a role in the immune-regulation in response to HTLV-1 infection. The homogeneity observed in 2B4 and PD1 co-expression may identify similarly exhausted population. Improvement in function demonstrates a role in inhibition of Tcell response and may pave way for translation into therapeutic intervention.

F.55. NKT-cell Activation by Lipophosphoglycan (LPG) During *Leishmania mexicana* Infections of BALB/c Mice

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Leishmania mexicana can show diverse clinical manifestations, ranging from a simple cutaneous ulcer “Chiclero´s ulcer” to a dissemination of the disease. Although the cause of disemination remains unknown, IFN-γ has been shown to confer host protection. One of the innate cells capable of producing IFN-γ is the NKT cell, which can produce both IFN-γ and IL-4 after recongizing glycolipids presented by CD1d in dendritic cells (DC). The role of NKT cells has not been analyzed in *L. mexicana* infections.We here analyzed IFN-γ and IL-4 production by NKT cells of cervical lymph nodes in BALB/c mice infected in the earlobes with *L. mexicana.* Furthermore, we analyzed whether *L. mexicana* LPG is presented by CD1d in DC cells of the lymph nodes after *in vivo* infections. We found a 5 fold (2.5%) increase of NKT cells in lymph nodes after 3 days of infection, as compared to non-infected controls (0.5%). The number of NKT cells producing IL-4 was higher (8%), as compared to IFN-γ-producing cells (1%). The number of CD11c^{+} CD1d^{+} cells presenting LPG in lymph nodes on days 1 y 3 post-infection with *L. mexicana* was 8% (CD11c^{+}, CD1d^{+}, LPG^{+}). With electron microscopy we proved co-localization of CD1d and LPG in DC after *in vitro* infection with *L. mexicana*.We propose that NKT cells can be activated by *L. mexicana* LPG presented by CD1d in DC and that IL-4 produced by NKT cells during *L. mexicana* infections contributes to the disease susceptibility of BALB/c mice. This work was supported by PAPIIT IN215212

F.56. Quantification and Characterization of CD45+ Cells in Human Healthy Dental Pulp

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Healthy dental pulp is a very dynamic tissue that has the ability to respond to external aggressions. However, there are many gaps in our current understanding of pulpal immune responses to caries. The aim of this study was to identify and quantify key cells of immune response among the CD45+ cells of healthy human dental pulp using cell flow cytometry. Healthy human third molars were collected from young patients after obtaining informed consent. The dental pulp tissues were removed and prepared in order to perform flow cytometry analysis (FACS), or immunostaining. We demonstrated by FACS that 1,04% ±0,76 of human dental pulp are CD45+. Among these cells, we quantified Natural Killer Cells 2.63% ±1.15, T lymphocyte 32.58% ±11 and B lymphocyte 1.65% ±0.89. Furthermore, we showed the presence of Treg and our data evidenced also cells with compatible DC-10 phenotype (CD45+, HLA-DR+, CD14+, CD16+, CD83+, CD163+). Confocal microscopy revealed expression of HO-1 or IL-10 on HLA-DR positive cells. These data may participate to improve knowledge of the immunopathologic mechanism of pulpitis. Additionnaly, we demonstrated the presence of cells having a phenotype compatible with Treg and DC-10 recently described in human tissues as cells with high immunoregulatory properties. Therefore, it may be interesting to characterize more precisely those cells in the dental pulp as they could represent potential targets for future therapeutic strategies.

F.57. Epstein-Barr Virus Infection of Humanized Mice Causes CD8⁺ T Cell Exhaustion

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Epstein-Barr virus (EBV) infection and EBV-associated malignancies are underrepresented fields of research despite affecting millions of people worldwide. While EBV infection mainly occurs asymptomatically in children in the third world due to earlier viral exposure, people in the Western world often contract EBV in their second decade of life or later as a severe primary infection called infectious mononucleosis (IM) that is slow to resolve and poses significant health risks. EBV⁺ individuals are at increased risk of malignancies such as Hodgkin's lymphoma and post-transplant lymphoproliferative disease. Poor understanding of the in vivo pathogenesis of EBV and the lack of treatments available present a significant unmet medical need, mainly due to the strict human tropism of this virus. EBV-specific CD8⁺ T cells from IM patient samples appear to express PD-1, a receptor that has been associated with poor immune control by T cells in a phenomenon known as exhaustion. We therefore wondered whether T cell exhaustion plays a role in EBV pathogenesis in vivo. We have examined human CD8⁺ T cell exhaustion in EBV infection of NOD/scid/γ-chain deficient mice that have been reconstituted with human CD34⁺ cells. These animals reconstitute most major human immune compartments. In response to EBV infection, they exhibit viral loads and expand CD8⁺ T cells. Interestingly, CD8⁺ T cells in infected animals upregulate multiple surface inhibitory receptors that have been associated with T cell differentiation or dysfunction, including PD-1, BTLA, KLRG1, 2B4, and Tim-3. Efforts to determine the role of these receptors in EBV infection and tumorigenesis are currently underway.

F.58. SAP Is Critical for Antigen-Induced Developmental Programming of Naive CD8 T Cells

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Mutations in *SH2D1A* gene that encodes SAP (SLAM-associated protein) cause X-linked lymphoproliferative disease (XLP), a primary immunodeficiency defined by exquisite sensitivity to Epstein-Barr virus (EBV) that results in fulminant, often fatal, infectious mononucleosis, massive expansions of EBV-infected B cells and malignant B cell lymphomas. However, the mechanism of how deficiency of SAP results in susceptibility to EBV remains unclear. Given the unique B cell tropism of EBV and the suspected role of SAP in regulating lymphocyte/lymphocyte interactions, we hypothesized that SAP expression within CD8 T cells was essential for mounting optimal responses to antigen-expressing B cells. To test this hypothesis, we compared activation of CD8 T cells from wild type and SAP-deficient (*Sh2d1a*$^{-/-}$) mice after stimulation by B cells and other antigen-presenting cells (APCs). Wild type and *Sh2d1a*$^{-/-}$ CD8 T cells responded strongly when stimulated *in vitro* with cognate antigen-coated whole splenocytes. However, compared to wild type CD8 T cells, *Sh2d1a*$^{-/-}$ CD8 T cells had greatly diminished proliferation and effector functions when cultured with antigen-coated B cells *in vitro* or challenged by antigen-expressing B

cell lymphoma *in vivo*. Together, our results indicate that SAP is required for the priming of CD8 T cells when B cells act as the primary APC. This CD8 T cell defect may be responsible for the selective immune dysregulation of XLP boys after exposure to EBV.

F.59. Novel Adjunctive Immunomodulatory Therapy Improves Lung Immunopathology and Survival of Mice with Severe Secondary Bacterial Pneumonia

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Among serious infections, acute lower respiratory tract infections are the leading cause of morbidity and mortality in the United States. Many patients progress from influenza infections to severe, secondary bacterial pneumonia (SBP) carrying a high case fatality rate despite adequate antibiotic use. The increased mortality observed in mouse models during bactericidal antibiotic treatment of SBP was previously attributed to the lung inflammatory response, aggravated by rapid release of bacterial cell wall fragments. We recently showed that adjunctive corticosteroid therapy could improve lung immunopathology and survival of mice with severe SBP. However, corticosteroids had drawbacks in the study related to decreased suppression of viral replication, putting their proposed use into question. Therefore, we tested the efficacy of a novel immunomodulatory pro-drug, 2,3-diacetyloxybenzoic acid (DABA), for treating SBP. Ampicillin plus mock treatment rescued only 60% of mice with mild SBP and could not rescue mice with severe SBP. However, adjunctive DABA therapy with ampicillin rescued all mice with mild SBP and significantly increased the survival rate of mice with severe SBP to about 70%. Furthermore, adjunctive DABA therapy did not worsen the morbidity of mice with mild SBP, implying a better treatment outcome compared to corticosteroids. Early DABA treatment during influenza infection did not increase the morbidity of influenza-infected mice suggesting a better safety profile in virus-infected hosts than does early corticosteroid therapy. Taken together, adjunctive DABA therapy is a promising approach to treatment of SBP. Investigating its mechanism of action will elucidate key processes regulating lung homeostasis during treatment of SBP.

F.60. Lack of IRAK4 Leads to Systemic Abscess Formation and Early Lethality But Does Not Impair Autoimmunity in Act1-Deficient Mice

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Act-1 deficient B6 mice develop a lupus-like disease characterized by elevated levels of serum anti-nuclear autoantibodies (ANA) and glomerulonephritis. Act1 is a negative regulator of BAFF- and CD40-mediated signaling, and hence controls B cell survival and activation processes. In this study we investigated if disease development in B6.Act1$^{-/-}$ mice was dependent on IL1/TLR-mediated signaling, as previously shown for BAFF-transgenic mice. We generated B6.Act1$^{-/-}$IRAK4$^{-/-}$ (DKO) mice and studied disease development in cohorts of mice up to 7 months of age. Surprisingly, we found that almost all double-deficient animals died within the first couple of weeks after weaning (90% mortality at 9 weeks of age). Already at 5-6 weeks of age, DKO mice presented with splenomegaly, accumulations of transitional B cells and elevated serum ANA similarly to, but not exceeding, levels seen in B6.Act1$^{-/-}$ mice. In addition, we found significant accumulations of granulocytes in spleen, bone marrow and the circulation of DKO mice. Interestingly, we observed abscess formation throughout the body of several DKO mice - a feature until then only observed in the oral cavity of B6.Act1$^{-/-}$ mice. Microbial analyses revealed *Staphylococcus and Streptococcus Spp.* in the majority of abscesses. Antibiotic treatment completely abrogated the development of abscesses and the accumulation of granulocytes, but had no effect on serum ANA levels and B cell abnormalities. Thus, IRAK4-deficiency failed to affect the development of autoimmunity in B6.Act1$^{-/-}$ mice, but precipitated an underlying primary immunodeficiency driven by Act1-deficiency.

F.61. Increased Urinary Tract Infection Susceptibility in Estrogen Receptor Alpha Knock-out Mice Involves Expression of TNFR 2 in the Bladder and Kidney

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Hormonal changes during menstrual cycle, pregnancy or menopause contribute to differential susceptibility to ascending urinary tract infections (UTI) including cystitis and pyelonephritis, suggesting estrogen as an important etiological factor. Using Dr adhesin bearing *E. coli* in an experimental ascending UTI model, our recent studies have shown an increased UTI susceptibility in estrogen receptor alpha (ERα) knock-out ERα$^{-/-}$ mice. ERα$^{-/-}$ mice showed significantly increased infection in bladder and kidneys than wild-type (ERα$^{+/+}$) mice, and delayed kinetics of pro-inflammatory cytokine production including TNFα at day 3 and day 7 post-infection ($P<0.05$). Role of TNF receptors (TNFR) 1 and 2 in UTI is not known. Therefore, we hypothesized that ERα regulates TNFR activation in the bladder and kidneys modulating UTI pathogenesis. Using immunohistochemistry, we investigated TNFRs 1 & 2 protein expression in the bladder and kidneys of mice at day 3 and 7 post-UTI. TNFRs 1 and 2 were differentially expressed on day 3 and 7 post-infection in ERα$^{-/-}$ vs ERα$^{+/+}$ mice. Transient increase in TNFR 2 expression was observed in bladder and kidney tissues of ERα$^{-/-}$ mice that remained significantly high compared to ERα$^{+/+}$mice, indicates its possible role in disease progression and histopathology seen in these mice. Thus, TNFRs 1 & 2 may serve as important markers for inflammation in ascending UTIs. This study provides the first in-vivo evidence indicating the importance of TNFR 2 expression in the urinary tract that may be regulated by ERα during ascending UTI. Selective ligands for TNFRs may serve as novel therapeutic agents for treating UTIs.

F.62. The Role of IL-17 in the Immune Response during Experimental Pulmonary Tuberculosis

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Tuberculosis is a chronic bacterial infection that produces immunological abnormalities. In man and mice cell mediated immunity is essential for tuberculosis protection and it has been reported that Th17 cells and interleukin-17 contribute to protection, but they can also produce tissue damage by excessive recruitment of neutrophils.The aim of this work is to define if IL-17 is related to protection or tissue damage using a murine model of progressive pulmonary tuberculosis.Groups of BALB/c mice were infected with virulent *Mycobacterium tuberculosis* strain H37Rv. Mice were evaluated for survival, and lungs were collected at different days post-infection for microbiological, histopathological and immunological studies determining the IL-17 expression and local production by RT-PCR and immunohistochemistry respectively. In a second part of the study, infected mice were treated with neutralizing antibodies against IL-17 at different days post-infection during the pre-determined period of increased production of this cytokine. Lungs were collected at day 21 post-infection for the same studies mentioned above.Infected lungs showed since the first day IL-17 production, essentially by airways epithelium and endothelium, at day 14 and 21 positives cells were detected in the perivascular and peribronchial infiltrates and in granulomas. The gene expression kinetics showed the maximal IL-17 transcription at day 14 post-infection. In comparison with control animals, infected mice treated with IL-17 neutralizing antibodies showed high bacilli burden and lesser inflammatory infiltrate. The production of IL-17 in these treated animals was reduced. These results suggest that IL-17 is related to protection in this model of progressive pulmonary TB.

F.63. Study of the Regulatory Response in a Murine Model of Progressive Pulmonary Tuberculosis

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Mycobacterium tuberculosis (Mtb) is the etiologic agent of tuberculosis (TB). Mtb infect one third of the world´s population and annually causes the death of 1.4 million persons. Many biological and immunological aspects of TB are not completely elucidated, such as the complex process of immunoregulation mediated by the enzymes hemoxygenase 1 (HO-1) and indoleamine 2,3-dioxygenase (IDO). When BALB/c mice are infected with a high dose of Mtb strain H37Rv by the intratracheal route a rapid progressive disease is developed. This model has two phases, the early phase characterized by activation of the innate (defensins) and acquired (Th-1) immunity, and the progressive phase that is characterized by a high production of Th-2 cytokines (IL-13, IL-4), IL-10 and TGF, which suppress the activity of Th-1 cells permitting high bacterial proliferation. In this work we studied the kinetics of HO-1 and IDO along the infection. In the lungs of tuberculous animals during the progressive phase there was high gene expression and production of HO-1 and IDO particularly in macrophages. In order to study the activity of HO-1 and IDO was inhibited using the specific inhibitors, zinc protoporphyrin-9 (ZnPP9) to suppress HO-1 and 1-

methyl-DL-tryptophan (1-MT) to block IDO. In comparison with control animals, mice treated with any of these molecules showed a significant decrease of bacilli loads improving survival but they developed higher lung inflammation. Thus, immune-regulation mediated by HO-1 and IDO is important to control excessive inflammation, but it decreases protective immunity, permitting bacilli proliferation and disease progression.

F.64. Antigen Excreted/Secreted from *Taenia crassiceps* Modulate the Development Experimental Colitis

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Colitis is an inflammatory disease characterized by a strong Th1 response. Interesting, helminth infections induce a response anti-inflammatory (Th2) which has been associated with different immunoregulatory effects. In this sense, in previous studies we described that infection with *Taenia crassiceps* decreases considerably the development of experimental colitis. The aim of this study was assess the antigens excreted/secreted by *Taenia crassiceps* (TcE/S) in in the development of experimental colitis. We induced colitis in mice through oral administration of dextran sulphate sodium (DSS) 4%, DSS+TcE/S, TcE/S was administered intraperitoneally and as negative control use only PBS. The mice were weighed daily and sacrificed 9 days, TNF-α levels were determined in serum, peritoneal cells were obtained to determine alternatively macrophages activated (AAM); also, the colon was removed to measure the length. On the other hand, tissue damage is assessed by histology, as AAM markers arginase-1, Ym-1 and TNF-α was determined by PCR, monocyte infiltration in the tissue was determined by flow cytometry. We found that mice with DSS reduce weight quickly and colon structure is lost, and there is a strong infiltration of neutrophils. Additionally, the levels of TNF-α increased. Whereas that, the mice DSS+TcE/S remained constant weight, colon preserved the structure without the presence of fibrosis and decreases slightly the $CD11b^{+}Ly6G^{+}$ neutrophils, meanwhile, the levels $CD11b^{+}Ly6C^{+}$ monocytes tends to increase in the tissue; also, we found markers arginase-1, Ym-1 of AAM. Moreover, in the peritoneum determine AAM. In conclusion, we identified that the antigen TcE/S decreased inflammatory processes in the experimental colitis.

F.65. Characterization of Biphenotypic B/Macrophage cells *in Vitro* and in Pneumonic *Francisella*

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Biphenotypic B/Macrophage cells are a unique cell type derived from B-cells that co-express both B and macrophage cell surface markers (B220, CD19, IgM, F4/80, and CD11b). Following treatment with M-CSF and GM-CSF, splenic B-2 B cells can be induced to transition into biphenotypic cells. As the cells transition, the expression of CD19 becomes down-regulated, CD11b and F4/80 become up-regulated, and B220 and IgM remain static. RNA microarray analysis of *in vitro* biphenotypic cells demonstrates the expression of several monocyte and lymphocyte chemotactic receptors. To evaluate the presence of these cells in an infectious model, B6 mice were intratracheally inoculated with the LVS strain of *Francisella tularensis,* a pulmonary pathogen. Biphenotypic cells within the pulmonary tissue were significantly elevated as compared to uninfected mice on days 10 and 14 ($p<0.0001$, $p<0.001$, respectively) post-infection, which corresponded to the resolution of disease. Further functional studies are underway to elucidate the role of biphenotypic cells in disease, which may provide the premise for devising therapies to enhance or attenuate their development.

F.66. Lymphopenia, Leukocytosis and Hypochromic Anaemia in the Pregnants with Syphilis

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Blood cells are the most important factors of favorable course and pregnancy maturation, normal functions of uteroplacental surface. Investigation of the indicators of hospital laboratory blood samples of 61 pregnant women with early latent syphilis has been carried out in terms 24-40 weeks of pregnancy in Lviv Clinical Regional Perinatal Center in 2007-2013. The basic investigative groups comprised 30 patients with untreated syphilis and 31 treated women. The control group comprised 30 healthy pregnant women in the same terms. The blood cells counts changes in the pregnants with syphilis have been found out. The Hgb levels have been 101,73+/-3,15 g/L ($P\leq0,05$) in untreated women, 110,88+/-2,15 g/L ($P\leq0,05$) in treated pregnants contra 117,84+/-2,2 g/L in healthy pregnants. The RBC counts were accordingly $3,23+/-0,16*10^{12}$/L ($P\leq0,05$),

3,34+/-0,08*10^{12}/L (P≤0,05), 3,7+/-0,07*10^{12}/L. The WBC counts were accordingly 11,12+/-0,76 *10^9/L (P≤0,05), 10,62+/-0,66 *10^9/L (P≤0,05) contra 8,83+/-0,38*10^9/L. Neutrophil granulocytes composed accordingly 73,54+/-1,36 %, 74,88+/-1,32 % and 74,84+/-1,59 %. Lymphocytes composed 16,57+/-1,2 % (P≤0,05) in untreated patients, 16,84+/-1,43 % in treated women contra 19,67+/-1,42 % in control group. Monocytes composed accordingly 1,77+/-0,48 %, 1,5+/-0,26 % and 1,62+/-0,3 %. Conclusion. Disturbance of blood cells counts have been designated in the pregnants with syphilis, which are displayed as relative lymphopenia, leukocytosis and hypochromic anaemia.

F.94. Prolactin regulates the cytokine immune response and signaling pathways triggered by *Mycobacterium bovis* in THP1 monocytes

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The immunomodulatory functions of prolactin (PRL) are well recognized. Augment PRL plasma levels were observed in patients with advanced tuberculosis. Recently, we have reported that LPS and *M. bovis* induce differential expression of prolactin receptor (PRLR) isoforms in THP-1 cells and bovine macrophages, respectively. The aim of this work was to determine whether PRL should be considered as a potential modulator of the signaling pathways and cytokine synthesis, induced by culture filtrate protein (CFP) from *M. bovis* in THP-1 monocytes. The THP-1 cells were stimulated by PRL (20ng/mL), *M. bovis* CFP (50 μg/mL). PRLR as well as, phosphorylated STAT3, STAT5, Akt1/2/3, ERK1/2 and p38 expression, were evaluated by western blot. IL1-β, TNF-α, IL-6, IL-12, IL-8 and IL-10 concentrations were measured by ELISA. Our results demonstrate that the expression pattern of PRLR short isoforms is induced by *M. bovis* CFP. PRL only activated the JAK2/STAT3-5 signaling pathway, while *M bovis CFP* induce phosphorylation Akt2, ERK1/2, p38, Stat3 and STAT5 pathways. However, when combined both stimulus, PRL significantly increased the p-STAT3-5 expression and downregulated the Akt2, ERK1/2, p38 phosphorylation. As expected, *M. bovis* CFP induced major amounts of IL1-β, IL-6, TNF-α, IL-8, IL-12 and IL-10; the PRL costimulus considerably decreased IL1- β, TNF- α and IL-12 secretion, however increased IL-10 production. This results suggest that up-regulation of IL-10 by PRL could be modulating the pro inflammatory response during a mycobacterial stimulus and may be mediated through the JAK/STAT-3 pathway.

Immunodefiency- Primary or Acquired

1108B. Human Hematopoietic Stem Cells with a Defined Immunodeficiency and Enteropathy Transfer Clinical Phenotype to a Novel Humanized Mouse Strain

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The development of immunodeficient mouse strains has facilitated human immune reconstitution in murine hosts. However, adaptive immune responses are generally weak in models lacking autologous fetal thymic grafts. We modified the widely utilized NOD.*Prkdc*^{scid}.*Il2rγ*$^{-/-}$ (NSG) strain to be deficient for murine MHCII and instead expressed human HLA-DR1 under the control of the murine MHCII promoter (NSGAb^{o}DR1) and tested whether adaptive immune responses are improved following reconstitution using human HLA-matched CD34$^+$ hematopoietic stem cells (HSCs). Furthermore, we reconstituted these mice using HSCs from a patient with IPEX syndrome, a well-defined immunodeficiency caused by a mutation in *FOXP3*, to see whether the clinical phenotype would transfer to NSGAb^{o}DR1 mice. **Results:** We found that NSGAb^{o}DR1 mice had an

increase in T cell clonotype diversity compared to NSG mice and displayed a delayed-type hypersensitivity response. NSGAb^{o}DR1 mice had an increase in mature B cells and antibody class switching. We reconstituted NSGAb^{o}DR1 and NSG mice using HSCs from a patient with IPEX syndrome and all NSGAb^{o}DR1 mice succumbed to systemic inflammation whereas NSG mice remained viable. IPEX mice developed lymphocytic infiltrate in lung, liver, and skin similar to scurfy mice, which also harbor a mutation in *Foxp3*. **Conclusions:** We demonstrate that human HSCs from a patient with a defined genetic immunodeficiency can transfer the disease phenotype to a novel humanized mouse strain. This strain does not require human fetal grafts nor is it restricted to autologous fetal HSCs permitting reconstitution using HSCs with defined genetics to study human immunopathologies *in vivo*.

OR.21. Human Lymphoid Development in the Absence of Common Gamma Chain Receptor Signaling

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Despite the power of model systems to reveal basic immunologic mechanisms, critical differences exist between species that necessitate the direct study of human cells. Illustrating this point is the difference in phenotype between patients with Severe Combined Immune Deficiency (SCID) caused by mutations affecting the common gamma chain (g_c) cytokine signaling pathway and mice with similar mutations. Although in both species, null mutations in either *IL2RG* (which encodes gc), or its direct downstream signaling partner *JAK3*, result in T and NK cell deficiency, an associated B cell deficiency is seen in mice but not in humans with these genetic defects. In this study, we applied recent data that have revised our understanding of the earliest stages of lymphoid commitment in human bone marrow, to determine the requirement for signaling through IL2RG and JAK3 in normal development of human lymphoid progenitors. Bone marrow samples from SCID patients with IL2RG (n=3) or JAK3 deficiency (n=2), which produce similar "T-NK-B+" clinical phenotypes, were compared to normal bone marrow and umbilical cord blood as well as bone marrow from children on enzyme treatment for adenosine deaminase deficient SCID (n=2). In both IL2RG- and JAK3-SCID patients, the early stages of lymphoid commitment from HSC were present with development of Lymphoid-primed Multipotent Progenitors, common lymphoid progenitors and B cell progenitors, and normal expression patterns of IL7RA and TLSPR, and DNA recombination genes *DNTT* and *RAG1*. Thus, in humans, signaling through the gc pathway is not required for pre-thymic lymphoid commitment or for DNA rearrangement.

W.43. A Novel Genetic Autoimmune Disorder of Chronic Pulmonary Disease and Arthritis Resulting from Impaired ER-Golgi Function

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Congenital genetic immune defects are often characterized by autoimmunity. Here we report a novel autoimmune syndrome characterized by chronic pulmonary disease with pulmonary hemorrhage and other autoimmune features such as arthritis and nephropathy. Using whole exome sequencing of affected individuals from three separate families we have identified distinct autosomal dominant mutations in a gene involved in protein transport between the Golgi and ER. We show, using primary patient cells and cell lines in which we recapitulate the mutations, that these loss of function mutants result in an increase in ER stress, a defect in autophagy, and an increase in the size of cellular endo-lysosomes. These cellular defects have been described in other autoimmune diseases, such as systemic lupus erythematosus and Chron's disease. Here we show, for the first time a causal relationship between a mutant protein involved in Golgi-ER transport and an induction of auto-immunity. In conclusion we propose that aberrant intracellular transport leading to ER stress triggers impaired autophagy, resulting in dis-regulation of immune function and an autoimmune disorder.

W.56. Identification and Characterization of Two Novel Splice Site Mutations in the *SERPING1* Gene in Two Non-related Families with Hereditary Angioedema

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Hereditary angioedema due to C1-inhibitor deficiency (HAE-C1INH) is a rare autosomal-dominant disease caused by mutations in *SERPING1* gene. The main clinical feature of C1INH deficiency is sudden spontaneous edema of the submucosal layers. More than 280 different mutations scattering the entire *SERPING1* gene have been reported. We identified and characterized two novel splice site mutations in *SERPING1* gene in two non-related Spanish families with hereditary angioedema: In the first family we found the c.685+2T>A mutation, which disrupts the donor splice site of intron 4 leading to the loss of exon 4 in mutant mRNA. We demonstrated that mutant mRNA is mostly degraded, probably by the surveillance pathway no-go mRNA decay. Bioinformatic modelling analysis indicated that the mutant protein, if produced, would be non-functional since the it lacks a stretch of 45 amino acids included in the functional RCL loop. Finally, we found a reduction of the wild-type mRNA expression in c.685+2T>A carriers. In the second family we found the c.1249+5G>A mutation, presumably also affecting the splicing process. Sequencing of the patient's cDNA did not showed only the wild type sequence was present. However, taking advantage of the presence of a heterozigous SNP in this coding region we demonstrated that the c.1249+5G>A mutation leads to haploinsufficiency presumebly by degradation of the mutant allele.

W.57. Identification and Molecular Characterization of Two New Mutations Causing C5 Deficiency in Two Un-related North-African Families

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Complement C5 deficiency (C5D) is a rare autosomal recessive defect associated to recurrent Neisseria spp infections. We report the cases of two unrelated patients of North African origin, (P1) and (P2) who had suffered of septicemia by uncommon serotypes (E29 and Y) of *Neisseria meningitidis*. C3 and C4 were normal but activation through the classical (CH50) and the alternative pathways (AP50) was reduced. In addition, neither C5 nor its fragment sC5b9 could be detected. Haemolytic activity was restored by the addition of purified C5 to patients' sera. Sequencing of C5 showed that P1 carried a homozygous deletion of 2 nucleotides in exon 21 (c.2607_2608delAA) leading to a frameshift and a truncated protein of 872 aa (compared to normal 1676 aa reference sequence) and P2 carried a homozygous deletion of 3 nucleotides in exon 9 (c.960_962delCAA) leading to deletion of one aa (Asn320). Both mutations were novel, and the parents of both patients were identified as heterozygous carriers. These results illustrate the molecular heterogeneity of this disorder. Systematic studies will be required to establish the real prevalence of this deficiency in the different populations including this little studied African group. As with other immunodeficiencies, the analysis of new cases and genotype phenotype correlations can contribute to understand better the role of complement in protection from infection.

W.58. *Gene Therapy for X-Linked Agammaglobulinaemia (XLA) using a Humanised Mouse Model*

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XLA is a primary immune deficiency resulting in an absence of B cells and antibody production due to mutations in the Btk gene. Treatment requires lifelong immunoglobulin replacement and carries significant morbidity making gene therapy an attractive possibility. The aim of this study is to directly test reconstitution B cell ontogeny in patient-derived $CD34^+$ cells in a humanized mouse model following lentivirus-mediated gene transfer. To date we have (i) established the minimum wild-type

CD34+ cell dose required to reconstitute the B cell compartment in NSG mice using Busulphan conditioning, (ii) constructed and functionally validated a lentiviral vector encoding the human Btk cDNA under the transcriptional control of the ubiquitous EF1a promoter, (iii) explored transduction conditions, including the optimal cytokine cocktail and vector multiplicity of infection (MOI), and (iv) undertaken an initial reconstitution experiment using BTK-deficient patient CD34+ cells. In this latter experiment we observed early evidence of B cell reconstitution, albeit at low levels. In parallel experiments we have identified a number of challenges that include loss CD34+ CD19+ progenitors during conventional cytokine stimulation and the need to ensure that the total number of gene-modified XLA patient-derived bone marrow progenitors reaches the engraftment threshold established in NSG mice with wild-type cells. In further studies we are exploring transduction conditions that retain CD34+ CD19+ progenitors, the relative contribution of this population to B cell reconstitution, and strategies for optimizing CD34+ cell transduction efficiency. These studies have the potential to generate valuable pre-clinical efficacy and safety data for a human clinical trial.

W.59. Combined Immunodeficiency Associated with Homozygous *MALT1* Mutations

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Background: Defining the genetic cause of human primary immunodeficiency disorders can be lifesaving for affected patients, and provides unique opportunities to study specific genes affecting the development and regulation of human immune systems. Combined immunodeficiency disorders are a spectrum of human diseases affecting cellular and humoral immune responses. They are often caused by defective lymphocyte activation, and can be clinically challenging in both diagnosis and treatments. Objective: To discover the genetic cause of a novel combined immunodeficiency associated with immune dysregulation and a hyper IgE phenotype. Methods: We combined whole exome sequencing with detailed functional immune analysis and genetic reconstitution assays to determine the cause of this novel primary immunodeficiency. Results: Immunological characterization was notable for: subtle alterations in T cell numbers but the absence of proliferation and IL-2 secretion following TCR stimulation; normal IgG, IgM and IgA levels with chronically elevated IgE; and very low peripheral B cell numbers with a developmental block in B cell maturation. Whole exome sequencing revealed the causal mutation in mucosa associated lymphoid tissue lymphoma translocation gene 1 (*MALT1*) that was associated with profound deficiency in MALT1 protein and loss of both paracaspase proteolytic activity and the molecular scaffold function central to the CARMA1–BCL10–MALT1 (CBM) complex formation. The patient's primary T cells had a defect in both IκBα degradation and NF-κB p65 subunit phosphorylation, and this ability to activate NF-κB was rescued by artificial expression of the normal MALT1 protein. Conclusion: Mutations in *MALT1* represent a novel cause of human combined immunodeficiency.

W.60. The Contributions of MicroRNA-205 and its Surrounding Long, non-coding RNA, MIR205.001, in the Specification of the Thymus, and their Connection to DiGeorge Syndrome

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DiGeorge syndrome comprises the cohort of 22q11.2 deletion syndrome patients with a defined T cell lymphopenia. The lymphopenia results from impaired specification of the thymic epithelial tissue during embryogenesis, resulting in a low T cell output. These developmental abnormalities occur within the pharyngeal apparatus, an embryonic region that forms the thymus, the parathyroid glands, and the great vessels of the heart. T-box transcription factor (Tbx1) is a key regulator of pharyngeal morphogenesis, and its haplopinsufficiency in patients with 22q11.2 deletion syndrome can result in cardiac anomalies, hypoplastic thymii and/or hypoparathyroidism. Our preliminary profiling of thymic tissues from these patients revealed a deficiency of miR-205. MiR-205, principally detected in epithelial cells of the thymus and skin, is embedded within a long non-coding RNA, MIR205HG. *In situ* hybridization and RT-PCR revealed its precise spatial temporal expression within the 3rd pharyngeal pouch and the developing thymus. A knockout of miR-205 causes a proliferative defect in epithelial cells, which results in embryonic and post-natal lethality. We will characterize the role of miR-205 and the long non-coding RNA in thymopoiesis using miR-205 and MIR205.001 conditional knockout mice. These lines are being crossed with the Foxg1-Cre

(pharyngeal pouch expression) and Foxn1-Cre recombinase (thymic epithelial cells) lines. We will relate the developmental abnormalities in these mice with the clinical presentations described for 22q11.2 deletion syndrome patients. Results from our study could reveal novel approaches to restore thymopoiesis in patients undergoing stem cell transplants and chemoablative therapies, and for the elderly, who have an age-dependent thymic atrophy.

W.61. Identification of Novel Genes in Human Primary Immunodeficiency Diseases Using Exome Sequencing

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Primary immunodeficiencies are rare in the human population, characterized by a broad clinical and genetic heterogeneity and their genetic etiology is very difficult to decipher. More than 150 genes have been linked to various forms of primary immunodeficiencies (PID) and most of these are the consequence of a monogenic defect and hence follow a simple mendelian inheritance. In this study we investigated two siblings from a consanguineous family who presented at an early age with prominent manisfestations of warts and cryptosporidium sclerosing cholangitis, and profound T cell lymphopenia. We performed exome sequencing on all the members of the family and analysed the RAW sequence data using Genome Analysis tool kit (GATK). We hypothesized an autosomal recessive mode of inheritance and utilized several filtering strategies to distinguish potentially deleterious mutations from other variants and identified a novel homozygous mutation in the splice site region of DOCK8 gene in the two affected siblings. Flow cytometry revealed marked reduction in DOCK8 protein expression in the two affected siblings. RNA-SEQ analysis of whole blood from the patients showed significant reduction in mRNA levels of DOCK8 gene as well aberrant splicing of DOCK mRNA in the two probands. More functional studies and in depth analysis of splice variants of DOCK8 gene in the affected siblings are underway. This finding further supports that whole exome sequencing has great potential for identifying novel genetic lesions in patients with primary immunodeficiency of unknown genetic etiologythat will help us better understand the biology of the immune system.

W.62. Immunodeficiency, Endocrinopathy, and Lymphoma Associated With a Novel ITK Missense Variant c. 329C>T Clinical Case Report

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The Tec family of non-receptor tyrosine kinases are implicated in modulating immune cell receptor signaling. The Tec family includes 5 family members including IL-2-Inducible T-cell kinase (ITK), TXK, BTK, TEC, and BMX. Mutations in other members of this family have been implicated in immunodeficiency. Here we report the first clinical description of a ITK missense variant associated with immunodeficiency, endocrinopathy and lymphoma. A 25 year old female presented to clinic with type 1 diabetes diagnosed at 18 months of age, history of seizures, immune thrombocytopenia purpura previously treated with splenectomy, and hypogammaglobulinemia with poor antibody responses to both polysaccharide and protein antigens prompting initiation of immunoglobulin replacement therapy. Recurrent lymphadenopathy ensued and the patient was noted to have CD8+ T lymphopenia, protracted Herpes Simplex Virus 2 genotype refractory to acyclovir, Human Papilloma Virus prompting cervical Loop Electrosurgical Excision Procedure, and Epstein Barr Virus (EBV) infection, with subsequent identification of EBV + Hodgkin Lymphoma lesions in the thoracic and lumbar spine and left iliac crest. Further work is necessary to clarify to role of ITK variants with mechanisms into immunodeficiency, endocrinopathy, and lymphoma.

W.63. Unraveling hFOXP3 Function in Physiological and Pathological Lymphoid Cell Development

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Peripheral immune tolerance is controlled by T regulatory (Treg) cells. Mutations in FOXP3 cause severe dysfunctions in Treg cells and lead to IPEX syndrome, a life-threatening autoimmune disease. Recent studies in IPEX patients suggest that FOXP3-mutated Treg cells can differentiate, although dysfunctional and unstable, and that also T effector cells can be dysregulated in the absence of FOXP3, possibly contributing to the disease pathogenesis. Aim of this work is to determine the role of FOXP3 in shaping human lymphoid cell development and define the best gene transfer strategy to be adopted to restore immune tolerance. We have established *in vitro* and *in vivo* models to study lymphoid development of hematopoietic stem/progenitor cells (HSCs) genetically modified to over-express or down-regulate FOXP3 expression. *In vitro* models, including OP9-DL1 co-culture, showed that FOXP3 constitutive expression affects HSC differentiation/proliferation and skews CD4/CD8 differentiation. By testing different strains and conditioning, we have shown that neonatal intra-liver injection of cord blood-derived HSCs into sublethally irradiated NOD/SCID common g-chain$^{-/-}$ (NSG) mice give rise to human hematopoietic system encompassing a significant fraction of CD3+ T cells, including naïve and memory T effector and T regulatory subsets. Preliminary *in vivo* data would confirm *in vitro* findings and further suggest a wide role for FOXP3 in T cell lineage commitment. These results will help understanding IPEX pathogenesis and assessing the feasibility of HSC-based therapeutic strategies for this disease. Moreover, the development of this disease model might serve as a pre-clinical model for autoimmune and lympho-proliferative disorders.

W.64. Targeting HIV Peptide/HLA Class I Complexes Using T Cell Receptor Mimics for Immunotherapy

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Despite advances in anti-retroviral therapy, Human Immunodeficiency Virus (HIV-1) infection and the resulting Acquired Immunodeficiency Syndrome (AIDS) remains a prominent global health concern. It has previously been demonstrated that inducing an HIV-1-specifc cytotoxic T lymphocyte (CTL) response represents a viable therapeutic course to prevent and reverse HIV-1 pathogenesis. As such, numerous HIV-1 CTL epitopes have been identified; however, we are not aware of any epitopes that have elicited long-term protective immunity in vaccine studies. With the aid of a mass spectrometry direct discovery approach, an HLA-A*11:01 presented peptide derived from Nef was identified and designated SR11. We then sought to generate a T cell receptor mimic (TCRm) antibody with specificity to the SR11/A*11:01 complex that demonstrated therapeutic potential. Balb/c mice were immunized against SR11/A*11:01, and B cell hybridomas were selected for target specificity by high throughput enzyme-linked immunosorbant assay (ELISA). Peptide pulsed target cells stained positively for the SR11/A*11:01-reactive antibody, suggesting that the TCRm was capable of recognizing an endogenously presented SR11 target. The resulting monoclonal antibody was further characterized by staining HIV-1 infected PBMCs and *in vitro* cytotoxicity assays. Overall, these results underscore the potential therapeutic value of TCRm technology against HIV-1 infection using directly identified epitopes within the context of the HLA complex.

W.65. New Case of Isolated C7 Deficiency. The Heterogeneous Disease (OMIM #610102)

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Complement deficiency is a rare disease account for 2.5% as extracted from Data collected from International Registries. C7 deficiency is even rarer. It is reported sporadically in the literature with no full genetic understanding. We are reporting a 19 year old Qatari male patient, referred to Immunology Service on April 2012 for history of recurrent bacterial meningitis. Meningococcal Infection sensitive to ceftriaxone was isolated from blood culture. Procalcitonin and lactic acid were also elevated in this admission. Initially patient was admitted to ICU. Fortunately he recovered completely from the infection. The

patient gave history of two meningitis episodes before. The former was at the age of 7 and the other was on 16 May 2011. No organism been identified in both admissions. One sibling died in 2009 with meningitis. CH50 and AH50 were requested and CH50 was low. Terminal Complements level and functional assay (not available in Qatar) requested and revealed C7 deficiency. Patient received two injections of Quadri-valent Conjugated Vaccines two months apart. Prophylactic antibiotics (azithromycin) orally are given once weekly. Patient and his family are not willing for any genetic study. To the best of our knowledge, no previous complement component C7 deficiency was reported from the Arabian Gulf Countries. Around 21 different molecular defects leading to total or subtotal C7 deficiency defects have been reported in the literature. This indicates that the molecular bases for C7 deficiency are heterogeneous, because different individuals have different molecular defects.

Immunodermatology

W.20. Phenotypic and Functional Characterization of Tissue-infiltrating B Cells in Cutaneous Neoplasia

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Lymphocyte homing to tissue sites is mediated via expression of tissue-specific homing receptors. Co-expression of CLA and CCR4 on T cells has been identified as essential for migration from the peripheral circulation to cutaneous sites. B cells have recently been shown to comprise the lymphocytic infiltrate in some chronic inflammatory skin diseases, as well as in cutaneous melanoma. We therefore aimed to investigate the phenotype of skin-homing B cells and their possible roles in melanoma pathogenesis. Using flow cytometry, we analyzed peripheral blood (PB) from healthy donors (n=45) where we identified a population of $CD20^+$ B cells that expressed the homing markers CLA and CCR4. B cells formed two distinct populations expressing either CLA or CCR4 alone. The CLA+ but not the CCR4+ population was found to be predominantly $IgG/IgE/IgA^+CD22^+CD27^+$, indicative of a population of class-switched mature memory B cells primed to home into the skin. Melanoma patients had a statistically higher proportion of PB CLA^+B cells (n=63) compared with healthy donors (n=45; p=0.02). Comparison of $CD20^+CLA^+$ B cell infiltrates in cutaneous metastatic melanoma biopsies and matched blood samples from the same patients (n=10) revealed a significantly enriched population (p=0.03) within cutaneous melanomas. In-vitro functional assays using peripheral blood mononuclear cells revealed that the CLA+ B cell subset produced interferon gamma (IFN-γ), and tumour necrosis factor alpha (TNF-α) when co-cultured with metastatic melanoma cells (A375), indicating their pro-inflammatory capabilities in the context of cancer. Our findings highlight previously unappreciated roles for B cells in cutaneous immune responses.

T.90. Improvement in the CDASI activity score of patients with dermatomyositis is associated with a better quality of life

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Dermatomyositis (DM) skin disease activity correlates with a worse quality of life. This prospective study assessed whether an improvement in quality of life, as measured by the Skindex-29 and patient-reported itch and pain on a 10-point visual analog scale (VAS), correlated with an improvement in cutaneous DM disease activity. Patients with a completed cutaneous DM disease area and severity index (CDASI) at two visits separated by at least two months were classified into responder (n=15) and non-responder (n=30) groups according to the point change in the CDASI activity scores between visits. Responders had at least a four-point improvement in CDASI, indicating clinically relevant improvement. The Wilcoxon rank sum test compared the change in each score between visits. For the responders, the median Skindex-29 emotions score decreased from 45 to 25 (P=0.004), functioning score from 15 to 4 (P=0.006), and symptoms score from 50 to 14 (P=0.002). Changes in the median Skindex-29 scores of non-responders were not statistically significant: emotions from 33 to 30 (P=0.76), functioning from 13 to 17 (P=0.13), and symptoms from 32 to 43 (P=0.65). The median itch score of responders decreased from 2.8 to 0.5 (P=0.03). There was no significant difference in itch scores for non-responders or in pain scores in either group. Variations between responders and non-responders in disease subtype, sex, race, age, and treatment for DM were not statistically significant.

This is the first study to demonstrate that the quality of life of patients with DM increased with improvement in their cutaneous disease activity.

Immunology of the Eye

T.86. IL-2/IL-2 Ab complex plus rapamycin ameliorate experimental autoimmune uveoretinitis associated with expansion of $CD4^+Foxp3^+$ regulatory T cells

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Purpose: To determine whether injection of IL-2/anti-IL-2 (JES6-1) complex (IL-2C) together with rapamycin is effective in reducing ocular inflammation in experimental autoimmune uveoretinitis (EAU). Methods: C57BL/6J mice were immunized with human interphotoreceptor retinoid binding protein peptide (h-IRBP1-20). Mice were injected i.p. with PBS, IL-2C (JES6-1), rapamycin, or IL-2C plus rapamycin on days 1, 2, 3, and 4 after immunization. Fundus examination was performed on days 9, 12, 15, 18, and 21 after immunization. The expression of Foxp3 on $CD4^+$ T cells from draining lymph nodes (LNs) was examined by flow cytometry on days 7, 12, and 21. Results: Injection of IL-2C plus rapamycin significantly reduced the clinical score of EAU on days 12, 15 and 18 compared to PBS, IL-2C, or rapamycin only treated mice. In addition, mice treated with IL-2C plus rapamycin showed decreased severity of EAU by histopathological analysis. Although the expansion of $CD4^+Foxp3^+$ regulatory T cells (Tregs) from draining LNs was observed on day 7 in IL-2C, rapamycin, and IL-2C plus rapamycin treated mice, the expansion of Tregs was most prominent in IL-2C plus rapamycin treated mice. The production of IFN-g and IL-6 in draining LNs was significantly reduced in IL-2C plus rapamycin treated mice. Conclusions: These results indicate that injection of IL-2C plus rapamycin is effective in reducing ocular inflammation in EAU and in inducing the expansion of $CD4^+Foxp3^+$ Tregs. In vivo expansion of T regs using IL-2C plus rapamycin may have clinical potential for the treatment of human refractory uveitis.

T.87. Analysis of Circulating Effector and Regulatory B Cell Subsets in Autoimmune Retinopathy Patients

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Autoimmune retinopathy (AIR) is clinically characterized by rapidly progressive vision loss distinct from retinitis pigmentosa. Laboratory results typically demonstrate an association with antibodies that react against multiple retinal proteins, suggesting that ocular immune tolerance has been compromised. Many patients have marked improvements to vision following immune suppressive treatments, but the pathogenic role of the immune system in AIR remains poorly understood. In the present study, the peripheral blood mononuclear cells of patients with AIR were analyzed for alterations in T and B lymphocyte subsets and responses to mitogenic stimulation. Although untreated AIR patients had remarkably similar levels of circulating $CD4^+$ and $CD8^+$ T cell subsets and total $CD19^+$ B cells compared to a panel of healthy controls, an increase of $CD27^+$ (memory) B cells was detected in the blood of AIR patients. This included a $CD27^+CD24^{low}$ subset of B cells that was nearly absent in controls but which can be detected at similar levels in the circulation of patients with multiple sclerosis. Response to treatment with immune suppression was associated with a relative increase of $CD24^{high}CD38^{high}$ regulatory B cells in the blood, and increased production of IL-10 from purified B cells following stimulation with CD40 ligand. Studies are ongoing to compare the presence or absence of B cell subsets with the titers of anti-retinal antibodies in patient serum and T cell responses to mitogenic stimulation.

T.88. Elevation of $CD14^+CD16^{++}$ monocyte subset is associated with systemic corticosteroid therapy in autoimmune uveitis

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Human peripheral monocytes have been categorized into three subsets: classical $CD14^{++}CD16^{-}$; intermediate $CD14^{++}CD16^{+}$; and nonclassical $CD14^{+}CD16^{++}$, based on differential expression levels of lipopolysaccharide receptor CD14 and Fcγ receptor III CD16. Accumulating evidence suggests that monocytes could trigger and polarize T cell responses during autoimmune conditions and infection. However, the distribution of human subsets of monocytes in non-infectious autoimmune uveitis is not well studied. Here we showed that patients with non-infectious autoimmune uveitis display a profound skewing towards $CD14^{++}CD16^{+}$ subset ($P<0.01$), compared to healthy controls. The skewing of monocyte subsets correlated with systemic corticosteroid therapy, but was independent of patient age, sex, associated systemic disease and uveitis activity. Furthermore, we demonstrated that $CD14^{++}CD16^{+}$ monocytes inhibited T cell proliferation induced by other subsets, and tended to induce memory $CD4^{+}$ T cells to produce more IL-10. Our results elucidated a clearly defined feature of $CD14^{++}CD16^{+}$ monocytes in regulating T cell function, and underline its potential value as a marker for evaluating the treatment effect in autoimmune uveitis.

T.89. IL17A+CCR6(low)CD4+ T cells Predict Steroid Resistance In Noninfectious Autoimmune Uveitis

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Glucocorticoids are used as immunosuppressive agents in autoimmune disease but 30% of patients fail to respond adequately. Previous studies have shown steroid resistant asthma is mediated by IL17A producing CD4+ T cells, these cells were also found to express CCR6. This study investigated the role of IL17A-producing CD4+CCR6+ T cells in 23 patients with noninfectious uveitis enrolled from the NEI clinic. If their intraocular inflammation was controlled at daily doses below 10mg oral prednisone monotherapy they were defined as steroid-sensitive (SS), if higher daily doses were needed to control their uveitis they were defined as steroid-resistant (SR). Peripheral blood mononuclear cells were isolated from whole blood and stimulated in anti-CD3/28 coated 6-well plates for 3 days. The ratio of IFN-gamma/IL17A on total CD4+ T cells was decreased in SR patients, indicating an increased proportion of IL17A-producing CD4+ T cells in SR. These CD4+ T cells were characterized by their CCR6 expression into a high and low group. Although levels of IL17A+CCR6(high)CD4+ T cells were not significantly different between SR and SS patients, IL17A+CCR6(low)CD4+ T cells were significantly increased in SR patients($p < 0.008$). IFN-gamma did not show a difference in SR and SS patients. In conclusion, CCR6(low)CD4+ T cells producing IL-17A are highly associated with the clinical characteristic of steroid resistance in noninfectious uveitis patients. The presence of IL17A-producing CCR6(low)CD4+ T could be used as a biomarker to predict which patients will respond less favorably to steroid treatment.

Immunoncology

1102A. A Novel Approach to Enhance and Redirect the Anti-Leukemia Properties of Invariant Natural Killer T Cells

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B-cell malignancies are among the commonest cancers affecting children and adults and occur more frequently in patients with immunodeficiency. To improve the outcome for patients with advanced B-cell malignancies, we are developing alternative therapeutic approaches that capitalize on the anti-tumor functions of invariant natural killer T cells (iNKTs), innate-type T lymphocytes that directly kill tumor cells and exhibit robust capacity to trans-activate the anti-tumor functions of dendritic cells (DC), natural killer (NK), T and B cells. Maximal tumor-directed iNKT cell responses require tumor cell expression of the antigen-presenting molecule CD1d. However, many tumors down-regulate CD1d and thus evade iNKT cell recognition. To circumvent this critical barrier, we have generated a novel soluble fusion protein to direct iNKTs to the site of B-cell cancers in a tumor antigen-specific yet CD1d-independent manner. This fusion protein is comprised of a human CD1d molecule joined to single chain antibody FV (scFV) fragment specific for CD19, a B cell antigen expressed on most B-leukemias. Once loaded with the iNKT cell lipid agonist alpha-galactosylceramide (aGC), the CD1d-CD19 fusion induces *in vitro* and *in vivo* activation of iNKTs and robust cytokine production. iNKTs activated by the aGC-loaded CD1d-CD19 fusion also potently induce T, B and

NK cell activation and DC maturation. Importantly, the aGC-loaded fusion induces robust iNKT cell lysis of CD19+ tumor targets, which are completely resistant to iNKT cell killing under normal conditions. Collectively, these data suggest that the aGC-loaded CD1d-CD19 fusion is capable of "linking" iNKTs and CD19+ targets in a therapeutically beneficial manner.

OR.8. Combined Immune Checkpoint Protein Blockade and Lymphodepletion as Immunotherapy for Myeloma

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Multiple myeloma is characterized by the presence of transformed neoplastic plasma cells in the bone marrow and is generally considered to be an incurable disease. Successful treatments will likely require multi-faceted approaches incorporating conventional drug therapies, immunotherapy and other novel treatments. We previously showed that a combination of transient lymphodepletion (sublethal whole body irradiation) and PD-1/PD-L1 blockade generated anti-myeloma T cell reactivity capable of eliminating established disease. We hypothesized that the anti-myeloma effect of transient lymphodepletion and PD-1/PD-L1 blockade would be increased by blocking other immune checkpoint protein interactions. A temporal phenotypic analysis of bone marrow and spleens from myeloma-bearing mice was performed to examine T cells for expression of immune checkpoint proteins and presence of immune regulatory T (Treg) cells. PD-1, 2B4, LAG-3, and TIM-3 were the most prominent immune checkpoint proteins present on T cells in myeloma-bearing mice. We found that Treg cells in the tumor microenvironment also had increased expression of PD-1 and other inhibitory receptors; this could potentially be related to enhanced suppressive function by these cells. Finally, we addressed the hypothesis that survival of myeloma-bearing mice treated with lymphodepleting whole body irradiation could be improved by blocking more than one immune checkpoint protein. When PD-L1 blockade was combined with blocking antibodies to LAG-3, TIM-3 or CTLA-4, synergistic or additive increases in survival were observed (survival rates improved from ~30% to >80%). These data indicate that partial lymphodepletion and blockade of multiple immune checkpoint proteins is a promising combinatorial strategy for myeloma and other hematologic malignancies.

W.9. Biomarker Discovery in Multiple Myeloma Using Monoclonal Lamprey VLR Antibodies

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The adaptive immune system of jawless vertebrates uses antigen receptors known as variable lymphocyte receptors (VLRs) which, unlike immunoglobulins, comprise leucine rich repeats as the basic structural unit. We hypothesize that VLR antibodies, due to their distinct protein architecture and evolutionarily distant origins, may allow us to target antigens to which conventional antibodies cannot easily be raised for structural or tolerogenic reasons. Here, we propose to use VLR antibodies for biomarker discovery in multiple myeloma (MM), a malignant disorder characterized by accumulation of monoclonal plasma cells in the bone marrow. Prognoses for MM patients are poor due to the near inevitability of tumour regrowth after initial therapy. A subpopulation of tumour cells have a stem cell-like phenotype in that they can resist chemotherapeutic agents and induce relapse (or tumour recapitulation on allotransplantation). Lamprey larvae were immunized with drug-resistant MM model cell lines, followed by extraction of lymphocyte RNA, generation of VLR cDNA libraries, and cloning into a yeast surface display vector. A series of enrichment steps were carried out to preferentially retain VLR clones specific for surface antigens on the MM cell lines; screening of the enriched libraries has resulted in a panel of monoclonal antibodies that bind the target cell lines and a variety of other lymphocyte cell lines and primary lymphocyte populations. Ongoing work with these antibodies focuses on correlating their binding patterns to clinical parameters in primary malignant samples and ultimately to identify their antigens, which would present potential targets for diagnostic, prognostic, or therapeutic purposes.

W.13. Increased Levels of Multiple Inflammatory Factors and Myeloid Derived Suppressor Cells (MDSCs) in Blood of Stage IV Breast Cancer Patients

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Bone marrow-derived myeloid immune cells are known to infiltrate malignant tumor sites in large numbers, and are often a prominent feature in the tumor-surrounding stroma. In addition to their role in the inflammatory response, recent studies have shown these cells to have important roles in driving crucial events in the metastatic process such as invasion, migration, and angiogenesis. Specific attention has been drawn to myeloid-derived suppressor cells (MDSCs), as linkers of inflammation, immunosuppression, and metastatic progression. The presence of MDSCs in the bloodstream of breast cancer patients is strongly associated with poor prognosis. We hypothesize that factors produced by metastatic tumors alter the host immune system leading to this systemic recruitment of specific populations of MDSCs. Using multiplex assays we have surveyed and measured over 80 different factors including cytokines and known cancer biomarkers in serum of stage IV breast cancer patients and of healthy donors. In these samples, levels of MDSCs [Lin(-/Lo)HLA-DR(-)CD33(+)CD11b(+)] were significantly higher in breast cancer patients than in healthy individuals. Although the number of patients is at this point is limited (19), our preliminary data show that stage IV breast cancer patients exhibited higher level of inflammatory markers compared to healthy individuals. Together with an increased level of circulating MDSCs, inflammatory factors or the combination of several of them may be of potential diagnostic and therapeutic value

W.14. Cancer-testis Antigen Expression is Shared Between Epithelial Ovarian Cancer Tumors

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Cancer-testis (CT) antigens have been proposed as potential cancer immunotherapy; however their presence is known to be heterogeneous with no single antigen universally expressed. Our objective was to evaluate the expression of a panel of 20 CT antigens in epithelial ovarian cancer (EOC) tumors and determine if there is antigen sharing between tumors, using Real Time-PCR analysis. Sixty two EOC specimens, 8 ovarian cancer cell lines and 3 benign ovarian tissues were evaluated. The majority of the specimens were: high grade (62%), serous (68%), and advanced stage (73%). Fifty eight (95%) of the EOC tumors analyzed expressed at least one of the CT antigens evaluated. The median number of CT antigen expressed was 4 (0-17). Higher grade tumors expressed 3 or more CT antigens (72% of grade 2/3 vs 25% of grade 1, p: 0.04). The most frequently expressed CT antigens were MAGE A4 (67%), SP17 (47%) and GAGE (47%). No single CT antigen was universally expressed. We found that: 9 tumors shared only one antigen with 62% of the specimens, 5 tumors shared up to 2 antigens with 74%, 11 tumors shared up to 3 antigens with 71%, and 37 tumors shared 4 or more antigens with 82%. 5 tumors expressed over 10 CT antigens, which were shared with 90% of the tumors. The degree of antigen sharing between tumors increases with the total antigens expressed. Based on our findings, we suggest that a allogeneic cell based vaccine will provide a multiepitope immunotherapy approach for treatment of ovarian cancer.

W.24. The Effect Of Obesity On The Peripheral Immune Signature Of Subjects With Renal Cell Carcinoma

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INTRODUCTION: For metastatic renal cell carcinoma (RCC), multiple immune-stimulatory therapies exist but complete response rates remain <10%. As obesity is an independent risk factor for poor prognosis in RCC, this suggests that obesity diminishes anti-tumor immunity. To examine the effect of obesity on immune responses in RCC, we compared multiple immune parameters, including exhausted T-cells and $CD14^{-}/CD11b^{+}/HLA\text{-}DR^{-}$ myeloid-derived suppressor cells (MDSCs)—a pro-tumorigenic population that suppresses T-cell function—in peripheral blood from obese (BMI ≥30) and non-obese (BMI <30) RCC patients both prior to and after tumor excision. METHODS: We used flow cytometry to measure T-cell and MDSC subsets in a cohort of RCC subjects +/- metastases (n=77) both pre-operatively and 30 days post-tumor excision along with age- and BMI-matched tumor-free controls (n=40). For statistical comparison, Mann-Whitney t-test was used. RESULTS: Obese subjects (n=52) had decreased frequencies of circulating MDSCs pre- and post-surgery compared to non-obese subjects (n=25; both $p<0.002$). Obese subjects with high grade tumors (Fuhrman grade III/IV; n=17) had higher frequencies of exhausted CD4+ T-cells post-excision than before surgery ($p=0.02$). These differences were not seen in BMI-matched tumor-free controls. CONCLUSIONS: Our results indicate obesity is associated with decreased frequencies of circulating MDSCs in subjects with RCC. We hypothesize further examination of excised tumors from obese subjects will reveal a local accumulation and sequestration of MDSCs, explaining the decreased frequency of these cells in the periphery. Furthermore,

the increased frequencies of exhausted CD4+ T-cells observed post-tumor excision may be due to difficult and prolonged wound healing related to obesity.

W.25. CD4⁺ T cell Activation in the Pathogenesis of Multiple Myeloma

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Multiple myeloma (MM) is a monoclonal plasma cell malignancy which is featured at multiple osteolytic lesions in the bone marrow. The interaction between malignant plasma cells (MPC) and their molecular and cellular microenvironment has been of the interests in the pathogenesis of MM. The aim of this study is to determine which cellular and molecular bone marrow microenvironment is able to facilitate MPC growth using *in vitro* cell culture and *in vivo* model. We found that bone marrow Th cells not only supported MPC *in vitro* survival and proliferation but also released several cytokines e.g. CCL2 and CCL4 which are among key growth factors for MPC. Reciprocally, MPC was able to produce substantial amount of VEGF, CCL2, CCL3 and etc. Polarised Th cells triggered MPC proliferation *in vitro*. Furthermore, significant proliferation of MPC could be only found in the NOD-scid IL2rgnull (NSG) mice injected Th cells with MPC, indicating Th cells help autologous MPC growth in NSG mice. These results highlight a new role for Th cells involved in pathogenesis of MM instead of restraining tumour growth.

W.28. Characterization of Regulatory T-cells and Anti-Tumor T-cell Responses in Urinary Bladder Cancer

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We have previously shown that tumor-reactive T-cells can be expanded from tumor draining sentinel lymph nodes and used for immunotherapy of Urinary Bladder Cancer (UBC). This prospective study aimed to characterize the regulatory T-cell subpopulations in patients with UBC, and correlate these data to actual tumor-reactivity of the T-cells and clinical outcome. Peripheral blood, tumor and normal bladder tissue were retrieved from both initial trans-urethral resections of the bladder (TUR-Bs) and cystectomies. Sentinel and non-sentinel lymph nodes were collected at the time of cystectomy. *Ex vivo* samples were analyzed with multicolor flow cytometry for effector T-cell and regulatory T-cell population and activation markers. T-cell reactivity towards tumor antigens was estimated by an adaptation of *Flow-cytometric* Assay of Specific Cell-mediated Immune response in Activated whole blood (*FASCIA*). We observed reactivity of both $CD4^+$ and $CD8^+$ T-cells coexpressing $HLA\text{-}DR^+CD45RO^+$ from single sentinel nodes in response to tumor but not normal bladder homogenate. The tumor infiltrating Tregs displayed a $CD4^+FOXP3^+CD25^+CD127^-$ phenothype and high expression of CD45RO, CTLA-4, HLA-DR and CD39. The majority of Tregs (63.6-80.6%) infiltrating the tumor expressed the very early activation marker CD69. In contrast, lymph node Tregs expressed much more varying degrees of these activation, memory and effector markers. UBC tumor tissue contains TILs with activated and more pronounced effector Treg phenotypes than sentinel node or blood derived Tregs. Our results support the existence of both $CD4^+$ and $CD8^+$ tumor-reactive T-cells in sentinel lymph nodes, confirming this site's potential in the study of anti-tumor T-cell responses and development of immunotherapies.

W.29. Genomics characterization of immune status activation in melanoma cell lines

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It is assumed that transcriptional signatures displaying a status of immune activation of cancer reflect genes expressed by infiltrating immune cells. However, it is possible that part of the immune signature is due to constitutive activation of cancer cells. The best evidence supporting this phenomenon is the constitutive activation of immune genes by pure cancer cell cultures in vitro. In the present study we focused on genomic characterization (gene expression profiling, variation of copy number and mutational status) of 15 melanoma cell lines to identify the genetic determinants responsible for the phenotype

(IRF-1, STAT1, Th1) associated with tumor suppression function. Transcriptional pattern (Affymetrix Human gene 1.0 ST arrays), copy number variation (Agilent aCGH) and mutational status (RNAseq, Illumina GAII technology) were correlated with constitutive levels of intracellular STATs and their relative activation form (pSTAT) by flow cytometry, Elisa, and Western blot analysis. Consistent tumor heterogeneity was observed across all 15 samples. 154 IFNG related genes (JAK1, JAK2, IFNGR1, IFNGR2, IL 15, IDO1, eg.) were found significantly differentially expressed between IRF1 positive compared to IRF1 negative cell lines (Student's T-test p2<0.05). Protein data also confirmed that the samples with strong up-regulation of IFNG related genes have pSTAT1 constitutively activated. Copy number variation for some interferon gamma related genes was observed. Novel fusion transcripts and mutations which could not be identified by conventional expression analysis were identified. These preliminary observations support the notion that immune activation of some tumors, at least in part determines by the intrinsic biology of cancer cells.

W.83. Surgical Resection of EMT6 Breast Cancer followed by Immunization of CD200R1KO Mice Results in long-term (>1year) Cure of Tumor Metastases

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Decreased CD200 expression by EMT6 breast cancer cells and BALB/c hosts, as well as of the receptor, CD200R, in host mice, decreased local tumor growth and metastasis in immunocompetent animals. We attempted to cure BALB/c CD200KO or CD200R1KO mice of tumors ≤1cm^3 in size by surgical resection of primary tumor, followed by immunization with irradiated EMT6 cells and the adjuvant CpG. Control animals still developed pulmonary and liver metastases within 30 days of surgery, while protection was observed in both CD200KO or CD200R1KO mice, with no macroscopic lung/liver metastases found in CD200R1KO mice at day 300. Despite surgical resection and immunization, draining lymph nodes from control mice contained tumor cells which could be cloned at limiting dilution in vitro before pulmonary and hepatic metastasis was seen. However, within the limits of detection of the assay used (sensitivity ~1 in 10^7 cells) limiting dilution failed to detect tumor cells in similarly treated CD200R1KO mice, with marked reductions also seen in CD200KO mice. Anti-CD4, and less so anti-CD8, mAb infused into surgically treated and immunized CD200R1KO mice decreased protection from both macroscopic (liver/lung) and microscopic (assayed by limiting dilution of DLN) metastasis. Lymphocytes from treated CD200R1KO mice transferred to surgically-treated control mice attenuated metastatic tumor growth, but protection was abolished by pretreatment of transferred cells with anti-CD4 mAb. Conclusion: CD200:CD200R attenuates a host breast cancer-protective CD4 response.

W.84. CD33$^+$CD14$^-$CD11b$^+$CD66b$^+$CD15$^+$VEGFR-1^{hi} Myeloid Derived Supressor Cells and their Clinical Relevance in Non-Small Cel Lung Cancer

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Lung cancer is the leading cause of cancer death worldwide. Myeloid-derived suppressor cells (MDSCs) are major contributors to tumor immune tolerance and targeting them can improve antitumor activity. We investigated the CD33$^+$CD11b$^+$CD66b$^+$CD15$^+$VEGFR-1^{hi} MDSCs frequency in 120 non-small cell lung cancer (NSCLC) patients (completed 1-year survival) analyzing its prognostic significance with respect to outcome analysis as well as its potential immunosuppression over CD8$^+$ cytotoxic T lymphocytes. We found a significant increase compared with controls in: 1) Percentage of CD33$^+$CD14$^-$CD11b$^+$CD66b$^+$CD15$^+$(10.4 ± 5.01% vs. 3.1 ± 1.7% p<0.0001); 2) Mean Fluorescence intensity (MFI) of VEGFR on MDSCs (p<0.001); 3) plasma levels of arginase-1 (p<0.01); 4) arginase-1 enzymatic activity (p<0.05); 5) plasma levels of TGF-β (p<0.0001), IL-10 (p=0.0027) and IL-6 (p<0.0001). We found a significant diminish compared with controls of: 1) plasma levels of IFN-γ (p<0.0001); 2) CD8$^+$ T cells (p<0.001); 3) CD8$^+$T cells IFN-γ production co-cultured with MDSCs (n=10; p<0.001) and 4) MFI of CD3ζ chain (n=10; p<0.05). The percentage of MDSC's was negatively related to percentage of CD8$^+$ T cells in the peripheral blood (n= 155, r= -0.3045, *P*= 0.0167). Finally, we found an inverse correlation between circulating MDSCs percentages and overall survival (p=0.09). Our study provided evidence of an increased pool of CD33$^+$CD11b$^+$CD66b$^+$CD15$^+$VEGFR-1^{hi} MDSCs in the peripheral blood of NSCLC patients. The suppressive effect of MDSCs

on CD8+ T lymphocytes, suggest their important role in mediating immunosuppression in NSCLC that should enable the development of novel biomarker and might thus represent a potential target for therapeutic intervention.

W.85. Suppression of Human Regulatory T Cells (Tregs) via TNFR2 Receptor Antagonism

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Human regulatory T cell (Treg) inhibition has the potential to enhance anti-cancer treatments by promoting antitumor immune responses. We developed a novel method for selectively inhibiting Tregs *in vivo* by targeting tumor necrosis factor receptor 2 (TNFR2) using newly identified monoclonal antibodies (moAb) or tumor necrosis factor (TNF) muteins. Effects of TNFR2 moAbs on Treg proliferation, signaling, phenotype, and function were studied by multiple *in vitro* assays using isolated fresh human CD4+ and $CD25^{hi}$ coexpressing T cells from >100 donors. Antibody performance was compared to standard Treg expansion methods and categorized as agonist, antagonist or neutral in activity. Six antibodies were identified that acted as TNFR2 antagonists and suppressed human Treg proliferation *in vitro*. Two TNF muteins that suppressed Treg activity were also identified. When Tregs were co-incubated with their target CD8+ T-cells, the antagonist-treated Tregs had negligible effects on CD8+ proliferation, in contrast to an agonist that suppressed proliferation. The findings show that certain TNFR2 ligands can act as antagonists on human cells regarding Treg proliferation, signaling, and target cell activity. Unlike many Treg approaches in cancer, the unique restricted tissue distribution of the TNFR2 receptor makes this an ideal therapeutic target with few systemic toxicity concerns. Selective inhibition of Tregs via targeting of the TNFR2 pathway has the potential to enable the development of new therapeutic regimens for oncology indications.

W.86. Peripheral Blood Immune Signatures and Survival in Stage IV Melanoma

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Data generated in a single center prospective study in Tübingen and validated in cohorts from 3 different European centers have shown that the presence of peripheral memory T-cells recognizing antigens derived from NY-ESO-1 or Melan-A, together with low frequencies of circulating myeloid-derived suppressor cells (MDSCs), confers a survival benefit in late-stage melanoma. Fluctuations in percentages and proportions of MDSCs under loco-regional IL 2 or systemic ipilimumab therapy seemed to correlate with clinical efficacy. Lower levels of antigen-reactive T cells were correlated with higher levels of MDSCs. Where T cell responses were seen, NY-ESO-1 was recognized more frequently by CD4+ than by CD8+ T cells, whereas Melan-A more often stimulated CD8+ T cells. Possession of NY-ESO-1-reactive T cells of any type conferred a survival advantage; however, recognition of Melan-A by CD4+ T cells was associated with poorer clinical outcome if these cells produced IL 4 or IL 17. At this stage of melanoma progression, the proportion and number of peripheral regulatory T cells was not informative. Thus, assessing specificity and function of peripheral tumor antigen-reactive T cells and their interactions with MDSCs can be informative in advanced melanoma and may provide insights into immune mechanisms still able to influence survival even in such late-stage patients and therefore potentially subject to clinical manipulation.

W.87. Identification of Immunogenic Danger Signals From Heat-Conditioned Tumor Cell Lysates for DC-based Immunotherapy: Preliminary Data

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We have previously described the importance of specific damage associated molecular patterns (DAMPs) belonging to heat-shocked melanoma cell lysates during the *ex vivo* process from immature to mature dendritic cells (DCs) for therapeutic use. However, the complete amount of DAMPs from tumor cell lysates, and their relative contributions to the observed mature phenotype of the DCs remain unknown. In this context, our aim was to characterize the proteomic profiles of different melanoma-derived cell lysates with and without heat activation, in order to identify new danger signal molecules from

biologically relevant candidates belonging to the lysates and appearing after heat activation. We performed high-throughput proteome analysis of melanoma-derived cell lysates to the depth of >5000 proteins. Our result showed significant differences of the proteome of tumor cell lysates with and without heat-shock treatment regarding previously described DAMPs like high mobility group box 1 (HMGB1), heat shock proteins (HSPs), and calreticulin (CRT). However, some other new proteins like the protein arginine methyltransferase 5 (PRMT5) also showed a significant increase in conditioned tumor cell lysates, and will be considered as new DAMPs for further functional analyses. Determination of new immunogenic danger signals present in cancer cells and their immune effects, will allow us to better understand the activation process of DCs during the immune response against tumors and, in turn assist the development of improved and more effective approaches in the cancer immunotherapy field

W.88. Characterization of Single Cells from Dissociated Solid Tumors

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The heterogeneous nature of solid tumors, coupled with the relatively small sample size of available biopsies, has led to an emerging need to glean as much information as possible from these valuable specimens. Current approaches to solid tumor analysis fail to completely reveal the diverse range of cellular compartments that comprise the tumor microenvironment. A comprehensive approach to tumor interrogation requires efficient tissue dissociation to facilitate analysis at the single-cell level. In contrast to current methods, single-cell analysis of tumor derived cell suspensions by flow cytometry has the potential to provide a more complete understanding of the many subpopulations within the tumor microenvironment and the cell to cell interactions that govern this space. Here we demonstrate an efficient workflow that enables comprehensive single-cell analysis of solid tumors from breast cancers. Using tumors from PDX mouse models and human samples, we evaluated different dissociation and processing techniques for their effects on cellular viability and surface marker expression. Solid tumors were dissociated into single-cell suspensions using a combination of mechanical dissociation and enzymatic digestion. Phenotypic distribution and morphology of cells within the tumor microenvironment was evaluated using flow cytometry and immunohistochemical/imaging studies. As this approach evolves, and a knowledge base of relevant surface markers is established, this technology has the potential to significantly impact how tumor biopsies are processed to get multiparametric information at a single cell level.

T.91. Overcoming Tumor Induced Chronic Inflammation in Melanoma Patients by Mimicking Immunoregulatory Mechanisms In LATE Pregnancy

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The systemic immune system of metastatic melanoma patients exists in a state of chronic inflammation/immune exhaustion (Th2 bias) which correlates to a poor prognosis. This state of Th2 biased systemic immunity is very similar to that of early normal pregnancy. However, unlike metastatic melanoma, an unknown mechanism during late pregnancy switches systemic chronic inflammation (tolerance) back to a normal, proinflammatory (Th1) state prior to labor. We hypothesize that the switch towards normalization of systemic immunity in pregnancy is due to haploidentical fetal cells leaking into maternal circulation causing a strong proinflammatory signal to the maternal immune system, promoting immune recovery and delivery (rejection) of the fetus (labor). We are testing this hypothesis in an ongoing clinical study of pregnant women and applying these findings towards a therapeutic strategy in metastatic melanoma (allogeneic lymphocyte transfusion). We have tested whether or not allogeneic stimulation (transfer from a genetically non-identical donor) can “recover” the function of peripheral blood immune cells in patients with metastatic cancer. Preliminary data suggests that in vitro exposure of peripheral blood immune cells from patients with untreated metastatic melanoma to allogeneic stimuli “recovers” proinflammatory characteristics of immune cells. We also looked at cellular proliferation in the presence sex hormones, which are strong drivers of immunity, to determine other important modulators that could be targeted to improve the acute immune response. This work will serve as the basis for an interventional phase I clinical trial using allogeneic lymphocyte infusion for the treatment of patients with metastatic melanoma.

T.92. High Numbers of Differentiated Effector CD4 T Cells are Found in Cancer Patients and Correlate with Clinical Response after Neo-adjuvant Therapy of Breast Cancer

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The role of $CD4^+$ T-cells in anti tumor immune responses is poorly known, especially in humans. Herein, we characterized a population of differentiated effector $CD4^+$ T cells defined by low levels of the IL-2 and IL-7 receptors ($CD25^-CD127^-$) that expands in several types of cancers to represent 2-20% of total $CD4^+$ blood T lymphocytes. Contrary to the similar minor (0.2-2%) subset found in healthy donors, these cells bear effector markers such as CD244 and CD11b and low levels of CD27. However, these cells do not cycle ($Ki67^{neg}$) nor secrete IL-10 or IL-17 but display cytotoxic features. This subset encompasses oligoclonal expansions and its increase parallels the expansion of effector $CD8^+$ T cells that include tumor antigen-specific T cells. During neo-adjuvant chemotherapy in breast cancer patients, the increase in $CD127^-CD25^-CD4^+$ T cells correlated with tumor regression, suggesting that $CD4^+$ T cells may include tumor antigen specific cells, which could be generated by or participate in tumor regression during chemotherapy. Altogether, these data support the hypothesis that $CD4^+$ T cells are involved in anti-tumor response in humans. Moreover, the number and the characteristics of these effector CD4 T cells in the blood might be predictive bio-markers for prognosis and staging before chemotherapy.

T.93. Impaired Neutrophils Maturation and Function in Non-Small Cell Lung Cancer

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A tumor-promoting impact of neutrophils has been demonstrated in some cancers. However, their phenotype and functionality in patients with non-small cell lung cancer (NSCLC) is unclear. The aim of this study was to investigate whether there are differences in the biological functions of neutrophils from patients with NSCLC and healthy donors. In addition, the phenotype of neutrophil granulocytes in peripheral blood was investigated (n=120). ROS production, in blood-derived granulocytes was investigated in patients with NSLC (n=10) and healthy controls after *in vitro* activation. Furthermore, the neutrophil expression of CD10, CD11b, CD16, CD15, CD33 and CD49d, in peripheral blood was studied. Our results showed a significant increase of circulating neutrophils in NSCLC patients compared with controls ($p<0.0001$). Neutrophils from NSCLC patients were to a larger extent $CD11b^{hi}CD16^{low}$ positive than healthy blood donors ($p<0.001$) and showed corresponding decreases in the $CD11b^{hi}CD16^{hi}$ ($p<0.05$) and CD11b-CD16- ($p<0.001$) populations and showed a delay in spontaneous apoptosis compared to healthy donors ($p<0.001$). Neutrophils from NSCLC patients showed increased capacity to produce ROS compared to healthy blood donors after *in vitro* activation with PMA ($p<0.001$). Frequently, human peripheral neutrophils have a short half-life and die by spontaneous apoptosis. Here we reported the reduction of spontaneous apoptosis that may partially explain its accumulation in bloodstream. Moreover, the increased percentage of $CD11b^{hi}/CD16^{low}$ cells indicates an immature neutrophil phenotype in NSCLC patients with advanced stages (III/IV) as well as functional alterations in ROS production. This study demonstrates the relevance of further research in these inflammatory cells in NSCLC.

T.94. Autocrine Induction of MIP-2 Secretion from Metastic Breast Cancer Cells is Mediated by CXCR-2 Receptor

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Background: MIP-2 is the mouse homologue of IL-8 which is a CXC chemokine. Effects of IL-8 are mediated by 2 receptors designated as CXCR-1 and CXCR-2. Previous studies demonstrated that IL-8 may contribute to tumor progression through regulation of angiogenesis, cancer cell growth and survival. The role of IL-8 and its receptors in breast cancer metastasis, however is not clear. The goal of the study is to characterize MIP-2 (IL-8) pathway in liver and brain metastatic subset of 4T1 murine breast carcinoma cells and to determine its significance on metastatic growth. Materials and Methods: 4THM cells were originally derived from myocardial metastasis of 4T1 cells. 4TLM (4T Liver Metastasis) and 4TBM (4T Brain Metastasis) cells were from liver and brain metastasis of 4THM cells respectively. MIP-2 levels in the conditioned medium were measured

from each cell line at different time points. CXCR2 and CXCR1 expression was measured using flow cytometry. CXCR2 antagonist SB225002 was used to evaluate the role of CXCR2 on cell proliferation and MIP-2 secretion. Results: Both brain and liver metastatic cells secreted significantly higher amounts of MIP-2 levels compared to parental 4T1 cells. In contrast to previous findings these cells predominantly expressed CXCR2. Inhibition of CXCR2 activity with SB225002 markedly decreased cell proliferation. Unexpectedly CXCR2 blockage significantly increased MIP-2 secretion.

T.95. The Influence of PDGF Stimulation on Proliferation and Metabolism of Colorectal Cancer

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Recent data demonstrate that the platelet derived growth factor (PDGF) plays a crucial role in proliferation and metabolism in several tumor entities. The aim of the study was to analyse the specific role of PDGF stimulation on tumor cell proliferation and metabolism in colorectal cancer (CRC). The human colon cancer cell line HT-29 was cultured and stimulated with PDGF or treated with PI3k/Akt/mTOR-pathway inhibitors in a time-dependent manner. Whole cell and RNA extracts were analyzed by Western Blot and RT-q-PCR for the PI3k/Akt/mTOR-pathway and components of cellular metabolism. To discover effects of PDGF on proliferation MTS proliferation assays were performed. Additionally gene levels of PDGF and metabolic factors in tumors from patients with CRC were analyzed by RT-qPCR. Stimulation with PDGF in HT29 cells resulted in increased proliferation compared to untreated controls. Blocking of Akt caused inhibition of pS6 and reduced tumor cell growth. Additionally, under stimulation a higher glycolytic rate was detected while oxygen consumption remains unaltered. Investigated tumor tissues showed a stage-dependent increase in PDGF expression and elevation in the glycolytic rate. Our results demonstrate a promoting effect of PDGF on HT-29 cell proliferation together with an altered glucose metabolism. An increased glycolytic rate in dependency of tumor cell progression may support accelerated cell proliferation and tumor growth. The growth reducing effect of Akt inhibition indicates the PI3K/Ak/mTOR- pathway to play a crucial role in CRC progression and therefore could be an important target in cancer therapy.

T.96. The Potential of PD-1/PD-L1 Signaling Inhibition Outlined from Clinical Analysis of Colorectal Cancer

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Recent data demonstrate the programmed death-1/programmed death ligand (PD-1/PD-L) pathway in T cell activation to play an important role in tumor evasion from host immunity. The aim of this study was to analyze the clinical significance of PD-1/PD-L1/L2 signaling in colorectal cancer (CRC). Gene and protein expression levels of PD-1, PD-L1, PD-L2, CD4, CD8 and Foxp3 were analyzed in tumors from patients with CRC (n=116 with completed 5-year follow up) by immunohistochemistry and RT-qPCR. Obtained data were analyzed for their prognostic significance with respect to outcome analysis. 90.5% of the investigated tumors showed T cell infiltration, with 58% of the patients demonstrating PD-1-positive T cells in their tumors. Increased PD-L1-expression levels were found in patients who developed PD-1-positive T cell infiltration compared to PD-1-T cell negative individuals. Statistical analysis showed ligand expression (PD-L1/PD-L2) in the cancer tissues combined with dense PD-1-positive T cell infiltration to be associated with poor prognosis in affected patients ($p<0.001$). Multivariate analysis demonstrated PD-L expression in the tumors to be an independent prognostic factor in CRC. The presented results from clinical cancers suggest for the first time for CRC that negative signaling of infiltrating PD-1-positive T cells through PD-L1 expression within the tumor is promoting tumor progression through downregulation of anti-tumor immunity. In conclusion, this study demonstrates the importance of strategies inhibiting negative PD-1/PD-L1 signaling in CRC.

T.97. Systemic Immune Response of Children with Acute Lymphoblastic Leukemia

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The immune system is a complex network designed to protect the host from both external and internal threats, such as malignant transformation. Cytokines are important mediators of immune response which allow integration of the cell's behavior in time and geographical location as the immune responses are generated. Acute lymphoblastic leukemia (ALL) is the most common cancer in children and the main cause of morbidity among childhood blood disorders. Mexico has an incidence of 3 to 4 patients per 100,000 people. 2,500 to 3,000 new cases in children are diagnosed every year. Aim. Determine the profile of inflammatory Cytokines in patients with ALL at diagnosis. Methodology. We included 78 patients with ALL and 37 healthy children. Interleukin (IL)-2, interferon (IFN)-γ, IL-4 IL-10, IL-8, MCP-1, IL-12p70, TGF-β and IL-1β, tumor necrosis factor-alpha (TNF-α) and IL-6 were measured in serum by xMAP (Multiplex Analyte Profiling) Technology by Luminex, according to manufacturer instruction´s. Results were analyzed by U Mann-Whitney test. Results. The concentrations of cytokines: TNF-α, IL-6, IFN-γ, IL-8, MCP-1 and IL-10 were found higher in the ALL patients group, exceeding 100 pg/ml compared to the control group with no more than 10 pg/ml p = 0.0001. IL-2, IL-4 and TGF-β cytokines showed similar production in both study groups. Conclusion. Pediatric patients with ALL had a predominant systemic inflammatory response to the diagnosis.

T.98. Targeting Soluble NKG2D Ligand Prevents Cancer Progression and Eliminates Metastasis in a "humanized' Spontaneous Pre-clinical Cancer Model

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Expression of the MHC I-chain related molecules A and B (MICA/B) on epithelial cell surface in response to transformation or DNA damage can signal the immune system of the abnormality and thus initiate active immune surveillance by Natural Killer (NK) cells and cytotoxicity T cells. We and others have shown that malignant tumor cells can shed MICA/B to down regulate NKG2D expression and negatively impact NK and CD8 T cell function in cancer patients. Since no MIC homolog was identified in rodents, we thus generated a humanized "MIC" transgenic animal model in which human native MIC was expressed concurrently with oncogenic transformation in an organ-specific manner. With this model, we not only recapitulate the biology in patients that cancer progression is associated with elevated serum sMIC, but also uncovered a novel cancer immune evasion mechanism that high levels of serum sMIC perturb NK cell homeostatic maintenance. These findings suggest that we have generated a bona fida pre-clinical model for cancer immunotherapy which enables us to take account of the impact of NKG2D ligands. These findings also suggest that sMIC is a therapeutic biomarker and target for advanced cancers. With a sMIC-specific antibody, we further demonstrated that targeting sMIC can effectively inhibit the progression of primary tumor and eradicate established metastatic tumors by restoring NK peripheral homeostasis and potentiate CD8 and CD4 T cell anti-tumor immunity. Together, our study suggests that the impact of sMIC should be accounted in current cancer immunotherapy trials and practice.

T.99. T cell Receptor Mimic Targeting of the CEA peptide/HLA-A2 Complex in a Metastatic Model of Breast Cancer

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Carcinoembryonic antigen (CEA), is a cell surface glycoprotein overexpressed in several cancers including 70% of breast cancers. The present study utilizes the T Cell Receptor Mimic (TCRm) monoclonal antibody (mAb), RL23C, generated against the altered CEA peptide "CAP-6D"$_{605\text{-}613}$/HLA-A*02:01 complex in a 4T1 murine model of metastatic breast cancer cells. We have demonstrated RL23C staining of altered "CAP-6D" and native CEA$_{605\text{-}613}$ peptide pulsed TAP deficient T2 cells, CEA+HLA-A2+ cancer cell lines and 4T1 cells stably transfected with a chimeric, single chain trimolecular MHC construct (CEA$_{605\text{-}613}$/HLA-A2/Db) designated (SCT-CEA). CEA protein expression was confirmed by Western blotting. CB6F1$^{(bxd)}$ Tg HLA-A2 mice were generated by breeding C57BL/6 Tg HLA-A2(H-2b) with BALB/c(H-2d). CEA/HLA-A2 presentation on CB6F1$^{(bxd)}$ Tg HLA-A2 normal mouse tissues and leukocytes, as well as 4T1 SCT-CEA and 4T1 tumors was determined by immunohistochemistry or flow cytometry using RL23C. RL23C, did not stain normal mouse tissues or leukocytes of CB6F1$^{(bxd)}$ Tg HLA-A2 mice. RL23C stained 4T1 SCT-CEA tumors, but not 4T1 parental tumors. As most cancer related deaths are due to metastasis, there is urgent need to study cancer therapeutics in a metastatic setting. For this purpose, we have created a syngenic metastatic breast cancer model, replicating stage IV, human breast cancer to study the effect of the TCRm, RL23C.

T.100. Targeting Breast and Ovarian Cancer Using a Humanized T Cell Receptor Mimicking (TCRm) Antibody
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Monoclonal antibody (mAb) targeting of tumor-associated peptide/MHC ligands has been shown to inhibit tumor growth in pre-clinical models. Additional proof-of-concept was established by our laboratory using a recently generated murine T cell receptor mimicking (TCRm) mAb designated RL21A, which targets a peptide/HLA-A*0201 (HLA-A2) ligand that was directly discovered in human breast and ovarian tumor cells. The naturally processed and presented peptide is derived from the protein, migration inhibitory factor 1-alpha (MIF). RL21A validated MIF peptide/HLA-A2 target expression in HLA-A2 positive human breast and ovarian tumor tissues and in murine tumor cells transfected to express the full-length HLA-A2 gene-product. The TCRm did not stain normal human tissue supporting the notion that the MIF peptide/HLA-A2 target expression is unique or over-expressed in human cancer cells. Further, RL21A inhibited primary tumor growth in human breast orthotopic and murine syngeneic tumor models, indicating potential therapeutic application for treatment of human cancer. To this end, humanized RL21A (hRL21A) was generated and found to have similar binding affinity (K_D = 24nM) and association/dissociation rates, sensitivity and specificity to the murine parent TCRm. The humanized TCRm demonstrated specific staining of MIF and HLA-A2 expressing tumor cells and human breast and ovarian tumor tissues with no observable cross-reactivity to normal tissues. Additionally, GLP-toxicology studies using MIF protein expressing HLA-A2 transgenic mice did not reveal adverse outcomes or immunopathology associated with administration of hRL21A. Pre-clinical validation studies are currently underway using primary and metastatic murine tumor models to assess hRL21A as a first-in-kind-therapeutic agent to treat human cancers.

F.83. PD-1 Expressing Foxp3$^+$ Regulatory T cells Appear Functionally Exhausted
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The immune response in tumors is characterized by a suppressed cellular infiltrate with enrichment of CD4$^+$CD25$^+$CD127$^-$ Foxp3$^+$ regulatory T cells (Treg) in the tumor microenvironment. It has been thought that tumor derived Tregs possess improved regulatory function owing to the presence of the co-inhibitory receptor PD-1 on their cell surface. Here, we isolated viable infiltrating lymphocytes from freshly resected tumors with matched peripheral blood of patients with glioblastoma and determined the functional efficacy of these cell populations. We observed increased expression of PD-1 and Tim3 on the surface of both Tregs and CD4$^+$ effector T cells infiltrating the tumor microenvironment. Tumor-derived effector cells displayed impaired proliferative responses to anti-CD3/CD28 stimulation while exhibiting robust cytokine responses. Though present in the tumor in increased numbers, tumor derived Tregs surprisingly exhibited less *ex vivo* suppressor function as compared to matched circulating Tregs. Investigation of PD-1$^+$ Tregs from the circulation of healthy controls revealed a similar phenotype with loss of *ex vivo* suppressor function associated with IFNg secretion. These data suggest the PD-1$^+$ infiltrating tumor derived Tregs are clonally exhausted and the impaired immune response in patients with glioblastomas is related to poor T effector cell function that may be amenable to anti-PD-1 therapy.

F.84. A noninvasive and sensitive preclinical model to monitor breast cancer progression in mice
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Breast cancer is the most frequently form of cancer and the second leading cause of death in Western women. Hence, there is an urgency to develop preclinical models for testing therapeutic drugs of this disease. The *MMTV-PYMT* mice are widely studied for breast cancer progression because the stages of breast cancer development largely recapitulates those of humans, as well as rapid tumor growth and enhanced metastatic potential to the lungs and bones. However, tumor progression of internal organs cannot be easily monitored and often can only be ascertain and examined after euthanizing the animal and completing an autopsy. Hence the current model is limited for a large-scale, non-invasive, longitudinal study necessary for monitoring drug efficiency. NFkB is a central coordinator of immune responses, and is often overexpressed in cancers. Hence we hypothesize that NFkB could serve as a marker for tumor progression. Therefore, we generate double transgenic mice by breeding MMTV-PYMT mice with reporter mice whose luciferase expression is controlled by NFkB, and monitored luciferase activity using IVIS. Luminescence was first observed in the abdominal mammary glands at 8 weeks even

before tumor masses were palpable. Later, luminescence was also observed in other mammary glands, with intensity increased as the tumor grew. At 21 week, the tumor masses were examined further by MRI and histopathology. The results indicated that tumor grades positively correlates with luminescence. Therefore, this double transgenic mouse model is a novel system capable of quantitating therapeutic efficacy in the treatment of breast cancer in longitudinal studies.

F.85. Activation of Human Invariant Natural Killer T Cell Immuno-stimulatory Functions by a Novel Recombinant DNA Derived Humanized Monoclonal Antibody

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Invariant natural killer T cells (iNKTs) are innate-type lipid-reactive T lymphocytes that directly kill tumor cells as well as exhibit robust capacity to trans-activate the anti-tumor functions of dendritic cells (DC), natural killer (NK), T and B cells. We recently characterized the iNKT stimulatory properties of a novel monoclonal antibody (NKTT320), which is specific for the human invariant T cell receptor (iTCR). Purified human iNKTs were cultured with or without varying concentrations (0.01-10.0 mg/ml) of plate-bound NKTT320 antibody. At varying times, culture supernatants were collected, cells were harvested, counted and viability assessed. iNKT cell activation (by examining for upregulation of activation markers [CD25, CD69], degranulation (measured by exposure of CD107 on the cell surface) and proliferation (by measuring CFSE dilution) was assessed by flow cytometry. Cell culture supernatants were evaluated for the secretion of a wide array of cytokines and chemokines using Luminex. Strikingly, immobilized NKTT320 antibody induced a robust dose-dependent iNKT cell activation, proliferation and degranulation. Additionally, iNKTs stimulated by the plate-bound antibody secreted increased levels of Th1 (IL-1b, IFN-g, TNF-a, IL-2, IL-6) and Th2 (IL-4, IL-5, IL-10) cytokines as well as chemokines in a dose-dependent manner. Our *in vitro* studies are consistent with *in vivo* data in Va24 transgenic mice, which express the human iTCR alpha chain. Dosing of NKTT320 in these animals led to iNKT cell activation, as well as incorporation of BRDU indicating *in vivo* iNKT cell proliferation. These studies provide a framework by which human iNKT cell functions could be enhanced for cell-based cancer therapeutics.

F.86. High Tumor Infiltration by CD8$^+$ and Absence of FoxP3$^+$ T Lymphocytes Correlates with Gallbladder Cancer patients Prolonged Survival

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Gallbladder cancer (GBC) is the leading cause of death in women over 65 years in Chile. The main risk factors are Amerindian ethnicity, and the presence of chronic local inflammation. Currently, surgery is the only effective treatment and the five-year survival rate in advanced stages is less than 10%. These facts make relevant to explore immunotherapy. Until now, immunogenicity of GBC has been poorly described. Here, we studied the relation between the immune response and survival of GBC patients based on the presence of tumor-infiltrating lymphocytes at different disease stages. Paraffin embedded tumor tissues from GBC patients were analyzed by immunohistochemistry for the presence of CD3$^+$, CD4$^+$, CD8$^+$ and Foxp3$^+$ T lymphocytes (TL) populations and the obtained results were associated with the clinical stage and patient survival. The majority of samples showed CD3$^+$ T cell infiltration that correlated with a better prognosis. Moreover, infiltration by CD8$^+$ TL, but not CD4$^+$ TL correlated with improved survival in early and advanced stages. Interesting, a CD4$^+$/CD8$^+$ ratio lower than 1 increases survival. Additionally, the presence of Foxp3+ TL correlated with a decreased survival, and a CD8+/ Foxp3+ TL ratio ≤ 1 associated with improved patients survival. The presence of CD8+ T cell populations and the absence of foxp3+ populations in tumor tissues correlate with improved survival of GBC patients, constituting potential markers for prognosis. The observed impact of the antitumor immune response in GBC patient survival make possible the testing of cell-based immunotherapy. *Financed by grants FONDECYT 1130320, 1130324; FONDEF D11I1036 and MIII P09/016-F.*

F.87. The Overexpression of CD47 Inhibit Phagocytosis of Neutrophils in Lung Cancer and Correlates with Overall Survival

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Lung cancer is the leading cause of cancer death worldwide. CD47 is a protein associated with integrins, which control cell communication, apoptosis, adhesion and proliferation. In cancer it has found increased and related with phagocytosis evasion mechanism. The aim of this study was to evaluate the expression of CD47 in neutrophils of peripheral blood cells and its possible clinical correlation and association in the augmentation of this cellular type in Non-Small Cell Lung Cancer (NSCLC). We included 50 NSCLC patients (stage IIIB and IV) naive to treatment and 25 healthy subjects. The immunophenotyping was performed with multiparametric flow cytometry. For the evaluation of phagocytosis, the neutrophils from NSCLC patients were co-cultured with circulating monocytes differentiated macrophages and THP-1 cells in the presence and absence of anti-CD47. The percentage of phagocytosis was assessed by flow cytometry. Our results showed an increase in patients with NSCLC compared to controls in: (1) Mean fluorescence intensity (MFI) of CD47 ($p<0.0001$); (2) Neutrophils percentage ($p<0.0001$) and (3) MFI of CD47 in neutrophils ($p<0.0001$). We found an increase in phagocytosis of neutrophils co-cultured with macrophages blocking CD47 ($p<0.001$) and with THP-1 cells ($p<0.05$). Patients in IIIB clinical stage had less MFI of CD47 compared to stage IV ($p=0.009$). The MFI of CD47 was negative correlated with the overall survival of patients ($p=0.02$). These findings suggest that this may be an important mechanism by which neutrophils are enhanced in this malignancy and their increased expression suggest that CD47 is a potential therapeutic target found in Lung Cancer.

F.88. Two Distinct Populations Distinguished by the Co-xpression or Absence of CD28 on Peripheral CD4⁺NKG2D⁺ T Cells Circulate in Patients with Invasive Cervical Carcinoma

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Introduction: NKG2D, an activating immunoreceptor, is primarily restricted to NK and CD8⁺ T cells. The existence of a rare cytotoxic CD4⁺NKG2D⁺ T cell population has been also found in patients with autoimmunity. However, contradictory evidence has implicated this population with immunosuppressive properties in patients with cancer. These confounding data have led to the proposal that two distinct CD4⁺NKG2D⁺ T cell subsets might exist. Objective: To characterize the immunophenotypic signature of CD4⁺NKG2D⁺ T cells in patients with cervical carcinoma. Methods: Patients with invasive cervical carcinoma and healthy individuals were enrolled in the study. Multicolor flow cytometry on peripheral blood was used to determine the expression of TCRalpha/beta, CD28, CD45RO, CD158b, CD107a, CD161, and HLA-DR in the gated CD3⁺CD4⁺NKG2D⁺ T cell population. A Luminex®-based cytokine kit was used to quantify pro- and anti-inflammatory cytokines on plasma. Results: We found an increased percentage of CD4⁺NKG2D⁺ T cells in patients when compared with controls. Interestingly, CD4⁺NKG2D⁺ T cells in patients showed a decrease of CD28 expression when compared with CD28 expressed on CD4⁺NKG2D⁺ T cells in controls. Accordingly with an increase of CD4⁺NKG2D⁺ T cells, we found decreased CD28 expression. The activating markers HLA-DR, CD161, and CD107a were heterogeneously expressed. The levels of IL-1beta, IL-2, TNF-alpha, and IL-10 were negatively correlated with the percentages of CD4⁺NKG2D⁺ T cells in patients with cervical carcinoma. Conclusion: This study revealed the existence of two separate CD4⁺NKG2D⁺ T cell subsets defined by the co-expression or absence of CD28, the latter more likely to be present in anti-inflammatory environments.

F.89. Functional Characterization of NK Cells in Non-Small Cell Lung Cancer

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Natural killer (NK) cells are important effector cells in control of infected, malignant, and tumor cells. The aim of this study was to investigate the activation state and cytotoxic potential of NK peripheral cells in patients with Non-Small Cell Lung Cancer (NSCLC). We further investigate the relationship between NK cells apoptosis and Fas expression. NK cell apoptosis, Fas and Fas-L, NKG2D, CD56 and KIR receptors were evaluated by multiparametric flow cytometry. We next evaluated the cytotoxic activity of NK cells and IFN-gamma expression. For this purpose, we analyzed simultaneously the loss of intracellular perforin and the surface expression of CD107a/b as well as the intracellular IFN-gamma expression. Our results showed that Fas-positive NK cells in lung cancer patients were higher than in healthy controls ($p<0.001$). These results also showed that up-regulation of Fas expression is related to increased apoptosis of circulating NK cells. Regarding the cytotoxic capacity, our results showed that upon PMA stimulation, the expression of surface CD107a/b and loss of intracellular perforin of NK cells from patients with NSCLC were not correlated indicating an impaired functional cytotoxic activity. Interestingly, we also found that, IFN-gamma ($p<0.005$) and NKG2D expression were also impaired significantly ($p<0.001$). These data together suggest a possible anergy state of NK cells. Our description will provide a mechanistic insight into tumor immune escape via negative regulation of NK cell innate function, however the underlying mechanisms remained to be addressed.

F.90. Detection of Exosome Associated HLA Biomarkers in Breast Cancer

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According to the American Cancer Society, more than 200,000 women will be diagnosed with invasive breast cancer each year and approximately 40,000 will die from the disease. Previously, our group developed T-cell receptor mimic (TCRm) monoclonal antibodies (mAbs) which recognize breast cancer specific peptide/HLA-A*02:01 complexes such as those derived from macrophage migration inhibitory factor (MIF $_{19-27}$) and NY-ESO-1 $_{157-165}$. Recently, membrane bound particles called exosomes shed by breast cancer cells have been shown to contain intact class I HLA molecules. These exosomes may act to suppress antitumor immune responses; however, they represent potentially relevant cancer biomarkers. In this work, we present a breakthrough biomarker screening system for cancer diagnostics incorporating T-cell receptor mimic monoclonal antibodies combined with a novel, label-free biosensor utilizing guided-mode resonance (GMR) sensor technology. We have demonstrated detection of shed MIF/HLA-A*02:01 complexes in MDA-MB-231 cell supernatants. Further, we are able to detect NY-ESO-1/HLA-A*02:01 complexes in spiked human serum. The impact of this work could revolutionize personalized medicine through development of targeted immunotherapies and disease diagnostics.

Inflammatory Bowel Disease

1109A. Expression of Blimp-1 in Dendritic Cells Modulate the Innate Inflammatory Response in DSS Induced Colitis

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A single nucleotide polymorphism of PRDM1, the gene encoding Blimp-1, is strongly associated with inflammatory bowel disease. Here, we demonstrate that Blimp-1 in CD103+ dendritic cells (DCs) regulates macrophage homeostasis in the colon. Dextran sodium sulfate (DSS)-exposed DCBlimp-1^{ko} mice with a deletion of Blimp-1 selectively in DCs exhibited severe inflammatory symptoms, more pronounced weight loss, high mortality, with robust infiltration of neutrophils in epithelial regions of the colon, an increased expression of proinflammatory cytokines, and a significant decrease in CD103+ DCs in the colon. Purified colonic macrophages from DCBlimp-1^{ko} mice expressed increased levels of matrix metalloproteinase 8, 9 and 12 mRNA. WT macrophages co-cultured with colonic DCs but not with BM-DCs from DCBlimp-1^{ko} produced increased MMPs in an IL-1b and IL-6 dependent manner. Treatment of DCBlimp-1^{ko} mice with anti-IL-1b and anti-IL-6 abrogated the exaggerated clinical response. We also investigated monocyte-derived DCs (MO-DCs) from PRDM1 IBD single nucleotide polymorphism (SNP) carriers. Compared to major allele carrier, DCs from risk allele carriers express lower level of Blimp-1 and higher level of proinflammatory cytokiens, IL-6, IL-12 and TNFa upon TLR stimulation. Overall, these data demonstrate that Blimp-1 expression in DCs can alter an innate inflammatory response by modulating the activation of myeloid cells.

OR.9. A Possible Role for IL-22BP-Proucing Eosinophils in Impeding IL-22 Protective Actions in Crohn's Disease but Not in Ulcerative Colitis

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In the gut, IL-22 production by T lymphocytes and innate lymphoid cells maintains barrier function through promoting wound healing and antimicrobial peptides (AMPs) release by epithelial cells. However, unregulated actions of IL-22 also sustain pathological conditions, justifying a tight control by IL-22BP, its soluble inhibitor. Strong levels of IL-22 are observed in both Crohn's disease (CD) and Ulcerative Colitis (UC). However their pathophysiological significance remains elusive. To clarify this, the present work studied the regulation of IL-22BP in colon of CD and UC, as compared to controls. qPCR and IFI analyses confirmed similar levels of IL-22 induction in both conditions. However, a strong up-regulation of IL-22BP was observed in CD only, suggesting more pronounced actions of IL-22 in UC than in CD. In agreement with this, expression of lipocalin-2 (an AMP) was significantly more induced in UC. *In vitro* stimulation of colon explants confirmed that IL-22BP inhibited the IL-22-induced expression of lipocalin-2. Moreover, expression of IL-18, a down-regulator of IL-22BP in colon, was higher in UC and negatively correlated with IL-22BP. Finally, IFI double stainings and qPCR analyses on FACS-sorted cells confirmed DCs as IL-22BP producers, a finding we previously published. However, and unexpectedly, the majority of IL-22BP$^+$ cells in CD and controls were HLA-DR$^-$ CD11b$^+$ CD11c$^+$ MBP$^+$ (Major Basic Protein) eosinophils. Thus, we propose that production of IL-22BP by eosinophils in CD but not in UC interferes with IL-22-induced AMPs production in CD. This may account for the more pronounced epithelial barrier permeability and bacterial translocation observed in CD.

OR.46. Th17 Cell Production of IL-21 Promotes Intestinal IgA Response

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CD4$^+$ Th17 cells are highly enriched in the intestines and crucial for epithelial protection, however, their role in regulating the immense mucosal IgA response to enteric microbiota during intestinal homeostasis is still not completely known. In this study, we found that repletion of T cell-deficient TCRβx$\delta^{-/-}$ mice with Th17 cells from CBir1 flagellin TCR transgenic mice, which are specific for a commensal bacterial antigen, increased levels of IgA$^+$ B cells and IgA production in the intestine. As Th17 cells produce mainly IL-17 and IL-21, mice with deficiency in IL-17 or IL-21 signaling had lower intestinal IgA than wild-type mice. IL-21, but not IL-17, is able to augment B cell differentiation to IgA$^+$ cells as mediated by TGFβ, and accelerate initiation of IgA class switch recombination. IL-21 and retinoic acid (RA) strongly induce IgA$^+$ B cell development and IgA production, and drives autocrine TGFβ production from B cells to initiate IgA class switch recombination. Thus, IL-21 from Th17 cells functions to strongly augment IgA production under intestinal environment. Furthermore, IL-21 is able to promote B cell homing to the intestine by inducing $\alpha_4\beta_7$ expression, alone and in cooperation with RA. Together, these findings reveal that microbiota-specific Th17 cells contribute to the robust intestinal IgA response by enhancing IgA$^+$ B cell differentiation, IgA production, and B cell trafficking into the intestine.

T.56. The Role and Regulation of CX3CR1 and CCR9 Interactions during Monocyte Recruitment to the Colonic Mucosa in Patients with Crohn's Disease

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Background: It is well established that blood monocytes relocate to the gut mucosa during inflammatory bowel disease (IBD), but the recruitment mechanisms remain unclear. We have previously shown that a monocyte subpopulation (CD14$^+$CD16^{lo}CCR9$^+$) is increased in peripheral blood of IBD patients during active colonic inflammation.(1) Here, we have

studied chemokine receptor interactions behind blood monocyte migration to the colon in patients with Crohn's disease (CD). Methods: We have included 10 patients with active CD and 20 healthy controls. Flow cytometry was used to analyze chemokine receptor expression levels in colon biopsies and peripheral blood. PCR array analyses (SA Biosciences) were carried out to assess local chemokine expression. Results: We present three main findings: **I)** Circulating $CD14^{lo}CD16^{+}CX3CR1^{+}$ monocytes show decreased CX3CR1 expression during CD; **II)** the selective ligands for CCR9 and CX3CR1, namely CCL25/TECK and CX3CL1, are produced at high levels in the colonic mucosa during CD; and **III)** two distinct macrophage populations that closely resemble $CD14^{+}CD16^{lo}CCR9^{+}$ and $CD14^{lo}CD16^{+}CX3CR1^{+}$) are present in the colonic mucosa during active CD. Discussion: These findings establish the roles of CCL25 and CX3CL1 in human colonic inflammation, and indicate that circulating $CCR9^{+}$ and $CX3CR1^{+}$monocytes represent phenotypically distinct subsets. These populations may give rise to separate macrophage populations in the colonic mucosa. Future studies will address the direct relationship between blood monocyte populations and their local counterparts. 1. Linton L, Karlsson M, Grundström J, Hjalmarsson E, Lindberg A, Lindh E, et al. HLA-DR(hi) and CCR9 Define a Pro-Inflammatory Monocyte Subset in IBD. Clin Transl Gastroenterol. 2012;3:e29.

T.57. Myosin Light Chain Kinase Induced by NF-κB is Involved in the Development of Colitis-Associated Carcinogenesis

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Background & Aim: It has been suggested that prolonged inflammatory bowel disease may promote carcinogenesis in the epithelia. Myosin light chain kinase (MLCK) has been reported to be essential to the permeability of epithelial barrier in the setting of colitis, but its role in the development of colitis-associated cancer (CAC) is still unknown. We therefore examined the expression of MLCK as well as the activation of NF-κB in an animal model of CAC. Methods & Results: Wild type C57BL/6 mice were treated three times with dextran sodium sulfate (DSS) to induce chronic colitis. Pro-tumorigenic cytokines such as IL-1β, IL-6 and MIP-2, which are known to be associated with tumor growth, were up-regulated in the colonic lamina propria by semi-quantitative polymerase chain reaction (qPCR). Western bloting (WB) showed slight up-regulation of MLCK in the colonic epithelia in association with up-regulated NF-κB. We next induced the CAC model by pretreatment with azoxymethane before three cycles of DSS administration. NF-κB and MLCK were further up-regulated in the tumor tissues compared to the non-tumor areas, as well as up-regulation of the specific receptors for tumor necrosis factor (TNF) assessed by qPCR and WB. Transmission electron microscopies showed that administration of ML-7, an MLCK inhibitor, as well as MP6-XT22, an anti-TNF mAb, restored the disrupted intercellular junctional complexes including tight junctions and suppressed tumor development. Conclusions: Our present studies suggest that permeability of epithelial layer in the CAC tissues is associated with MLCK up-regulation and susceptibility to pro-tumorigenic cytokines that potentially promote CAC growth.

T.58. Multiplexed Measurement of Th17 Cytokines in Healthy Subjects and Inflammatory Bowel Disease (IBD)

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Cytokines are humoral regulatory molecules that act together in immunologic pathways. Their analysis can identify the presence of an activated immune response or immune dysregulation. Inflammatory Bowel Disease (IBD) is a chronic inflammatory disorder of the gastrointestinal track. In this study, we evaluated the concentration in the plasma of 15 analytes measured by multiplex cytokine immunoassay: IL-1b, IL-10, IL-4, IL-6, IL-17A, IL-17F, IL-21, IL-22, IL-23, IL-25, IL-31, IL-33, sCD40L, IFN-g, TNFa, (Bio-rad Th17 kit) in healthy subjects (H), Ulcerative colitis (UC) and Crohn's disease (CD). Th17 cells from these donors were measured separately by immunophenotyping. Of the 15 cytokines studied, eleven did not show any significant differences when disease samples were compared to healthy plasma. Both, UC and CD showed increased levels of IFNg (469.5 pg/mL, 476.5 pg/mL respectively compared to 19.6 pg/mL in healthy) and sCD40L (1474.5 pg/mL, 1069 pg/mL compared to 846.5 pg/mL in healthy). When comparing UC to CD, two cytokines showed significantly different concentrations; IL-21 and IL-17F were higher in UC compared to CD. We looked at correlation between plasmatic cytokines concentration and Th17 cells, and we observed a significant negative correlation between the percentage of CD4+CD146+ and the concentration of sCD40L. Th17 cells have been described as pathogenic in IBD, and these observations demonstrate that Th17

inflammatory cytokines elevation can be measured in IBD compared to healthy. The measurement of IL-21 and IL-17F could be used to differentiate UC from CD, and this may suggest different models of Th17 pathogenic activities in these two diseases

T.59. R391 is a Potent Inhibitor of Syk and is an Orally Administered, Topical Treatment Effective in Preventing Colitis in Rodent Models of Intestinal Inflammation

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The spleen tyrosine kinase (Syk) is a key signaling molecule in multiple immune and non-immune pathways. High-throughput screening and optimization for suppression of IgE signaling in primary cultured human mast cells (CHMC) generated a series of small molecule compounds, several of which are highly potent inhibitors of Syk. One of these, R391 (Syk K_i ~ 9nM), is related to the previously described Rigel compound R788, and inhibits CHMC degranulation with an IC_{50} of 19 nM. Pharmacokinetic studies revealed that R391 was absorbed in the intestines but was not systemically available. In a 14-day toxicology study in rats, there were no findings during the in-life portion or in clinical chemistry or hematology parameters. R391 was tested in murine (DNFB/DNS) and rat (DNBS) models of colitis. These models are characterized by weight loss, altered stool consistency, and inflammation of the colon. R391 administered orally twice a day was able to improve all of these parameters relative to vehicle control. In addition, R391 prevented colonic patch hypertrophy and reduced TNFa levels in the mouse model. In summary, R391 is a potent Syk inhibitor which can prevent colitis in two different rodent models of intestinal inflammation. It is an orally administered, topically available compound, which has almost no systemic exposure. R391 may thus be a more specific and safer alternative for treating inflammatory bowel disease (IBD) or irritable bowel syndrome (IBS) than commonly prescribed steroid treatments.

T.60. A Novel Anti-Inflammatory Nanoparticle with Potential Applications in Treating Inflammatory Bowel Disease

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Inflammatory bowel disease (IBD) is a group of disorders that cause sections of the gastrointestinal tract to become severely inflamed and ulcerated. Currently there is no cure for IBD. The goal of contemporary IBD treatment is to reduce the inflammation that triggers the signs and symptoms of the disease. Although the cause of IBD remains elusive, multiple lines of evidence indicate that the abnormal recognition of commensal microorganisms and products of tissue damage by pattern recognition receptors (PRRs) of the innate immune system plays a central role in disease pathogenesis. Therefore novel anti-inflammatory agents targeting PRR signaling may represent a new therapeutic approach for these diseases. In seeking innovative approaches to treat damaging inflammation in IBD, we developed a unique anti-inflammatory nanoparticle, made of a gold nanoparticle core and a peptide shell. This nanoparticle potently inhibited both arms of Toll-like receptor (TLR) 4 signaling cascade (i.e., MyD88- & TRIF-dependent pathways) by blocking the activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kappa B) and interferon regulatory factor 3 (IRF3). By blocking pro-inflammatory transcription factors, the nanoparticle also inhibited the secretion of a variety of chemotactic cytokines. Preliminary *in vivo* studies showed that the nanoparticle significantly reduced weight loss, improved the overall disease activity index, and ameliorated colonic inflammation in a murine model of intestinal inflammation. These results suggest that this nanoparticle may represent a novel anti-inflammatory therapeutic approach for IBD.

T.61. Thiopurines Deplete NK Cells and Enrich NKT Cells in IBD

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The mechanism by which thiopurines azathioprine and 6-mercaptopurine suppress the immune system to control inflammatory bowel disease (IBD) remains obscure. Expression profiling of blood leukocytes revealed genes expressed by cytotoxic lymphocytes were reduced in IBD patients taking thiopurines, suggesting they selectively deplete NK cells. We performed flow cytometry upon blood and biopsies from IBD patients on or off thiopurines, as well as blood from age and gender-matched controls without IBD or medication. In blood, fewer CD3-negative lymphocytes were CD56+, CD16+, CD161+ NK cells in matched IBD patients on thiopurines than off (p=0.0032), or than patients without IBD (p=0.0022). Conversely, more CD3+ lymphocytes were CD56+ NKT cells in patients on than off thiopurines among integrin a4-b7- (p=0.033) and a4+b7- cells (p=0.012), although this difference was not seen among gut-homing a4+b7+ cells (p=0.52). The latter may reflect intramucosal sequestration of NKT cells, because in colon biopsies (matched for inflammation, non-thiopurine IBD medications, and IBD type) a significantly higher fraction of CD3+ lymphocytes were again CD56+ NKT cells (p=0.017). However, thiopurine use correlated with no difference in CD3-negative intramucosal NK cells relative to other lymphocytes (p=0.89). No other differences in lymphocyte populations, including effector and regulatory T cell subsets, were observed. The presence of NK cells or absence of NKT cells may thus be a valuable pre-treatment biomarker to identify good candidates for thiopurine therapy.

T.62. *Brugia Malayi* Recombinant Cystatin as Potential Therapeutic Agent for Ulcerative Colitis

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The concept of hygiene hypothesis has directed us to investigate anti-inflammatory and therapeutic effects of *B. malayi*recombinant cystatin (rBmCys) in dextran sulfate sodium (DSS) induced ulcerative colitis (UC) in BALB/c mice. rBmCys reduced the *in vitro*release of NO and cytokines IL-6, TGF-β and TNF-α by the PEC of BALB/c mice. Administration of rBmCys to the BALB/c mice with DSS induced UC led to decreased weight loss and significant reduction in the magnitude of disease parameters viz., disease activity index (DAI) ($p \leq 0.05$), colon length ($p \leq 0.005$) and mucosal edema ($p \leq 0.005$). Conserved histology of colon was observed in rBmCys administered group of mice with significantly decreased histopathological score and neutrophil mediated inflammation measured in terms of myeloperoxidase activity ($p<0.05$). This curative effect of rBmCys was in dose dependent manner. Associated with this curative effect, there was significant ($p<0.05$) increase in IL-10 and decrease in IL-5, IFN-γ, TNF-α levels in culture supernatants of splenocytes of rBmCys treated colitis mice followed by stimulation with rBmCys. In another set of experiments, mice were pretreated with rBmCys and later on induced for colitis to assess whether rBmCys had preventive effect on the colitis development. Significant protective effect was observed in rBmCys pretreated group of mice reflected from reduction in weight loss and decreased DAI ($p<0.05$), myeloperoxidase level ($p=0.005$), mucosal edema ($p \leq 0.001$) and histopathological score. Amelioration effect in UC observed in this study, followed by treatment with rBmCys either before or after induction of colitis implicates rBmCys as promising therapeutic agent for this debilitating disorder.

Innate Immunity

OR.10. RORγt Regulated BTLA Controls γδ T Cell Homeostasis and Activation Restricting Dermatitis

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γδ T cells are an innate lymphocyte population reactive to infectious and inflammatory stimuli, and which drive inflammation in affected tissues. Here, we show that retinoid-related orphan receptor gamma-t (RORγt) and interleukin (IL)-7 influence γδ T cell homeostasis and function by regulating expression of the inhibitory receptor, B and T Lymphocyte Attenuator (BTLA). RORγt directly represses BTLA transcription through canonical binding sites conserved in the mouse and human BTLA promoter. The activating function-2 domain of RORγt is required to repress BTLA transcriptional activity, while IL-7 increases surface levels of BTLA. BTLA limits γδ T cell numbers and sustains the normal distribution of γδ T cells by restricting IL-7 responsiveness and expansion of the $CD27^{-}ROR\gamma t^{+}$ IL-17-producing subset. Additionally, BTLA regulates γδ T cell production of IL-17 and TNF-α. Consequently, BTLA-deficient animals exhibit enhanced disease in a γδ T cell dependent model of

dermatitis, while activation of BTLA with agonistic antibodies suppressed γδ T cell function and inflammation. Therefore, by coordinating expression of BTLA, both RORγt and IL-7 balance suppressive and activation stimuli to regulate γδ T cell homeostasis and inflammatory responses. Moreover, our work shows that BTLA control of γδ T cells suppresses autoimmune pathogenesis, revealing a mechanism of how inhibitory receptors in innate cells may limit human disease.

OR.43. Choline Acetyltransferase+ T Lymphocytes of Unique Ontogeny Relay Neural Signals

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The nervous system and the immune system intersect by neural reflexes that control the response to infection and injury. Electrical signaling in the vagus nerve activates lymphocytes in spleen to release acetylcholine, the neurotransmitter which interacts with alpha7 nicotinic acetylcholine receptors (a7nAChR) on target immune cells. Acetylcholine inhibits cytokine release, and regulates the innate immune response to endotoxin and other pathogen- and damage-associated molecules. The fundamental nature of the acetylcholine-producing T lymphocytes that relay signals in this efferent neural pathway was previously unknown. Here, we describe that choline acetyltransferase-expressing (ChAT+) T lymphocytes are a unique lymphocyte population that arise early in T cell development and activate a7nAChR to regulate innate immunity. Isolated ChAT+ T cells can sustain the effect of immune-regulatory nerve signals, because transfer of ChAT+ T lymphocytes from murine donors subjected to vagus nerve stimulation confers the cytokine-inhibitory signal of vagus nerve stimulation to 1) dendritic cells in vitro, but not in the absence of a7nAChR and 2) recipient mice in vivo. Furthermore, we provide evidence for ChAT+ T lymphocytes in human blood. In light of these findings, ChAT+ T lymphocytes occupy a specific regulatory niche at the intersection of the nervous system and the immune system with a potential significance in humans.

W.4. Molecular Mechanism Responsible for Differences between Neonatal and Adult Dendritic Cell Responses

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Introduction: The neonatal immune system is different from that of adults. It is unable to mount rapid protective immune responses against a wide range of infectious agents. Recent findings suggest that many of the age-dependent differences in immunity between newborn and adult are cell extrinsic. However, previous studies had shown that purified dendritic cells (DCs) have cell-intrinsic differences as well. To delineate which aspects of the age-dependent difference in innate immunity are cell intrinsic vs. extrinsic we searched for global differences to Toll-like receptor (TLR) stimulation in purified adult vs. neonatal DC populations. We hypothesize that very few selected cell-intrinsic differences in gene expression of key immune genes between these two age groups exist, and the bulk would be due to cell-extrinsic differences. Methods: Human classical (cDC) and plasmacytoid (pDC) dendritic cells were isolated from cord blood (neonates) and peripheral blood (healthy adults) and stimulated with various TLR ligands. RNA was collected at different time points to contrast age-dependent differences in gene expression profiles. Results and Conclusion: We detected age-dependent differences in the expression of several genes involved in the immune response at baseline already. Upon stimulation, we identified a substantially larger fraction of age-dependent differentially expressed genes in cDCs than in pDCs. Pathway overrepresentation analysis indicated that important immune pathways were significantly differentially expressed only in cDCs between the two age groups. These differences thus led to differences in the response to TLR stimulation, which suggest that there are cell-specific as well as pathway-specific intrinsic differences in gene expression.

W.15. Licensing of NK Cells Confer Increased Capacity for Co-activation of CD4+ T Cells

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Background and Aim: Natural Killer(NK) cells are innate lymphocytes with important roles in mucosal host defense and immune regulation. NK cells expressing specific inhibitory receptors differentiate into licensed subsets which are characterized by enhanced cytolytic and cytokine producing abilities. Licensing process is mediated mainly by Killer immunoglobulin receptors (KIRs) in humans and Ly49 receptors in mice. Murine NK cells expressing specific inhibitory Ly49 receptors become licensed upon interaction of the receptor with its cognate MHC class 1 ligand. Previous studies in mice have shown that bulk NK cells augment $CD4^+$ T cell proliferation in a co-culture setting. We aim to show that the degree of $CD4^+$ T cell proliferation correlates with strength of NK cell licensing. Methods and Results: We used C57BL6 mice which have both licensed and unlicensed NK cells. The effect of licensed $Ly49C/I^+$, unlicensed $Ly49A^+$, and mixed (licensed and unlicensed) population of NK cells on $CD4^+$ T cell proliferation was analyzed following a co-culture period. Licensed $Ly49C/I^+$ NK cells had the strongest effect on $CD4^+$ T cell proliferation, and this was dependent on density of NK cells. Unlicensed $Ly49A^+C/I^-$ NK cells were shown to have the least proliferative effect. Conclusion: Licensed NK cells are known to be immunologically more active than unlicensed cells, however their effect on $CD4^+$ T cells have not been previously investigated. Here we show that licensed NK cells have the strongest effect on $CD4^+$ T cell proliferation, and that this effect is dependent on the density of licensed NK cells.

W.22. Human Mast Cells Engulf and Store Exogeneous IL-17A

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Objective: IL-17A plays an important role in numerous immune-mediated inflammatory diseases. Direct analysis of inflamed human target tissues revealed an abundant presence of IL-17A-positive mast cells. As mast cells are not known to produce IL-17A in mice, we aimed to investigate the mechanism of IL-17A expression by human mast cells. Methods: IL-17A, IL-17F and RORC mRNA and protein expression was assessed ex vivo and after PMA/ionomycine stimulation in primary human mast cells sorted from tonsils. Internalization of exogenous IL-17A was assessed by Western blot, imagestream, live imaging and confocal microscopy. Results: Immunohistochemistry and western blot analysis confirmed the presence of IL-17A protein in primary human mast cells. In contrast to T cells, however, mast cells did not express RORC protein, the exclusive transcriptional factor controlling IL-17A expression. Accordingly, IL17A, IL17F, and RORC gene expression was readily detectable in sorted T lymphocytes but not in mast cells, even after ex vivo stimulation. Given the discrepancy between the presence of IL-17A protein and absence of its transcriptional machinery, we investigated the uptake of GFP-fused or 6xhistidin-tagged recombinant IL-17A. Imagestream and Western blot analysis indicated that both primary mast cells and mast cell lines engulf and store exogenous IL-17A. Live imaging and confocal microscopy revealed that internalized IL-17A is stored in endocytic vesicles and that this uptake can be blocked by inhibiting receptor-mediated endocytosis. Conclusion: Human mast cells do not produce IL-17A but engulf and store exogenous IL-17A from the inflamed milieu. Molecular pathways of IL-17A uptake and eventually release are under investigation.

F.67. Unfolding Mechanisms of Antiviral Defense: Regulation of Viral-Induced Apoptosis by XBP1 and IRE1α

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Many viruses activate the unfolded protein response (UPR), a cellular pathway to detect and alleviate stress in the endoplasmic reticulum. We hypothesized that the UPR could provide cell-intrinsic innate protection against viral infection, in a manner independently of canonical pattern recognition receptors. Deficiency of the key UPR component XBP1 conferred impaired resistance to both an RNA (vesicular stomatitis virus; VSV) and DNA (herpes simplex virus; HSV) virus, independent of type I IFNs. XBP1 deficient cells lacked the ability to undergo virus-induced apoptosis and host cell apoptosis directly limited infection with both viruses. XBP1 deficient cells were not only resistant to virus-induced apoptosis, but also failed to undergo the intrinsic pathway of apoptosis, while the extrinsic apoptotic pathway and necrotic cell death remained intact. XBP1 deficiency conferred apoptosis resistance via an indirect mechanism involving feedback activation of its upstream activating nuclease IRE1α, which could be reversed with IRE1α silencing. We observed IRE1α-dependent degradation of mRNAs encoding essential components of the intrinsic pathway of apoptosis, which may account for apoptosis resistance. This pathway appears to regulate susceptibility to viral infection in vivo, as titers of HSV were increased in XBP1

deficient mice infected intravaginally. These findings demonstrate that activation of the UPR component IRE1α blocks intrinsic and viral induced apoptosis. We propose that the IRE1α pathway represents an ancient form of cellular protection from apoptosis, and could be targeted for tissue protection.

F.68. miR-24, miR-30b and miR-142-3p Regulate Phagocytosis in Myeloid Inflammatory Cells
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MicroRNAs (miRNAs) are small non-coding RNAs that regulate various biological pathways. As their role in phagocytosis remains poorly understood, we investigated their impact on phagocytosis in myeloid inflammatory cells. Overexpression of miR-24, miR-30b and miR-142-3p in monocyte-derived primary macrophages (Mφ) dendritic cells (DCs), monocytes and peripheral blood mononuclear cells (PBMCs) significantly attenuate phagocytosis of bacteria (E. coli, S. aureus) as well as the secretion of inflammatory mediators including TNF-α, IL-6, and IL-12p40. Changes in cytokine profiles were observed at transcriptional and/or post-transcriptional levels. Pathway focused PCR array data identified several genes associated with phagocytosis that were altered in both Mφ and DC transfected with miRNA mimics. We further show that miR-142-3p directly regulates protein kinase C alpha (PKCα), a key gene involved in phagocytosis. Overall, these results demonstrate that miR-24, miR-30b and miR-142-3p regulate phagocytosis and associated cytokine production in myeloid inflammatory cells through modulation of various genes involved in the pathway.

F.69. MiR-24, miR-30b and miR-142-3p Regulate Myeloid Inflammatory Cell Survival
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MicroRNAs (miRNAs) are small non-coding RNAs that regulate diverse aspects of human biology, from the earliest events of stem cell differentiation to the final fate of apoptosis. We have profiled miRNA expression during the differentiation of monocytes to macrophages (Mφ), dendritic cells (DC), and osteoclasts (OC), and identified miRNA-sets that are cell-type specific, as well as 'core' miRNAs whose expression is found in all. We have found miR-24, miR-30b and miR-142-3p to be regulators of myeloid inflammatory cell survival. Transfection of Mφ and DC with miRNA inhibitors increased cell survival. Pathway focused PCR array data identified several genes associated with apoptosis that were altered in Mφ and DC, including members of the protein kinase C (PKC), Ras, and phospholipase C families. Overall, these results demonstrate that miR-24, miR-30b and miR-142-3p regulate survival in myeloid inflammatory cells.

F.70. The Identification and Functional Characterization of Pulmonary Neutrophil Subsets *in vivo*
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Neutrophils, the dominant innate immune cell, are typically considered a homogeneous population. However, unique subsets of neutrophils have been postulated. In particular, neutrophils in the pulmonary circulation are known to form a marginated pool. We have observed, using flow cytometry and confocal lung intravital microscopy, that the pulmonary vasculature contains significant numbers of steady state neutrophils. Using stabilized pulmonary confocal intravital microscopy, we observed diverse neutrphil cellular behaviors, suggesting different neutrophil functions and perhaps differnet cell types. In particular, some neutrophils slowly crawled and surveyed the vessel wall, while others rapidly reentered circulation. Here, we hypothesize that subsets of pulmonary neutrophils can be identified through unique cell surface markers and that subsets may perform unique host-defensive functions. Using flow cytometry, we screened multiple cell surface receptors on neutrophils and identified two distinct populations, marked by the expression or absence of CD11c. Moreover, we have observed an increase

of double positive CD11c and MHCII neutrophils upon stimulation with LPS *in vivo*. These cells were enriched in the lung capillaries compared to the peripheral blood. Interestingly, recent reports have demonstrated that some neutrophils can become professional antigen presenting cells, or so called "neutrophil-dendritic cell hybrids". Hence, the lung may act as an organ where active antigen presentation occurs within the vasculature mediated by double-positive neutrophils, thereby linking the innate and adaptive immune systems within the microvasculature. Our future goal is to characterize and establish the function of these double-positive neutrophils *in vivo* using stabilized pulmonary confocal intravital microscopy.

F.71. Role of Blimp-1 Expression in Dendritic Cells in Autoreactive T Cell Receptor Repertoire

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A polymorphism of B lymphocyte induced maturation protein-1 (Blimp-1) has been identified as a SLE risk factor in a genome wide association study (GWAS). However, its functional role is not fully understood. In a previous study, mice carrying dendritic cell (DC)-specific deletion of Blimp-1 (Blimp-1 CKO) spontaneously develop lupus like symptoms in a gender dependent manner. Female Blimp-1 CKO mice have enhanced germinal center (GC) formation and an increased number of follicular helper T cells (TFH) in the spleen. Based on these observations, we asked whether development of autoreactivity is due to the increased frequency of TFH cells or a different repertoire of TFH cells? To address this question, T cell receptor (TCR) v beta chains from TFH cells were cloned and sequenced. In both groups of mice, v beta gene usage is compatible and the V beta transcript exhibit similar CDR3 length. However, comparison of CDR3 sequence revealed that the vast majority of sequences are expressed uniquely in one strain only. This data suggests that TFH cells differentiated in female Blimp-1 CKO mice actually express a different TCR repertoire. We investigated whether Blimp-1 deficiency reduces or increases any antigen-presentation machinery. Catheptins (Cts) are molecules that regulate antigen presentation, and the level of Ctss was significantly increased in DCs from Blimp-1 CKO mice. This finding is the first observation that not only the number of TFH cells but their repertoire might be critically involved in autoimmune diseases. Moreover, Blimp-1 might play a critical role in antigen presentation by DCs, determining TCR repertoire.

F.72. Duox2 and Mitochondria-induced Antiviral Innate Immune Response after Influenza A Virus Infection in Human Nasal Epithelium

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Rationale: The interferon (IFN) signaling system is perhaps the most critical pathway for antiviral defense and protective actions of IFNs rely on signaling through IFN receptors, transcription factors (Stat) and IFN-stimulating genes or antiviral cytokines requiring for degradation of virus and suppression of viral transcription or translation. Our goal is to explore the role of IFN signaling in nasal epithelium, especially Stat and IFN-stimulating genes and to investigate the molecules for regulating IFN-signaling after influenza A virus (IAV) infection. Material and Methods: We performed endonasal brushing and ALI culturing human nasal epithelial cells (NEC) from normal volunteers. Results: Microarray results showed that mRNA levels of Stat1, Stat2 and IFN-stimulating genes, such as Mx1, OAS1, IFIT1 and CXCL10 were highly induced after IAV infection. We found that mRNA levels of Mx1, OAS1, IFIT1 and CXCL10 were higher in NEC until 3 days post of infection (PI). Similarly, phosphorylation of Stat1 and Stat2 increased after PI 1 day. Interestingly, IFN signaling was down-regulated in case of scavenging ROS generated by Duox2 and mitochondria. Both Stat1 and Stat2 phosphorylation were significantly decreased after inhibition of mitochondria respiratory chain reaction and was also suppressed after knock-down of Duox2 gene expression. Inhibition of mitochondrial and Duox2-induced ROS generation attenuated mRNA levels Mx1, 2,5 OAS1, IFIT1 and CXCL10, resulting in increasing viral titer highly. Conclusion: Our findings suggest that IFN-signaling be primarily responsible for controlling IAV infection and both mitochondria and Duox2 might be important molecules for regulating antiviral innate immune response in nasal epithelium.

F.73. Influence of Killer Immunoglobulin-like Receptor Gene Repertoire on Predisposition to Lymphocytic Leukemias

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Background:Natural Killer (NK) cells, often referred to as the 'third lymphocytes' are critical to early control of viral infections and immune-surveillance of tumors and leukemias. NK cell activity is regulated by one or more HLA class-I specific inhibitory receptors, (chiefly, of the Killer immunoglobulin-like receptor (KIR) family), in a way that it can only be triggered when the inhibitory signals are either diminished or are overridden by activating signals. Given the remarkable genetic diversity demonstrated by the KIR gene cluster, it is hypothesized that different KIR gene repertoires characterized by differences in activating and inhibitory gene content could be associated with differential capacities of NK cells (and certain T-cell subsets) to eliminate leukemic target cells. Methods: A total of 218 subjects including healthy individuals (n=124) and patients of myelogenous (n=62) and lymphocytic (n=32) leukemia respectively were analyzed for KIR gene repertoire distribution using Luminex™ based assay. Differences in average incidences of individual KIR genes and haplotypes among all three groups were tested for significance. Results: Significantly reduced incidences of activating KIR2DS3 (p=0.009; OR=5.7) and KIR3DS1 (p=0.01; OR=3.1) were observed in patients suffering from lymphoid malignancies and not in patients suffering from myeloid malignancies. KIR gene repertoires containing ≤1 activating KIR were significantly more often represented by the lymphocytic leukemia patients (p=0.03; OR=3.5) as compared to controls who most often presented ≥5 activating KIR. Conclusions: This finding supports the hypothesis that the absence of appropriate activation signals may provide lymphocytic leukemias an escape mechanism from immune surveillance by NK cells and/or certain T-cell subsets.

F.74. Minimally Modified Low-Density Lipoprotein Triggers the Inflammatory Response in Human Monocytes Subsets

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Atherosclerosis is a chronic inflammatory disease, which are involved different innate immune cell types including to monocytes. Several studies have described three subsets of monocytes based on the expression of CD14 and CD16. The subset monocytes are described as classic, intermediate and non-classical. The antigenicity of minimally modified LDL (mmLDL) has been demonstrated i*n vitro* using cultures of monocytes from healthy subjects, which secrete inflammatory cytokines in response to mmLDL. However, the response of subsets of monocytes to this molecule is unknown. The aim of this research was to demonstrate that the mmLDL induces the secretion of cytokines differentially in subsets of monocytes. The subsets of monocytes were distinguished by surface expression of CD14 and CD16. Human monocytes were stimulation with mmLDL. As controls of activation were used LPS and Pam3CSK4 and culture medium as negative control. Cytokine levels were determined intracellularly. Total human monocyte secreted tumor necrosis factor (TNF)-alpha, IL-6 and IL-10 in response to mmLDL, Meanwhile, the classical monocytes expressed low levels of TNF-α. In contrast, the subsets of monocytes intermediate and nonclassical expressed an increased production of TNF-α. On the other hand, the classical and intermediate monocytes expressed low levels of IL-6. However, nonclasical monocytes expressed higher levels of IL-6. Furthermore, the three subsets monocytes expressed low levels of IL-10 The mmLDL induces the expression of pro-inflammatory cytokines preferentially in classic and intermediate monocytes. These findings suggest that the activation of these subsets of monocytes by mmLDL could be promote to the inflammatory process in the atherosclerosis.

F.75. Prenyl Pyrophosphate Stimulation of Human Vγ2Vδ2 T Cells Requires the Intracellular B30.2 Binding Face of Butyrophilin 3A1 but not its Extracellular IgV Binding Site

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Although γδ T cells were discovered 29 years ago, the antigens recognized by their TCRs and the presentation mechanisms of known antigens have remained elusive. The major subset of human γδ T cells express Vγ2Vδ2 TCRs and play roles in immunity to pathogens and cancer immunotherapy by monitoring isoprenoid metabolites such as (*E*)-4-hydroxy-3-methyl-but-2-enyl-pyrophosphate and isopentenyl-pyrophosphate. Although the chemical requirements for prenyl pyrophosphate stimulation are defined, the exact mechanism by which they stimulate is not. Recently, the immunoglobulin superfamily protein, butyrophilin 3A1 (BTN3A1), was shown to be required for prenyl pyrophosphate stimulation. However, the mechanism of action of BTN3A1 is controversial. We proposed that prenyl pyrophosphates bind to its intracellular B30.2 domain, perhaps

in conjunction with a second protein, resulting in a conformational change in the extracellular BTN3A1 dimer. A second study proposed that prenyl pyrophosphates bind to the BTN3A1 IgV domain and that the Vγ2Vδ2 TCR recognizes the complex. To distinguish between these two possibilities, we mutagenized residues in the domains and tested mutant BTN3A1 proteins for their ability to mediate prenyl pyrophosphate stimulation of Vγ2Vδ2 cell proliferation and TNF-α release. Mutagenesis of residues in the IgV prenyl pyrophosphate binding site had no effect whereas mutagenesis of residues within the basic pocket and surrounding V loops of the B30.2 domain diminished or abrogated prenyl pyrophosphate stimulation. The large binding footprint delineated by the mutations suggests that the B30.2 domain interacts with a second protein or homodimerizes. Thus, these findings support intracellular binding of prenyl pyrophosphates rather than extracellular presentation.

F.76. Nucleotide-binding and Oligomerization Domain 2 (NOD2) Knock-in Mice Carrying a Mutation Associated with Blau Syndrome Show Reduced Amounts of NOD2 Protein and Decreased Muramyl Dipeptide (MDP)-induced Inflammatory Responses

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Blau syndrome is an autosomal dominant disorder caused by mutations in NOD2 and characterized by arthritis, dermatitis and uveitis. Prior in vitro studies reported that NOD2 containing Blau mutations caused enhanced activation of NF-kB, suggesting a gain of function in mutated NOD2 caused Blau syndrome. We tested this hypothesis in vivo by creating a knock-in mouse where a point mutation resulted in a change of arginine [R] to glutamine [Q] at position 314 (R314Q) of NOD2 (position 314 in mice corresponds to 334 in humans). R314Q heterozygous (+/m) and homozygous (m/m) mice did not spontaneously develop arthritis or dermatitis. Compared to wildtype (+/+) mice, bone marrow derived macrophages (BMDM) from (+/m) and (m/m) mice showed a reduction in full length NOD2 protein levels, increased levels of a smaller NOD2 protein fragment that was confirmed by mass spectrometry. PCR and sequence analysis indicated this was not a splice variant. Treatment of BMDM with muramyl dipeptide (MDP) from R314Q mice showed reduced cytokine levels and ubiquitination of RIP2 protein. Mutant mice treated with either intraperitoneal or intravitreal MDP had reduced cytokine levels in the serum of the (+/m) and (m/m) mice. These data indicate that R314Q-NOD2 mice do not demonstrate a gain of function of the NOD2 pathway. Rather, R314Q causes a deficiency of NOD2 and raises the possibility that Blau syndrome may fall within the spectrum of an immunodeficiency disease.

Organ Transplantation

OR.27. Characterization of Human Clinical Grade Tolerogenic Dendritic Cells Used in The One Study Clinical Trial

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Even if the use of immunosuppressive drugs to treat transplant recipients has markedly reduced the incidence of acute rejection, such treatments have numerous adverse side effects and fail to prevent chronic allograft dysfunction. In this context, therapies-based on the adoptive transfer of regulatory cells are promising strategies. Our pre-clinical studies have demonstrated that administration of autologous tolerogenic dendritic cells (TolDC) prolongs graft survival. As part of the ONE Study clinical trial project, we have developed and characterized human monocytes-derived TolDC. Identification of human TolDC was done by a large phenotype analysis using surface and intracellular markers. By microarray analysis, we highlighted that TolDC express genes associated to endocytosis, antigen processing/presentation and chemotaxis pathways. As we described previously in rats, human TolDC also express the tolerogenic markers HO-1 and EBI-3. About the function of these cells, we showed their ability to resist to maturation stimuli and to suppress autologous T cell proliferation induced by mature allogeneic DCs. As TolDC will be derived from transplant recipients, we did a comparative study of the generation of clinical grade TolDC in healthy volunteers and renal insufficient patients receiving or not dialysis. Our results showed that TolDC generated from patients have similar phenotype and *in vitro* function as those generated from healthy controls. Before TolDC administration, we then in-depth characterized the purity/contaminant cells of our preparation, their stability and their absence of any chromosomal abnormalities by karyotype analysis. These autologous TolDC will be administered in kidney transplant patients in association with a minimized immunosuppressive regimen.

OR.41. Detection of non-HLA antibodies in cardiac transplant patient with a multiplex bead array

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We developed a multiplex bead array to identify endothelial specific non-HLA antibodies which may contribute and/or indicate rejection in cardiac transplant recipients. We identified 66 candidate targets by screening rejection sera with a protoarray. A subgroup of these targets was selected to develop a 12 protein non-HLA multiplex bead set. This protein multiplex was used to screen two adult cohorts of cardiac transplant patients. Each cohort had 3 groups of patients; those with antibody mediated rejection (AMR), those with acute cellular rejection (ACR) and those that had no rejection pathology in the first year. A novel set of non-HLA antibodies were identified in a high percentage (72%; 99 of 137) of patient sera screened with the multiplex bead set and in the serum of 41% (23 of 55) of patients prior to transplantation. In the combined cohort, non-HLA antibodies were identified in a higher percentage of patients with ACR (82%) as compared to AMR patients (66%, p=0.06) and patients who had no rejection over the first year (63%, p=0.06) Antibodies against a unique protein target were found in a higher percentage of AMR patients than non-rejection patients in the combined cohorts (40% versus 22%, p=0.11). AMR patients that had non-HLA antibodies along with donor specific HLA antibodies (DSA) had a higher average number of AMR incidents within the first year compared to patients that had non HLA antibodies or DSA alone (3 versus 1.7, p=0.14). This assay could potentially be used to identify patients at a higher risk of rejection.

W.8. Mechanisms of AMR Mediated by DSA: Activation of Endothelial Notch Pathway Triggers M1/M2 Polarization in Human Cardiac Transplants

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Notch signaling is a major pathway in cell fate decisions and it is considered as an important player in vascular homeostasis through the control of cell differentiation, proliferation and apoptosis. However, the contribution of the Notch pathway to vascular injury and endothelial dysfunction in transplantation remains unclear. This study investigated the contribution of Notch receptors and ligands to vascular injury associated with donor-specific antibody (DSA)-mediated rejection (AMR) in human cardiac allografts. Regulation of Notch receptors (Notch1- 4) and ligands (Jagged1, Dll4) was analyzed by quantitative PCR and by immunohistochemistry in cardiac biopsies from patients with stable graft (n=13) or with AMR (n=8). The impact of Notch activity was further investigated by modulating Dll4 in cellular co-culture models using endothelial cells (EC) isolated from donor transplants and monocytes. Our results show that AMR is characterized by an up-regulation in Notch1, -2, Dll4 and Jagged1 transcripts (respectively, 3.7-, 4.7-, 4.5- and 2.1-fold increase *versus* controls, $p<0.01$) and a drastic down-regulation of Notch4 (5.1-fold decrease *versus* controls, $p<0.01$). A Notch4/Dll4 imbalance, at endothelial level, upon AMR was confirmed by immunohistochemistry on biopsies and also observed in cellular models. We also found that overexpression of Dll4 in EC strongly induces Notch activity in monocytes, reflected by increased Hes1 expression. Moreover, Dll4 enhances IL-1β, IL-6, IL-8 and TNF mRNA expression in monocytes and induces a phenotypic switch of monocyte markers. Overall, our findings provide the first evidence that impaired Notch activity in graft EC is a key event associated with AMR triggering EC-mediated monocytes/macrophages polarization.

F.77. Soluble CD22, a Potential Marker of B-Cell Activation, Serves as an Exclusive Factor of Tolerance in Kidney Transplantation

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Long-term immunosuppressive therapy represents a huge burden on transplant recipients, and the use of nonspecific immunosuppressive drugs comes with many side effects. Improving long-term transplant outcome by immunosuppressive drugs minimization or withdrawal may be achieved in patients who have developed tolerance towards their grafts. Therefore, biomarkers that can identify tolerant recipients may provide potential useful tools to select recipients who are potentially tolerant. CD22 is a membrane protein present on the surface of B cells, which plays an important role in inhibiting B-cell activation. By enzyme-linked immunosorbent assay (ELISA), we attempted to detect the soluble extracellular domain of CD22 molecule, soluble CD22 (sCD22), in the serum of kidney transplant recipients. We found that the levels of sCD22 were elevated in a portion of rejective recipients and stable recipients, whereas the levels in tolerant recipients were the same as normal controls. The concentration of sCD22 less than 2.2ng/ml revealed a sensitivity of 100% in identifying tolerance, showing that sCD22 concentration more than 2.2ng/ml could serve as a potential exclusive factor of tolerance in kidney transplantation. Our study suggests that sCD22 may be useful in selecting patients who are potentially tolerant and eligible for immunosuppressive drugs minimization or withdrawal. Moreover, we raised a hypothesis that sCD22 serves as a potential marker of B-cell activation and might be useful in predicting antibody mediated rejection.

F.78. Temra CD8 T Cells are Highly Cytopathic Cells that Escape from Costimulatory Base-Therapy

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Background & Aim of the study. We reported that the accumulation of highly differentiated TEMRA (CD45RA+CCR7-) CD8 T cells in patients with a stable graft function is associated with an increased risk of kidney graft dysfunction. To understand how TEMRA CD8 T cell responses are regulated would be important for the rational design of immune-based strategies to treat transplant recipients. Thus, we characterize the phenotype and the signals that activate the function of TEMRA CD8 T cells in healthy volunteers.Results. High expression of transcription factors related to effector (T-bet), differentiation (Blimp-1) and memory (Eomes) processes were observed in TEMRA CD8 T (CD45RA+CD28-) cells as compared to early EM (CD45RA-CD28+). In contrast to early EM, TEMRA CD8 T cells are characterized by the production of cytotoxic molecules (Perforin, GZM-b) and pro-inflammatory cytokines (IFN-g). TEMRA CD8 T cells successfully respond to IL-2, IL-7 and IL-15 cytokines as exemplified by the phosphorylation of STAT5, enhanced proliferation and decreased apoptosis level. Of interest, in absence of CD28-related costimulation, the stimulation through the TCR of TEMRA or early EM CD8 T cells results in their proliferation whereas naïve CD8 T (CD45+ CD28+) cells failed to proliferate. Conclusion. TEMRA CD8 T cells constitute a preformed effector that can be efficiently activated using TCR stimulation alone or in combination with common-gamma chain cytokines. Given their lack of CD28 expression, highly differentiated TEMRA CD8 T cells will escape from costimulatory based therapy.

F.79. Investigating the Function of CD28 on Belatacept-Resistant Human Th17 Memory Cells

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Pathogen primed memory T cells can cross-react with allogeneic antigen and mediate graft rejection, a process termed heterologous immunity. Th17 cells are a pro-inflammatory $CD4^+$ lineage that provides immunity to bacteria and fungi but can drive pathologic immune responses. We found that Th17 memory cells are uniquely resistant to the CD28/CTLA-4 blocker belatacept and are associated with acute rejection in renal transplant recipients, but the mechanism of this resistance remains unclear. Our data demonstrate that $CD4^+$ Th1 and Th17 memory cell subsets constitutively express the costimulatory receptor CD28, leading us to investigate whether Th17 cells are sensitive to selective CD28 blockade with domain antibodies (dAbs) that leave coinhibitory CTLA-4 signaling intact. Selective CD28 blockade inhibited Th1 cells but surprisingly augmented Th17 responses. Conversely, CD28 ligation inhibited Th17 populations. CD28 ligation induced similar upregulation of the activation markers CD69 and CD25 on Th1 and Th17 cells, but induced significantly greater amounts of coinhibitory CTLA-4 on Th17 cells. Relative to Th1 cells, Th17 cells also expressed lower levels of the transcription factor FOXO3, which bind upstream of the *CTLA-4* gene, suggesting a repressive role for this molecule in the expression of CTLA-4. These results demonstrate that the CD28 receptor has distinct functions on Th1 and Th17 memory cells and suggest novel mechanisms of costimulation on Th17 memory cells. This study has identified differences in costimulatory pathways of $CD4^+$ memory subsets, and demonstrates that the heterogeneity of pathogen-derived memory has implications for the immunomodulation of pathologic T cell responses.

F.80. Peripheral Phenotype and Gene Expression Profiles of Combined Liver and Kidney Transplanted Patients

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The beneficial influence of one graft on the other has been reported in combined transplantation. Nevertheless, the mechanisms of such a process have not been established yet. We analyzed the phenotype, the gene expression (messenger RNAs (mRNAs) and microRNAs (miRNAs)) patterns in blood from combined liver kidney transplanted (CLK) recipients compared to patients with liver (L-STA) or kidney (K-STA) graft alone under classical immunosuppression and patients who operationally tolerate a liver (L-TOL) or a kidney graft (K-TOL). An extensive phenotype, a signature of 46 immune-related mRNAs and 754 miRNAs were analyzed in blood from 243 patients (57 CLK, 106 L-STA, 62 K-STA, 9 L-TOL and 9 K-TOL) enrolled in a multicenter study (Barcelona (Spain), Nantes (France), Rennes (France) and San Francisco (USA)). We found that CLK patients harbor a higher proportion of peripheral $CD19^+CD24^+CD38^{Low}$ memory B cells than L-STA ($p<0.05$) and a higher proportion of $Helios^+$ Treg than K-STA ($p<0.01$). Among 542 expressed miRNAs, 35 and 8 miRNAs were significantly differentially expressed in CLK vs. K-STA and CLK vs. L-STA, respectively ($p<0.05$). In addition, principal component analyses based on the expression of the miRNAs associated with CLK showed a superposition of the CLK with L-STA and L-TOL whereas it segregated from K-STA and K-TOL. Altogether, CLK patients display an intermediary blood phenotype between those of kidney and liver transplanted recipients. The CLK patients are characterized by a transcriptional profile closer to the L-STA and L-TOL profiles than to those of kidney transplanted patients.

F.81. Increased Frequency of Pathogenic IL-17 Secreting $Foxp3^+$ Tregs in Patients with *de novo* Autoimmune Hepatitis

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Up to five percent of liver transplant recipients develop a form of chronic allograft hepatitis termed de novo autoimmune hepatitis (dAIH). However, the etiology and immune cell types involved in the development of dAIH remain largely unknown. We have earlier identified the presence of $FOXP3^+$ T cells co-staining for IL-17 within inflammatory infiltrates of dAIH liver biopsies. As the subset of IL-17 producing $FOXP3^+$ regulatory T cells (Tregs) is potentially pathogenic and is associated with other autoimmune diseases, we wanted to characterize this cell population in detail and investigate the role of IL-17 producing $FOXP3^+$ Tregs in the development of dAIH. Transcriptional analysis of human $IL\text{-}17^+$ $FOXP3^+$ Tregs revealed a specific molecular signature discriminating them from conventional Th17 and Treg cells and led to the identification of novel markers for this cell subset in humans. The analysis of $CD4^+$ T cells isolated from peripheral blood of pediatric patients with dAIH compared to pediatric liver transplant recipients without dAIH and healthy children revealed that Tregs of dAIH patients displayed increased expression of $IL\text{-}17^+$ $FOXP3^+$ Treg cell specific markers. In line with this, Tregs of dAIH patients secreted significantly more IL-17 compared to Tregs from liver transplanted and healthy controls. These findings indicate that an increased Th17-Treg plasticity is potentially associated to the pathogenesis of de novo autoimmune hepatitis. The mechanisms, which lead to the increased IL-17 production of $FOXP3^+$ Tregs in dAIH patients and how this relates to the disease remains to be elucidated and is currently under investigation.

F.82. Multi-parameter Immune Cell Flow Cytometry and Gene Expression for the Characterization of Multiorgan Dysfunction Syndrome (MOD) after Mechanical Circulatory Support Device (MCSD)

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Introduction: A main cause of death in Advanced Heart Failure (AdHF) after Mechanical Circulatory Support Device (MCS) is the Multiorgan Dysfunction Syndrome (MOD). We aimed to incorporate multi-level immune cell flow cytometry and genome wide transcriptome analysis to improve phenotype characterization. Methods: PBMC from 11 patients were obtained at days -1, 1, 3, 5 and 7 before and after MCS surgery, and from 3 healthy volunteers at similar timepoints. Immunophenotyping (IP) was performed with multi-color monoclonal antibody T cell panel on an LSR Fortessa™. Whole-genome transcriptome profiling was assessed by Next-gen mRNA Sequencing. Patients were grouped by AdHF risk using the Intermacsclassification and MOD was quantified by routinely obtained organ function parameters. Bioinformatics analysis was performed. Clustered heatmaps were constructed for visualization. Results: We found significant differences in IP and gene expression profiles of patients with increasing AdHF severity and organ dysfunction. Compared to lower risk patients (InterMACS level 3, n=5) high risk patients (levels 1 and 2, n=6) showed IP profile of chronically activated CD57+PD-1+ CD8 T cell subsets with high frequencies of memory and terminally differentiated (TEMRA) cells before MCS-surgery and across all 5 time-points. Genes involved in the establishment of these IP showed significant differential gene expression ($q < 0.05$). Combination of gene expression and IP- supervised marker successfully predicted the patients with higher risk of MOD after MCS surgery. Conclusion: Simultaneous assessment of IP surface marker and gene expression are useful to improve characterization and risk prediction in patients with AdHF who undergo MCS.

Other

W.5. Respective Regulatory Properties of CD4+ AND CD8+ T Cells during Experimental Aristolochic Acid Nephropathy

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Experimental aristolochic acid nephropathy (AAN) is characterized by acute tubulointerstitial injuries (ATI) (i.e. proximal tubule necrosis and inflammatory cell infiltrate) followed by chronic lesions (i.e. fibrosis and atrophy). We investigated the role of T-cell subpopulations in an acute AAN model by using selective depleting antibodies (Ab). Mice were injected with AA and depleting $CD4^+$ T-cells Ab (AA+αCD4) or control Ab (AA+ctrl). As compared to the AA+ctrl group, a significant increase in plasma creatinine (pCr), blood urea nitrogen (BUN) and tubular necrosis was observed in the AA+αCD4 group. The TNF-alpha and MCP-1 mRNA renal expressions were also increased. We next compared a regulatory T-cells ($CD4^+CD25^+Foxp3^+$ T-Regs) depleted group (AA+αCD25) with the AA+αCD4 group. Surprisingly, pCr, BUN and tubular necrosis were not aggravated in AA+αCD25 as compared to AA+ctrl group. In another group, mice were injected with depleting $CD8^+$ T-cells Ab (AA+αCD8). As compared to AA+ctrl group, a significant increase in pCr, BUN and mRNA renal expression of TNF-α was found in this group, while tubular necrosis and mRNA renal expression of MCP-1 tended to increase. Within the myeloid-derived population, we noted a significant increase in the proportion of particular macrophages $CD11b^{hi}F4/80^-$ upon depletion of either $CD4^+$ or $CD8^+$ T cells. In conclusion, our results suggest that some $CD4^+$ and $CD8^+$ T-cells could afford protection against AAN-induced ATI. Interestingly, this T-cell mediated protection was associated with a decreased proportion of $CD11b^{hi}F4/80^-$ macrophages. Therefore, the regulatory functions of T cells and their interplay with renal macrophages will be investigated during our AAN model.

W.41. Neuronal Regulation of Antigen Trafficking in Peripheral Lymph Nodes

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Antigen travels into the lymphatic system through lymph vessels and into draining lymph nodes. These lymph nodes are innervated with motor and sensory neurons, however the role of neuronal input in mediating antigen trafficking via lymph nodes is yet unknown. Here we describe a role for neuronal regulation of antigen trafficking. Antigen injected into a mouse hind foot flows first through the popliteal lymph node, then to the sciatic lymph node, before continuing up the lymphatic system. Interestingly, in mice immunized to Keyhole-Limpet Hemocyanin (KLH), antigen trafficking of IrDye-labeled KLH was

restricted in the popliteal and sciatic lymph nodes. Quantification of the fluorescent intensity of labeled KLH one hour after administration in the hind foot, revealed a significant decrease in sciatic/popliteal signal in sensitized mice compared to naïve animals. Blocking of the neuronal activity with bupivacaine at the popliteal and sciatic lymph nodes in immunized animals resulted in antigen trafficking patterns similar to naïve animals. Conversely, in naïve animals, direct activation of neuronal signals at the popliteal lymph nodes using monopolar electrical stimulation resulted in significant decrease of antigen trafficking compared to sham stimulated controls. Taken together these studies reveal an important role of neuronal input in the regulation of antigen trafficking. Supported by a grant from DARPA (W911NF-09-1-0125) to KJT

W.51. Elucidation of Immune Subsets using Comparative Analysis of Mass Cytometry Data Through Cytobank
TJ Chen and ***Nikesh Kotecha****. Cytobank, Inc, Mountain View, CA*

The development of new technologies for high-parameter data has resulted in a surprising bottleneck: the rate limiting step for identification of immune subsets is often single-researcher-driven, focusing on post-acquisition analysis of immune subsets. Identification of such cell populations in flow cytometry has primarily focused on manual analysis, whereas new computational tools have proven useful for high-parameter and cross-sample comparisons. Reproducibility of analyses relies on sharing of analysis and data, requiring unified storage, processing, and iterative and collaborative analysis of the data. Adoption of these new tools for immune subset discovery requires thorough collaborative investigation and validation of identified cell populations. To this end, in this study we compare the ease of discovery of immune subsets by comparing analysis through the use of two visualization tools: the sunburst hierarchy and the SPADE tree. The sunburst hierarchy is a visual and interactive representation of traditional manual gating, whereas the SPADE tree is a semi-automated clustering and visualization tool for identification of cell subsets. In this study, we collaboratively investigate harder-to-find immune subsets and demonstrate the ability to elucidate these populations using Cytobank via an iterative analytic approach.

W.52. Exploring the Immune Population Hierarchy using Novel and Interactive Visualization Tools: Sunburst and Spade
Tiffany J. Chen *and Nikesh Kotecha. Cytobank, Inc, Mountain View, CA*

The development of new technologies for high-parameter data has resulted in a surprising bottleneck: the rate limiting step for identification of immune subsets is often single-researcher-driven, focusing on post-acquisition analysis of immune subsets. Identification of such cell populations in flow cytometry has primarily focused on manual analysis, whereas new computational tools have proven useful for high-parameter and cross-sample comparisons. Reproducibility of analyses relies on sharing of analysis and data, requiring unified storage, processing, and iterative and collaborative analysis of the data. Adoption of these new tools for immune subset discovery requires thorough collaborative investigation and validation of identified cell populations. To this end, in this study we compare the ease of discovery of immune subsets by comparing analysis through the use of two visualization tools: the sunburst hierarchy and the SPADE tree. The sunburst hierarchy is a visual and interactive representation of traditional manual gating, whereas the SPADE tree is a semi-automated clustering and visualization tool for identification of cell subsets. In this study, we collaboratively investigate harder-to-find immune subsets and demonstrate the ability to elucidate these populations via an iterative analytic approach.

W.89. Generation and Characterization of *IgM*, *Rag1* and/or *ILR2g* Knockout Immunodeficient Rats
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The objectives of this work were to generate new immunodeficient rat models. The rat is an important experimental model for studying human physiopathology and testing novel therapeutic approaches. Thus, a readily available, non-commercial source of immunodeficient rats would be useful.*IgM* KO rats showed undetectable serum levels of IgM, IgG, IgA and IgE. B cells ($B220^+$) in spleen and bone marrow were 99% and 90% reduced vs. controls and had a pro-B cell developmental block. Both *Rag1* and *IL2Rg* KO rats showed drastically reduced thymus, lymph nodes and spleen and the number of cells in these organs and in bone marrow were decreased between 98 and 77%. In spleen and lymph nodes, *Rag1* KO rats showed > 92 % reduced numbers of T $CD4^+$, T $CD8^+$ and B cells with conserved numbers of NK cells and macrophages. In *IL2Rg* KO rats, the proportion of T $CD4^+$, $CD8^+$ and NK cells were reduced > 90%, whereas B cells were reduced by 70%. Serum IgG, IgA and IgE were inhibited > 70% in *Rag1*- and in *IL2Rg*-deficient rats. Allogeneic organ transplantation in *Rag1* KO rats showed significant delay in rejection vs. controls and similar experiments are under way with *IL2Rg* KO rats. Human tumor growth was observed in *IL2Rg* KO rats and similar experiments are under way in *Rag1* KO rats.*IL2Rg*- and *Rag1* KO rats are being crossed to obtain profoundly immunosuppressed double KO animals for studies in areas such as stem-cell medicine, transplantation and cancer biology.

W.90. The Mechanism Underlying the IGA Enhancement of Lactobacillus Pentosus Strain b240 in the Gut Immune System

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Mucosal surface is a major site for the invasion of pathogenic microorganisms following ingestion or breathing. Thus, strengthening mucosal immune function is thought to be important for maintaining health. Secretory immunoglobulin A (SIgA) plays a pivotal role in the immunosurveillance by the mucosal immune system. In the multi-steps for the production of SIgA, Peyer's patches (PPs) act as an important inductive site for the generation of IgA-committed B cells in the intestine. Using a PP cell culture system, we previously screened 150 strains of lactic acid bacteria and found that the heat-killed *Lactobacillus pentosus* strain b240 (b240) had the highest activity to enhance IgA production from PP cells. Consistently, oral administration of b240 promoted IgA production in the intestine of mice and also accelerated salivary IgA secretion in healthy adults and elderlies. These findings clearly indicate that b240 has a prominent ability to enhance IgA production in both human and mice; however, the mechanism underlying IgA enhancement by b240 has remained elusive. In this study, we aimed to obtain cellular and molecular insights into the immunobiological activity of b240. We found that orally administered b240 were present at the subepithelial dome region of PPs, where they induced interleukin (IL)-6 production from PP dendritic cells (DCs) via toll-like receptor 2 (TLR2)-mediated pathway and consequently activated B cells to produce a large amount of IgA. Our findings open up a possibility that the heat-killed b240 is a stimulator of TLR2-mediated DC-activation in the PPs for enhancing IgA production in the intestine.

W.91. Rapid Image Cytometry Method for Measuring Concentration and Viability of Primary Cells used in Cellular Therapy

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Cellular therapy has become a major clinical research field that creates tailor-made medical treatments for many human diseases. Primary cells obtained from patients and mouse models often contain nonspecific particles such as red blood cells (RBC), platelets, and cellular debris, which can make the cell sample analysis difficult. To remove nonspecific particles that can interfere with analysis, a ficoll gradient separation or RBC lysis is routinely performed. Measurements of concentration and viability of the cell sample are necessary for clinical researchers to qualify the collected patient samples for research and downstream processing. In this work, we validated a fluorescence-based image cytometry method using acridine orange (AO) and propidium iodide (PI) to rapidly measure concentration and viability without the need for tedious purification protocols. Using the Cellometer Vision instrument we performed the viability and concentration analysis and validated our results using the traditional hemacytometer method for samples including peripheral blood mononuclear cells, mononuclear cells, Leuko Pac, bone marrow, cord blood, whole blood, bronchoaveolar lavage, and primary murine samples. These primary human samples may potentially contain red blood cell residue, platelets and other debris, but was measured correctly using

fluorescence-based analysis. This image-based cytometer method can increase clinical research efficiency by eliminating the need for purification steps and for manual counting. Furthermore, it can eliminate the user-to-user variation, thus improving the accuracy of the cell analysis.

W.92. Differences in the Polarization of the Inflammatory Response of Patients with Chronic Obstructive Pulmonary Disease (COPD) Secondary to Smoking and to Biomass Smoke Exposure

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Background. Smoking and exposure to biomass smoke cause the release of pro-inflammatory mediators, as well as the activation of T helper (Th) cells. This inflammatory response can lead to the development of COPD; however, there are different clinical features between patients with COPD secondary to smoking (sCOPD) and to exposure to biomass smoke (bsCOPD), and these may be due to the kind of predominant Th response. Methods. Thirty-seven patients were recruited, 20 sCOPD and 17 bsCOPD; we included 20 healthy controls (HC). Through flow cytometry the proportion of $CD4^+$ T cells subpopulations were defined. The serum cytokines were quantified through Luminex system. The differences between groups were evaluated with a Kruskal-Wallis test, and a post-hoc analysis was performed through a U of Mann Whitney test. Results. A prevalence of Th17 cells was observed in the sCOPD group (10.3±3.4%), when compared with bsCOPD (3.5±0.9%) and HC (0.9±0.4%) $p<0.001$. Th2 cells were predominant in the bsCOPD patients (4.4 ± 1.3 %), in comparison with sCOPD (2.5±0.8%), and HC (1.1±0.4%). IL-4 and IL-10 are in a higher concentration in bsCOPD versus sCOPD. Conclusions. Our data show a polarization of Th17 cells in sCOPD, and of Th2 cells in bmCOPD. There are differences in the bmCOPD and the sCOPD cytokine profiles, which may explain some of the clinical features observed in the COPD clinical phenotypes. Cell plasticity, together with the conditions of the microenvironment may exert a critical role in cellular polarization. To adress this questions, some functional assays must be performed.

W.93. Metalloprotease from *Naja naja* (Indian cobra) Snake Venom Inhibits Platelet Aggregation by Binding to α2β1 Integrin and Cleaving GPVI

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Snake venoms are depots of target-specific toxins found to interfere strongly with human hemostatic system. Enzymatic and non-enzymatic components of snake venom effectively alter platelet function and therefore, have been extensively studied with a view to develop effective antithrombotic agents of therapeutic value. We isolated a novel metalloprotease, *Naja naja* protein fraction-3 (NN-PF3) from the venom of *Naja naja* (Indian cobra), which is endemic to Indian Subcontinent. NN-PF3 is of high molecular mass, non-toxic and anticoagulant, and it also strongly interferes with platelet function. NN-PF3 completely inhibited collagen-induced human platelets aggregation and partially inhibited ADP-induced aggregation, but not thrombin-, arachidonic acid-, or ristocetin-induced aggregation. Inhibition of platelet aggregation both by enzymatic and non-enzymatic mechanisms was confirmed using EDTA-inactivated NN-PF3. NN-PF3-treated platelets lost the ability to bind collagen-coated surface in the microtiter wells. In contrast, NN-PF3-treatment did not alter platelet binding to fibrinogen and fibronectin. The major collagen receptor of platelets, integrin $\alpha2\beta1$and GPVI bind to collagen exposed following vascular injury leading to activation and aggregation of platelets. NN-PF3 binds to integrin $\alpha2\beta1$ and GPVI, and binding of NN-PF3 cleaves only GPVI but not integrin $\alpha2\beta1$. Further, GPVI downstream signaling phosphotyrosine protein is inhibited by NN-PF3. The enzymatic activity of NN-PF3 is selective to fibrinogen substrate. However, neither the proteolytic cleavage of fibrinogen nor its degradation products contributed to the collagen-induced platelet aggregation inhibition. Thus, NN-PF3 may serve as template to develop new generation of thrombolytic agents to treat clinical conditions associated with the cardiovascular diseases including strokes.

W.94. Screening for Potential T-cell Epitopes in a Therapeutic Protein using Peptide Microarrays

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Hemophilia A patients lack functional circulating factor VIII (fVIII), a protein cofactor that accelerates blood coagulation. Approximately ¼ of hemophilia A patients infused with therapeutic fVIII develop anti-drug antibody responses leading to bleeding that is difficult to control. Our laboratory has mapped several immunodominant HLA-restricted T-cell epitopes in fVIII using HLA-DR tetramers, but as fVIII is a large protein (>2,000 amino acid residues), methods more efficient than systematic mapping using overlapping peptides spanning its sequence would be useful. Because MHC-peptide binding is a prerequisite for T-cell stimulation, an initial screening method based on binding of fVIII peptides to soluble extracellular domains of recombinant HLA-DR proteins was tested. Microarrays containing triplicate sets of fVIII peptides immobilized on glass slides via a flexible linker were incubated with 10 HLA-DR proteins at several dilutions, followed by incubation with a fluorescent-labeled anti-DR antibody, and the slides were washed and read on a microarray scanner. Between 20-40% of the fVIII peptides produced fluorescent signals for each HLA-DR protein tested, including several peptides containing T-cell epitopes that had been verified by using tetramers to isolate fVIII-specific T-cell clones and lines. The intensities of fluorescent signals from the triplicate sets of spots were quantified and analyzed. For subsets of these peptides, the signal strengths were compared to (a) affinities from peptide-MHC binding assays, and (b) computer-predicted T-cell epitopes in fVIII. Non-MHC-binding peptides were eliminated as T-cell epitope candidates and the remaining peptides prioritized for further testing based on measured and predicted MHC binding.

W.95. Identification of New Invariant T Cells by Next Generation Sequencing

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Known invariant T cells occur frequently in the human population and have T cell receptors (TCRs) that are characterized by an α-chain that is build up from specific V and J gene segments, and having a modest amount of random insertions. Known invariant T cells are Natural Killer T (NKT), mucosal-associated invariant T (MAIT) cells, and germline encoded mycolyl-specific(GEM) cells. In this study we searched for new invariant T cells, using the characteristics of known invariant T cells. We focused on the TRAV1-2 repertoire, because two of the invariant T cells, GEM and MAIT, both have TRAV1-2 in their alpha chain. From 6 individuals, the TCRα repertoires of TRAV1-2/CD4+ and TRAV1-2/CD4- sorted T cells were sequenced (GS FLX, 454/Roche). Rearrangements with simple V/J joints, present in at least half of the samples, were selected as potentially new invariant T cells. MAIT sequences were removed and the remaining sequences were grouped by the presence of a similar CDR3 sequence (1 amino acid difference allowed). Our analysis identifies 17 new invariant T cell rearrangements of which 11 were replicated in independent datasets. MAIT cells are highly expanded in the 6 individuals. Within the total repertoire, the new invariant cells are medium to highly expanded (comparable to GEM cells). Of interest, 2 of the α-chain rearrangements, TRAV1-2 combined with J12 or J20, were recently also identified as MAIT cells by Reantragoon et al (2013) using MR1-tetramer sorting. Subsequent work will focus on isolation and functional analysis of the identified invariant T cells.

W.96. Characterization of Innate T Cell Frequencies In 100 Individuals

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With their unique ability to both recognize conserved antigens and rapidly secrete effector T cell cytokines, innate T cells have challenged the classical paradigm of distinct innate and adaptive immunity. We sought to quantify the circulating frequencies and compositions of invariant natural killer T (iNKT), mucosal-associated invariant T (MAIT), and γδ-T innate T cells in 100 healthy individuals. We analyzed peripheral blood by flow cytometry and identified iNKT, MAIT, and γδ-T cells by their restricted T cell receptor (TCR) expression patterns. We further sub-typed MAIT and iNKT cells by CD4 and CD8 expression. Across the cohort we found highly variable innate T cell frequencies with respect to total T cell number (MAIT 0.1-15.4%; iNKT 0.0-1.1%; γδ-T 0.8-23.4%). We used multivariate regression to identify significant ($p<0.05$) correlations between the observed cell frequencies and subject demographics. Aging was associated with decreases in MAIT ($p=5.57\times10^{-5}$) and γδ-T ($p=4.68\times10^{-}$

^{4}) cell frequencies. Conversely, we observed an age-correlated increase in the percentage of Vδ1^{+} γδ-T ($p=1.3x10^{-3}$) and CD8^{+} iNKT ($p=0.04$) cells with respect to their parent populations. We quantified a positive correlation between γδ-T cell frequency and the proportion of Vδ1^{+} γδ-T cells ($p=2.68x10^{-8}$) and an inverse relationship between MAIT cell frequency and CD4^{+} MAIT cell proportion ($p=4.82x10^{-7}$). Lastly we observed elevated γδ-T cell frequencies in male subjects ($p=0.02$). The findings of this project will facilitate further insight into the functional roles of innate T cells and the consequences of their dysregulation in the human immune response.

W.97. Normal Human Renal Microvascular Endothelial Cells Express Autoimmune Regulator (AIRE) Protein
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Humans, but not rodents, normally express high levels of MHC class II on renal microvascular endothelial cells (REMC). Prior studies from our lab indicate that the gamma-interferon inducible form of CIITA is responsible for the HLA-DR expression of RMEC although normal physiologic concentrations of interferon are sufficient to maintain the DR expression in cultured RMEC. CD80 and CD86 are not normally expressed on RMEC. The role of the high levels of HLA-DR without associated inflammation remains unexplained. Based on these observations, we hypothesized that RMEC HLA-DR may contribute to peripheral tolerance in humans. A possible mechanism could involve expression of tissue specific antigens under the direction of AIRE. Using multicolor flow cytometry of normal human kidney we now identify high levels of AIRE expression in RMEC, suggesting a mechanism for peripheral tolerance in humans similar to the central tolerance conferred by AIRE in the thymus. In humans, cardiac and gut microvascular endothelial cells also express high levels of HLA-DR under normal conditions. Hence, it is possible that microvascular endothelial cells form a peripheral tolerance network in humans.

W.98. Functional Analysis of FCP: A FYVE Domain Containing Protein of *Plasmodium Falciparum*
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One of the highlights of *Plasmodium falciparum* genome sequencing was the presence of several putative signaling molecules like protein kinases in the parasite. Despite this information, the role of signal transduction events in the development of malaria parasite was very limited. Especially, information about molecular machinery involved in carrying out signaling events was scarce. Subsets of these endosomal proteins possess a highly selective PI3P binding zinc finger motif belonging to the FYVE domain family. We have identified a single **F**YVE domain **C**ontaining **P**rotein in *Plasmodium falciparum* which we term FCP. Expression and mutagenesis studies demonstrated that key residues are involved in specific binding to PI3P. In contrast to FYVE proteins in other organisms, endogenous FCP localizes to a lysosomal compartment, the malaria parasite food vacuole (FV), rather than to cytoplasmic endocytic organelles. Transfections of deletion mutants further indicate that FCP is essential for trophozoite and FV maturation and that it traffics to the FV via a novel constitutive cytoplasmic to vacuole targeting pathway. However, full length FCP deletion mutant were lethal for *P. falciparum*. Alternatively, we examined the function of this gene in yeast system. Two FYVE domain containing homologs were identified in yeast S. Serevacie (ScPEP7 and ScVps27p). Complementation using codon optimized FCP into yeast deletion mutant (ScPEP7 and ScVps27p) is under study. Further we expressed and purified full length as well as different deletion mutant of FCP protein from *E.coli* in order to study the role of FCP in hemozoin formation. We propose that inhibitor of FCP function can be a potential therapeutic negotiator to treat malaria.

W.99. Induction of Specific Immune Tolerance to Encephalitogenic Antigens in BALB/c Mice via Anterior Chamber-associated Immune Deviation
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Anterior chamber-associated immune deviation (ACAID) is a form of antigen-specific immune tolerance that is induced by administration of antigens into the anterior chamber of the eye. We tested whether ACAID could be induced in BALB/c mice

following the anterior chamber inoculation of myelin oligodendrocyte glycoprotein (MOG_{35-55}) and myelin basic protein (MBP). Since ACAID is known to impair host delayed-type hypersensitivity (DTH) responses, DTH assays and local adoptive transfer (LAT) assays were performed to test for MOG_{35-55}/MBP-induced ACAID. Both antigens were shown to induce $CD8^+$ T cell-mediated ACAID following their anterior chamber injection in BALB/c mice. Eye-mediated immune tolerance could be used for therapy of MOG_{35-55}/MBP-mediated autoimmune diseases such as multiple sclerosis.

W.100. Generation of CD11b(+)Ly-6C(+)Gr-1(+) Granulocytes Induced by the Vaccine Adjuvant

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The vaccine adjuvants have been extensively used to enhance the immunogenicity of specific antigens in vaccination. Although oxidation is implied in connecting the innate immune response and MHC I antigen presentation, its role in vaccine adjuvanticity remains poorly understood. The objective of this study was to examine the roles of the granulocytes in the production of the reactive oxygen species (ROS) induced by the vaccine adjuvant treated animals. The C57BL/6J mice were immunized at 6-8 week of age with the vaccine adjuvant, consisting of Pluronic L121, Tween 80, and squalane in PBS, and the ovalbumin (OVA) model antigen. Cells from bone marrow and spleens were harvested on day 10 and analyzed by flow cytometry. To determine the effect of antioxidants, the bone marrow cells were co-cultured with the adjuvant-pulsed EL4 cells in the presence of the antioxidants, and the production of reactive oxygen species (ROS) in the cells was detected by 2', 7'-dichlorofluorescene diacetate (DCFDA) and hydroethidine (HE). Our results showed that L121-adjuvant significantly stimulated the generation of SSC(+)CD11b(+) Ly-6C(+)Gr1(+) granulocytes in bone marrow and spleens of the immunized animals, notably the CD11b(+)Gr-1(hi) subpopulation. The adjuvant also induced the ROS production, including H_2O_2 and $\cdot O_2^{\infty\bullet}$, in the granulocytes, including both the CD11b(+)Gr-1(hi) and CD11b+Gr-1(lo) subpopulations, which was reduced upon the treatment with the antioxidants. Results obtained in this study demonstrated a significant generation of granulocytes and the percentage of the Lin(-)c-kit(+)Sca-1(+) cells in the bone marrow of vaccinated animals, implicating the potential roles of granulocytes in cross-presentation of exogenous antigens.

Reproductive Immunology

OR.35. Maternal Cell MicroRNA as a Biomarker to Predict Adverse Pregnancy Outcome

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Background: Prior to the end of the first trimester, pathogenic mechanisms may commit pregnancies to adverse outcome such as preeclampsia and miscarriage. A long-term search for bio-markers predicting these adverse outcomes has not identified any that reliably succeed prior to the beginning of the second trimester. MicroRNAs, with their important role as regulators of signaling and metabolic pathways within living cells, may offer a new approach. Methods: qRT PCR for a panel of microRNAs was performed on 39 patients (19 healthy deliveries, 12 preeclampsias, 7 late onset and 5 early onset, and 8 miscarriages) using standard Taqman real time PCR and Fluidigm dynamic array. Peripheral blood samples were collected during the first trimester. MicroRNAs were quantified and results scored and their predictive values assessed.Results: MicroRNA assessment predicted miscarriage and late preeclampsia with a p value of $p<0.0001$, and demonstrated a fitted ROC area of 0.90 for miscarriage and 0.90 for late preeclampsia. Performance characteristics of microRNA quantification are compared to similar characteristics of each of several cell-based immune tests. Our data demonstrate the superior power of maternal blood cell microRNA to predict pregnancy outcomes. Cconslusion: MicroRNA quantification of maternal blood cells offers the clinician a single quantitative test result that is simple to interpret. In addition, it can successfully predict late preeclampsia. Identification of important maternal cellular microRNA during the first trimester may improve our understanding of the feto-maternal dialogue during the formative period of the placenta and benefit patients from therapy.

T.54. Lymphocyte Expression and Activity of the Co-Stimulatory Molecules CD226 and Tight in Decidual Tissue and Umbilical Cord Blood

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A breakdown in immune tolerance to the semi-allogeneic conceptus plays a role in the pathogenesis of pre-eclampsia. A recently described T cell co-stimulatory pathway important for immune regulation involves the competitive binding of CD155 on APCs with the co-stimulatory molecule CD226 or the inhibitory receptor TIGIT. CD226 has been putatively identified as a candidate susceptibility gene for multiple autoimmune diseases, whereas TIGIT possesses intrinsic suppressive effects on T cells and decreases trans-epithelial migration of lymphocytes. Therefore, we sought to better understand the role for these receptors on T cells during pregnancy and placental development. We characterized the expression of these markers on decidual lymphocytes isolated from healthy term placental tissue. Flow cytometric analysis demonstrated constitutive CD226 expression on $CD4^+$ T cells (MFI=5999+412), while TIGIT expression was limited to 8.9+0.62% of CD4+ T cells (MFI=160+6, N=3). To assess the immunoregulatory function of these receptors, we FACS isolated and expanded $CD4^+CD25^+CD127^{-/lo}$ Tregs from cryopreserved umbilical cord blood samples. Treg subsets were further segregated based on CD226 or TIGIT expression and analyzed for co-expression of FOXP3 and Helios and the degree of demethylation at the Treg-specific demethylated region (TSDR). After expansion, CD226 was constitutively expressed (MFI=8978+2534) whereas TIGIT was identified on 73% (MFI=1940+446). At a 1:1 Treg:Teff ratio, suppression of CD4+ T cell proliferation was observed (mean=42%) and this correlated with TIGIT expression (r^2=0.82, n=4). These studies provide novel insight into this co-stimulatory axis during pregnancy. Further studies will determine if an imbalance in this pathway plays a role in pregnancy related pathologies.

T.55. Effect of Epigallocatechin Gallate as an HIV-1 Microbicide on Mucosal Immunity

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Topical microbicides applied to the vaginal mucosal tissue to prevent HIV-1 infection are potential preventive methods for HIV-1 infection. Previously our laboratory has shown that the green tea catechin, epigallocatechin gallate (EGCG), inhibited attachment of gp120 to the CD4 receptor on T cells thus, preventing HIV-1 entry. To test EGCG as an HIV-1 microbicide, cervicovaginal cultures (derived from human ectocervical cells), in simulated vaginal (SVF) and semen (SSF) fluids to simulate an *in vivo* mucosal environment were utilized. Cultures were exposed to EGCG (0 - 100uM) or control catechin in basal medium or simulated fluids tested separately or combined 1:4 (SVF: SSF) and tissue viability was measured by MTT assay. Cultures were subject to pH transition with pH adjusted RPMI or simulated fluids (pH 4.2 or 7.7). Fluids were A disk diffusion assay assessed the effect of EGCG (0 -75uM) on the lactobacilli (*L. gasseri* and *L. crispatus*) found in the female reproductive tract (FRT). The cultures exhibited no significant decrease in viability when exposed to EGCG (0 - 100uM) at 1 and 24 hours ($p<0.001$). There was no significant cytotoxicity due to pH transition from pH 4.2 (SVF) to pH 7.0 (SVF: SSF) (50 uM EGCG; $p=0.005$). Compared to ampicillin and vancomycin controls, no zones of inhibition were seen with either strain of lactobacillus when exposed to EGCG (0-75uM). In conclusion, EGCG is not cytotoxic on human cervicovaginal cultures and would be a safe and well tolerated component of a potential HIV-1 microbicide.

Therapeutics/Pharmacology

OR.15. Synthetic Nanoparticle Vaccines for the Induction of Antigen-Specific Immunological Tolerance

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Current treatments to control pathological or unwanted immune responses often employ broadly immunosuppressive drugs. New approaches to induce antigen-specific immunological tolerance that control both cellular and humoral immune responses are desirable. Here we describe the development of tolerogenic nanoparticle (tNP) vaccines for the antigen-specific inhibition of undesired immune responses. These self-assembling, biodegradable poly(lactide-co-glycolide) (PLGA) nanoparticles containing either protein or peptide antigens and a tolerogenic immune modulator are capable of inducing durable antigen-specific tolerance that control adaptive immune responses and withstand multiple immunogenic challenges with antigen even in the presence of toll-like receptor agonists. We demonstrate that administration of tNPs through multiple routes (e.g. subcutaneous and intravenous) inhibits the activation of antigen-specific T cells and B cells while inducing antigen-specific Tregs. These effects are dependent on the presence of the encapsulated immunomodulator, as an equivalent dose of the free immunomodulator was ineffective. Tolerogenic nanoparticles effectively prevented anti-FVIII inhibitory antibody development in a mouse model of hemophilia A. Remarkably, despite multiple challenges, FVIII-specific immune tolerance

was sustained for at least 166 days after the last treatment. A therapeutic protocol using hemophilia A mice pre-sensitized with FVIII also led to inhibition of anti-FVIII titers. Tolerogenic NP therapy represents a potential novel approach for the treatment of treatment of allergies, autoimmune diseases, and prevention of anti-drug antibodies (ADA) against biologic therapies.

OR.18. Inhibition of TYK2 and JAK1 Ameliorates Imiquimod-induced Psoriasis-like Dermatitis by Inhibiting IL-22 and IL-23

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Psoriasis is a chronic autoimmune disease affecting the skin and characterized by scaling due to aberrant keratinocyte proliferation and function. Proinflammatory cytokines released by infiltrating immune cells play important roles in psoriasis and in particular, IL-23 and IL-22 have emerged as critical components in the pathogenesis of psoriasis. Cytokine signaling is dependent on the JAK family of protein tyrosine kinases, making Janus kinase inhibition an appealing strategy for the treatment of psoriasis. IL-22 signaling is dependent on TYK2 and JAK1, while IL-23 signaling is predominantly mediated by TYK2. Using a small molecule inhibitor with specificity for JAK1 and TYK2, we investigated the role of TYK2 and JAK1in an imiquimod-induced psoriasis model in mice. Compared to TYK2 knockout mice, dual inhibition of JAK1 and TYK2 resulted in a striking decrease in disease pathology, reduced activation of keratinocytes, and lower proinflammatory cytokine levels, including IL-22 and IL-23. Of note, neither TYK2 nor dual TYK2/JAK1 inhibition resulted in a decrease in CD4+ cell numbers in the skin, but did result in a decrease in IL-17 production and Vγ3+ cells, supporting a role for gd T cells in the pathology of psoriasis. Overall, inhibition of both TYK2 and JAK1 represents a viable therapeutic target in the treatment of psoriasis.

T.26. Surrogate Antibody for a Humanized Anti-IL-12/23p40 Mab, CEP-37248, Shows Potent Activity in Multiple Models of Inflammatory Bowel Disease

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Inflammatory bowel disease (IBD) is a general term used to describe conditions, such as Crohn's disease and ulcerative colitis (UC), which share similar clinical symptoms, but differ in pathophysiology. Central to both IBD-subtypes is the role of Th1/Th17-type cytokines, IL-12 and IL-23. To further characterize a humanized anti-IL-12/23p40 Mab, CEP-37248, a surrogate antibody was generated (105D10) that bound the same epitope on mouse IL-12/23p40 as CEP-37248 does on human. Induced, pre-symptomatic adoptive T cell transfer model mice were dosed with 105D10 Ab at 3, 10, 30 mg/kg, i.v., 2x weekly for 28 days and compared against whole mouse IgG (mIgG) and vehicle controls. Acute (5 day-exposure) DSS-colitis induced model mice were dosed with 105D10 at 0.1, 1, 10 mg/kg, i.p., 2x weekly for 10 days. For the T cell adoptive transfer model significant improvements over mIgG control were observed for 3, 10, 30 mg/kg groups in colon weight (36.4%, 40.0%, 40.0% respectively, $p<0.01$) and colon length; this correlated with improvement in body weight, survival and histology for area of involvement ($p<0.0001$), hyperplasia ($p<0.0001$), and cellular infiltration ($p<0.01$) vs. mIgG control. Similar results were observed in the DSS-induced colitis model for 1 and 10 mg/kg groups with improvements in colon length and end body weight (14.0%, 16.2% respectively, $p<0.01$). This report here shows that targeting a domain different than that of ustekinumab (D1 domain of p40 subunit; surrogate Ab blocks D3 domain) results in effective cytokine blockade and significant disease suppression in mouse models of IBD.

T.27. Orally Active, Janus Kinase 2 (JAK2) Inhibitor, CEP-33779, Ablates Disease in Multiple Models of Experimental Autoimmune Encephalomyelitis (EAE)

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Multiple sclerosis (MS) is a chronic human autoimmune disease caused by an inflammatory reaction to myelin antigens in the central nervous system leading to demyelination and neurodegeneration. Experimental autoimmune encephalomyelitis (EAE) shares many of the clinical and histopathological features of MS and is the accepted animal model for MS. CEP-33779 is a highly selective, orally active, small-molecule inhibitor of JAK2 which was evaluated in three models: chronic EAE induced by myelin oligodendrocyte glycoprotein (MOG_{35-55}), acute EAE induced by spinal cord homogenate (SCH), and relapsing remitting EAE induced by proteolipid protein ($PLP_{139-151}$). Pre-symptomatic animals were treated orally with CEP-33779 at 3-10, 30, 55-60 or 100-110 mg/kg, p.o, q.d or b.i.d for 30-60 days, depending on the study; all compared to vehicle controls. CEP-33779 could inhibit group mean scores of MOG-induced EAE at 30, 55 mg/kg, b.i.d and 110 mg/kg q.d (90.5%, 99.5%, 98.5% respectively, $p<0.001$), SCH-induced EAE at 60 mg/kg, 30 mg/kg, b.i.d ($p<0.05$), and PLP-induced EAE at 100 mg/kg b.i.d, q.d, 60 mg/kg b.i.d, q.d and 30 mg/kg b.i.d (87%, 60.9%, 65.2%, 39.1%, 43.5% respectively, $p<0.01$) compared to vehicle controls. Spinal cord histology matched in-life data as CEP-33779 at 30, 55 mg/kg b.i.d, and 110 mg/kg q.d could also significantly reduce the level of scored parenchyma inflammation ($p<0.0001$) and white/gray matter demyelination ($p<0.01$) as determined by specialty stains. CEP-33779 demonstrated efficacy across multiple models of EAE with a minimal efficacious dose of 30-60 mg/kg. These data demonstrate the potential use of selective JAK2 inhibitors in the treatment of MS.

T.29. Serum soluble ST2, IgG Subclass, hsCRP and eSOD Biased By Chronic Statin Exposure

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Statins are the most common drugs used to lower LDL cholesterol. In addition, statins alter immune and inflammatory responses. In this era of new and evolving infections, vaccine development, autoimmune diseases, and patients with compromised immunity, it will be prudent to predict whether statins might, either beneficially or detrimentally bias the treated population's response to vaccinations and disease. This observational study included 42 volunteers: 21 adults having continuous statin exposure of at least 2 years with 21 statin-naive demographically-matched statin-naïve controls. The aim was to determine whether chronic statin exposure is associated with a pattern of immunoinflammatory responses distinguishable from that of controls, based on IgG subclass antibody production (pattern of humoral immune response to antigenic exposures, high-sensitivity C-reactive protein (marker for inflammatory response), erythrocyte superoxide dismutase (marker for antioxidative capacity), and soluble ST2 decoy interleukin-33 receptor (marker for immunoinflammatory processes associated with cardiovascular disease). Evaluable results support significant anti-inflammatory effects that likely contribute beneficially to reduce cardiovascular disease risk and bolster immunity against extracellular pathogens. It is also possible that bias toward TH2 rather than cellular immune response could risk worse outcomes in some circumstances. For example, in treatment of NRTI (e.g., stavudine) -provoked dyslipidemia with statins, the clinical implication of an incidental TH2 bias for HIV-infected patients has not been evaluated in controlled trials.

T.30. Human Primary Bronchial Epithelial Cells With Allergen Stimulation Differentially Express Cytokines in the Presence of a Steroid and LABA or Their Combination

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Background: Different allergens *in vitro* might induce separate cytokines, and could be suppressed with formoterol *vs.* mometasone in synergistic combination. Methods: Primary bronchial epithelial cells were cultured *in vitro* until confluence with either ragweed (500 mcg/ml) or dust mite antigens (1000 AU/ml) with or without the addition of formoterol (1.3 x 10-7 M) or mometasone (1 X 10-7 M) or their combination. Supernatants were harvested after 24 hrs . Samples were shipped on dry ice to Assaygate (Ihamsville, MD) for ultrasensitive human ELISA cytokines assays. Results: Interferon γ was significantly

suppressed with either formoterol or mometasone alone but not with the combination. Mometasone decreased IFN-γ levels more than formoterol (P<0.05) with ragweed allergen. IL-12P70 was suppressed with either formoterol or mometasone alone but not the combination with ragweed allergen. (P<0.05).IL-12 and IL-23 share the IL-12p40 molecule. IL-7 was only significantly reduced with mometasone (P<0.05)and IL-13, was only significantly reduced by either formoterol or mometasone (P<0.05) but not the combination in the presence of ragweed allergen. IL-5 was suppressed by either formoterol, mometasone or the combination in the presence of ragweed allergen but there was no statistically significant differences among these treatments.There was no change in IL12P70, TNF, IL-5, IL-13 and only mometasone alone suppressed IL-2 significantly, compared to formoterol alone or the combination (P<0.05) in the presence of dust mite allergen. Conclusions: The ability of combinations of formoterol and mometasone to suppress pro-inflammatory cytokines may depend on the specific type of allergen used to stimulate *in vitro* bronchial epithelial cells.

T.31. Optimizing the Timing of Biomarker Measurement in Response to Immune Therapies (Rituximab and Abatacept) in Type 1 Diabetes

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There is an urgent need to discover early biomarkers of therapeutic response in T1D in order to expedite clinical trials. Recently, Pescovitz et al[1] reported a temporal relationship between change in a biomarker of B-cell depletion and change in C-peptide. Specifically, the difference between treatment groups in the number of CD19+ cells explained more of the difference in C-peptide at 12 months the farther back in time the immunological change was measured. In this study, we sought to determine whether this finding would be replicated in a co-stimulation blockade model. The TrialNet Abatacept study[2], a placebo controlled trial of CTLA4-Ig with 127 patients randomized, measured immunologic changes in the T-cell repertoire with FACS over a 2-year treatment period. In the abatacept treated group, changes in central memory T-cell populations at 6 months explained more of the variation in 1 year C-peptide changes than did central memory changes measured at the same time (1 year) (R^2=9% vs. R^2= 4%). Although this difference failed to reach statistical significance, it should be noted that the Rituximab findings were also not tested for significance. Our findings therefore empirically replicate the delayed effect. We speculate that immunotherapies that impact on autoimmune response require a definable time period for effective changes to occur in the islet inflammatory milieu. Events during this time period might include removal/depletion of inflammatory cells; “wash-out” of inflammatory mediators; and recovery of beta cell function. This new insight indicates that there may be optimal time-points for discovery of biomarkers for these processes.

T.32. Inhibition of Foxp3 in Cancer Cells Induces Apoptosis of Thyroid Cancer Cells

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Forkhead box P3 (Foxp3) is a classical marker of regulatory T cells (Tregs). An increase of Foxp3+ Tregs was found in peripheral blood and intra thyroid lesion of human thyroid cancer and these Tregs facilitate the growth and invasion of thyroid cancer. Recently, there are increasing publications showing that Foxp3 can also express in cancer cells but its function remains inconsistent. Very limited information is available on Foxp3 expression in thyroid cancer cells. In this study, we first found that the expression of Foxp3 was higher in thyroid cancer cells that in normal thyrocytes. Depletion of Foxp3 by its shRNA resulted in the decrease of cell proliferation and migration, but an increase of cell apoptosis, suggesting a positive role of Foxp3 in the progression and migration of thyroid cancer cells. Interestingly, the inhibition of Foxp3 could lead to the elevated expression of PPARg protein and enhanced PPARg activity determined by Western blot and luciferase reporter assay respectively. In addition, the inhibition of Fox3 downregulated the expression of NF-κB subunit p65 and cyclin D1 but upregulated the level of caspase-3. These molecular changes are in line with Foxp3 shRNA-mediated alteration of cell functions. In conclusion, our study demonstrates that human thyroid cancer cells express a high level of functional Foxp3 and that the inhibition of this Foxp3 suppresses the proliferation and migration but promotes apoptosis in thyroid cancer cells. The findings suggest that targeting Foxp3 in thyroid cancer cells may offer a novel therapeutic option for human thyroid cancer.

T.33. The intestinal mucosa immunomodulatory effect of *Osmanthus fragrans*

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Owing to its special aroma, the flower of *O. fragrans*, called *Kwai-fah* in China, has been used as a beverage and as an additive for foods. *Ben Cao Hui Yan*, traditional Chinese medical literature, describes the usefulness of the flower of *O. fragrans* for arrest of dysentery with blood in the bowel, and stomachache and diarrhea treatment. Many studies have reported that intestinal inflammations that damage intestinal epithelial cells and secrete IL-8 are important cause for inflammatory bowel disease (IBD). The immunomodulatory activity maintenance in intestinal environment will be the important issue in IBD therapy. Previously, anti-allergic effect of OFE (ethanol extract of *O. fragrans* flowers) has been reported, but the immunomodulatory mechanism has not been studied. This study is focused on the intestinal immune modulatory activity of *Osmanthus fragrans* extract and its related compound on human intestinal epithelial cell line. At the first, the IL-8 inhibitory activities of OFE and its related compound *in vitro* after H_2O_2 administration were evaluated. The NF-κB signal pathway dependent or independent by this inhibition is also been evaluated. *In vitro* results showed OFE and its related components can suppress IL-8 secretion even after H_2O_2 administration and this suppression is NF-κB dependent. Furthermore, the maturation of mouse bone marrow derived dendritic cells was evaluated. OFE and its one major compound could not promote the maturation of dendritic cells. However, OFE can suppress the maturation of ovalbumin treated dendritic cells. More investigation for the immunemodulatory mechanism of OFE is needed in the future.

T.34. Nanobodies: A Versatile Advanced Therapeutic Platform

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While the field of therapeutic antibody design and development technology has advanced rapidly in the past few decades, several limitations have also become apparent, such as the ability to generate multi-specific drugs, vary PK/distribution, develop highly selective drugs to certain molecular classes such as ion channels and GPCRs, and deliver via alternate routes of administration. Overcoming these limitations has particular relevance when developing drugs in the fields of inflammation, host defense, and immunotherapy, where multiple different pathways/molecules/epitopes need to be targeted using drugs with tailored pharmacokinetics and modes of administration. Novel approaches to extend beyond the basic antibody structure are therefore needed. Single domain antibody fragment technology represents one potential avenue of addressing some of these issues. As a leader in the field with over 30 active drug programmes, Ablynx has world-leading insight and experience in the use of single domain antibody fragments, which we term Nanobodies, that spans product conceptualization through discovery, preclinical and advanced clinical development. Using both preclinical and clinical stage compounds as case studies in the fields of inflammation, host defense, and cancer immunotherapy, I will illustrate how the use of Nanobodies can successfully address a range of concrete and distinct limitations of conventional antibody technology, effectively delivering clinical benefit beyond what is feasible with the standard antibody structure.

T.35. Diverse rResponses of Scleroderma Fibroblasts Against Anti-fibrotic Agents - Implication for "Personalized Medicine" in mmune-induced Diseases

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Systemic sclerosis (SSc) is a heterogenous immune-mediated fibrotic disease. Disability of daily life in SSc patients is largely depending on skin fibrosis starting in distal site of a limb. However, there is no RCT proven therapies for skin fibrosis of SSc. The effect of anti-fibrotic drug, including TGF-beta Ab, PPAR-gamma agonist, and Imatinib was various between patients. In the clinical situation, it is crucial to know the best drug protocol for a specific patient facing rather than to know the result of RCT. We, therefore, explanted fibroblasts from SSc forearm skin, which were cultured with multiple anti-fibrotic drugs, such as Imatinib or PPAR-gamma agonists exclusively, since fibrosis is fundamentally a result of collagen deposition released from fibroblasts. The anti-fibrotic drug responses were different between patients' fibroblasts: some patients' fibrosis were preferentially blocked by PPAR-gamma agonists more than Imatinib in vitro; however the other patients were effectively blocked by Imatinib. Thus, it is possible to test the anti-fibrotic effects of candidate agents on patients' own fibroblasts just before the patient adopts their individualized anti-fibrotic drug. Recently, drug selection based on gene signature has been

named as “personalized medicine”, potentially providing best drug selection. We now propose a new sight into personalized medicine, which includes in vitro results on targeted cells in addition to gene information. Likewise, this in vitro agent-cell culture approach may offer a novel translational approach for the other immune diseases with a single cell target as well.

T.36. Suppression of Inflammation and Hyperglycemia in Murine Models of Type 1 Diabetes by Emetine - a Novel Therapeutic Agent that Targets Pigment Epithelium Derived Factor

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Pigment epithelium derived factor (PEDF) is a 50kDa secreted protein with multifunctional properties including anti-angiogenesis, neurotropism, and inflammation. Elevated serum levels of PEDF are significantly associated with multiple diseases including type 1 and type 2 diabetes. Our previous studies have shown that PEDF induces macrophage activation in concentration dependent manner. PEDF administration to naïve animals results in the development of insulin resistance. Additionally, neutralization of PEDF with anti-PEDF antibodies improves the clinical outcome in animal models of type 2 diabetes. Our recent studies identified a clinical drug candidate that attenuates PEDF mediated inflammation. Using a high-throughput screening approach, murine macrophages were challenged with PEDF in either the presence or absence of clinical drug candidates, and levels of TNF released into the supernatant were analyzed. To evaluate the effect of drug compounds on viability, cell death was assessed using MTT assay. From 1,600 clinical candidate compounds screened, three lead compounds were identified, one being emetine, that dose dependently reduces PEDF induced macrophage activation without toxicity. Surface plasmon resonance analysis revealed that emetine binds to PEDF with high affinity (kD<25nM), and inhibits PEDF binding to its proinflammatory receptor adipose triglyceride lipase. Moreover, emetine reduces endocytosis of PEDF into macrophages. Administration of emetine to murine models of type 1 diabetes improves hyperglycemia, suppresses circulating levels of anti-insulin antibodies, and reduces pancreatic TNF and PEDF. Our studies suggest that emetine attenuates PEDF induced inflammation, and has a significant potential for the treatment of type 1 diabetes.

T.37. B16 Mouse Melanosomes Can Serve as a Chemoattractant for Macrophages

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ABSTRACT:Previous research in the Rosenthal lab has shown that the inhibitor of differentiation-4 (Id4) stimulates pigment production, *in vivo,* resulting in histiocyte recruitment and tumor necrosis. Histiocytes are tissue macrophages derived from the monocyte lineage. However, the chemotactic factor that attracts macrophages to the melanoma has not been described. Two mouse macrophage cell lines (RAW264.7 and IC-21) were therefore incubated with different concentrations of conditioned medium (CM) containing melanosomes obtained from B16F0 mouse melanoma cells. HaCaT immortalized human keratinocytes were used as a negative control. After 3 hours of incubation with CM, macrophages were collected and melanosome uptake was measured by absorbance at 340 nm. Significant melanosome uptake was observed in both macrophage cell lines when compared to negative control, indicating that macrophages readily phagocytose melanosomes present in CM. A trans-well migration assay was performed to further examine the interaction between RAW264.7 and IC-21 macrophages and pigment-producing B16F0 melanoma cells. Our initial observation suggests a trend toward increased migration of macrophages toward pigment-producing melanoma cells and thus merits further investigation. Since Id4 expression was required for melanin synthesis *in vivo*, we investigated the effect of ectopic Id4 on the production of tyrosinase, the rate-limiting enzyme in controlling the production of melanin. However, no association was detected after 48 hours. Further time points, may be required to determine if Id4 alters tyrosinase production in B16 melanoma cells.

T.38. Low Dose Methotrexate Induction Treatment Induces Long-Lived, Antigen-Specific Immune Tolerance through the Induction of Regulatory B cells

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Biologic drugs, including enzyme-replacement therapies (ERT), can stimulate the development of anti-drug antibodies (ADA) that may interfere with drug efficacy and impact patient safety. In an effort to control the development of ADA, we focused on identifying a regimen of immune tolerance induction that would be readily applicable for clinical use. Previously, we discovered that three brief cycles of low dose methotrexate provide long-term tolerance to alglucosidase alfa and agalsidase beta, two ERTs. Subsequently, successful immune tolerance induction coupled with an improvement of drug efficacy have been reported using this low-dose regimen of methotrexate along with Rituximab and optional IVIG in treatment-naïve infantile-onset Pompe patients that experience poor clinical outcomes linked with ADA. We now provide additional murine data suggesting that even a single cycle of low dose methotrexate can induce a long-lived reduction in algulcosidase alfa-specific ADA. The methotrexate-induced reduction in ADA is maintained through alglucosidase alfa rest and re-challenge and the tolerance induction appears to be antigen-specific. IL-10-expressing regulatory B cells are expanded following methotrexate treatment and can transfer immune tolerance to alglucosidase alfa-naïve mice. We hypothesize that low-dose, methotrexate induction treatment may successfully tolerize patients to biologic therapies, representing a readily available, benign and cost-effective means of promoting immune tolerance in at-risk patient populations.

T.39. Scavenger Receptor CD36: a New Therapeutic Target for Chronic Kidney Disease Progression

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Chronic kidney disease (CKD) affects 20 million Americans. Many patients treated with current standard-of-care Renin-Angiotensin-Aldosterone System inhibitors still progress to end-stage disease. New therapeutics are needed. CD36 is associated with many risk factors for CKD, such as dyslipidemia and metabolic syndrome. To determine the role of CD36 in CKD, we compared WT vs. CD36 knockout (KO) mice in a model of CKD. We further tested a CD36 antagonist peptide (5A) in vivo. Sixteen-week-old C57BL/6 WT and KO mice were subjected to 5/6 nephrectomy (Nx) with continuous infusion of Angiotensin II (AngII) via osmotic minipump, which is required for progression of CKD in this model. WT mice subjected to 5/6Nx without AngII infusion were used as controls. Blood pressure was measured weekly by radio telemetry in KO5/6Nx+AngII and WT5/6Nx+AngII groups (N=6/group). Albuminuria was measured weekly; serum creatinine (Scr), BUN, histological damage and mRNA expression of cytokines measured in the remnant kidney at 4 weeks (N=11-19/group). WT5/6Nx+AngII mice developed a substantial decline in kidney function with histological damage, albuminuria, and a metabolic profile that accompanies CKD. Kidney function was protected in the KO5/6Nx+AngII and WT5/6Nx+AngII+5A groups, with improved histology and metabolic profile, and decreased albuminuria. There was no difference in mean blood pressure between the groups. 5A decreased mRNA expression of cytokines (IL-6, CXCL-1, IL-1beta) in the kidney. Conclusions: CD36KO and WT mice treated with 5A are protected from CKD progression, and this effect was independent of blood pressure. CD36 is a novel therapeutic target for CKD.

T.40. Therapeutic Application of Targeted Synthetic Vaccine Particles (*t*SVP) in HPV Cancer Model: Long-term CTL Activity, Adjuvant Sparing and Relief of Injection Site Reactions

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We have earlier reported that targeted Synthetic Vaccine Particle (*t*SVP) technology, which enables nanoparticle co-encapsulation of antigens and adjuvants leads to induction of strong humoral and cellular immune responses with minimal systemic production of inflammatory cytokines. Such cellular responses provided for significant activity against OVA-expressing tumor cells in vivo and generated long-term central and effector CTL memory. Here we extend these findings using *t*SVP immunization for management of TC-1 epithelial tumor, which expresses HPV-16 oncogene E7. Therapeutic treatment with *t*SVP-E7.I.49 containing dominant MHC class I peptide from E7 protein in combination with TLR7/8 or 9 agonists lead to profound suppression of tumor growth after subcutaneous or metastatic seeding and thus to 3-5 week delay in animal

mortality. A single injection of *t*SVP-E7.I.49 carrying TLR9 agonist CpG resulted in strong and consistent levels of CTL activity as early as 4 days and as late as 31 days after particle inoculation. *t*SVP-encapsulated CpG, *t*SVP-CpG, provided superior CTL induction and therapeutic benefit when compared to equal or higher amounts of free CpG. Moreover, identical levels of therapeutic activity and CTL induction were observed whether *t*SVP-entrapped CpG contained a natural nuclease-labile phophodiester backbone (PO-CpG) or modified nuclease-resistant phosphorothioate backbone (PS-CpG), which is commonly used in experimental vaccination and clinical trials employing CpG-based adjuvants. Notably, repeated multiple injections of therapeutically active *t*SVP-PO-CpG as needed for maintenance of anti-tumor activity in vivo did not result in local tissue inflammation, which was observed when PS-CpG was utilized.

T.105. Candidiasis in Secukinumab-Treated Subjects Is Non-serious and Transient: A Pooled Analysis of Data From 10 Phase 2 and 3 Clinical Trials in Psoriasis

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Background: Physiologically, interleukin (IL)-17A is involved in immune surveillance of mucocutaneous tissue, and genetic deficiencies in IL-17A function are associated with increased susceptibility to *Candida* infection (Puel A. *CurrOpinAllergyClinImmunol*. 2012;12:616-22). Consequently, anti–IL-17A therapies may confer increased risk of mucocutaneous candidiasis. Secukinumab, a fully human anti−IL-17A monoclonal antibody, demonstrated strong efficacy/tolerability in clinical studies of subjects with moderate-to-severe plaque psoriasis. Methods: To evaluate mucocutaneous *Candida* infection risk, we pooled data from 10 randomized, blinded, phase 2/3 studies in which subjects received secukinumab 300mg (n=1410; 1178 subject-years of exposure), secukinumab 150mg (n=1395; 1142 subject-years), etanercept (n=323; active comparator; 294 subject-years), and placebo (n=793; 201 subject-years) for ≤52 weeks. Results: The overall incidence of candidiasis in the pooled dataset was low. Exposure-adjusted incidence (per 100 subject-years) was greater in the 300mg (3.6) group, while incidence with 150mg (1.9) was similar to placebo (1.0) and etanercept (1.4). All *Candida* infections with secukinumab were localized to skin or mucosa and non-serious; two etanercept-treated subjects experienced severe cases. There were no chronic infections; all infections either self-resolved or responded to standard treatment, and none necessitated study treatment discontinuation. Conclusions: *Candida* infections with secukinumab in this pooled analysis were all mucocutaneous in origin, non-serious, localized, and either self-resolving or responsive to standard treatment. Long-term data are needed to fully understand the impact of anti–IL-17A therapy on candidiasis risk. The data presented here suggest that therapeutic IL-17A inhibition with secukinumab does not confer any serious biologic consequences typically observed in individuals genetically deficient in this cytokine.

T.106. Incidence of Major Adverse Cardiovascular Events With Secukinumab: A Pooled Analysis of Data From 10 Phase 2 and 3 Clinical Trials in Psoriasis

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Background: Psoriasis is associated with heightened cardiovascular risk and an increased frequency of major adverse cardiovascular events (MACE). Secukinumab, a fully human anti–interleukin-17A monoclonal antibody, demonstrated strong efficacy/tolerability in clinical studies of subjects with moderate-to-severe plaque psoriasis. Methods: To evaluate cardiovascular safety, we pooled data from 10 randomized, blinded, phase 2 and 3 studies in which 1410 subjects received secukinumab 300 mg (1178 subject-years of exposure), 1395 received secukinumab 150 mg (1142 subject-years), 323 received etanercept (active comparator; 294 subject-years), and 793 received placebo (201 subject-years) for up to 52 weeks. Results: Overall, 13 MACE were reported, with an incidence per 100 subject-years of: 300 mg, 0.51 (6 cases); 150 mg, 0.44 (5 cases); etanercept, 0.34 (1 case); placebo, 0.50 (1 case). No dose-dependence was evident. To verify potential MACE, an independent, blinded Cardiovascular and Cerebrovascular Safety Adjudication Committee was established. Two cases (1 for 150 mg [moyamoya disease], 1 for 300 mg [myocardial infarction]) did not meet prespecified adjudication criteria (the 300-mg dose level event initially reported as a myocardial infarction was reclassified as an electrocardiographic abnormality). The exposure-adjusted rate of confirmed incident MACE was thus 0.42 for 300 mg and 0.35 for 150 mg. All confirmed MACE

occurred in subjects with prior/active cardiovascular disease or risk factors. Conclusions: MACE incidence, regardless of the adjudication outcome, was infrequent and comparable among each secukinumab dose level, etanercept, and placebo.

Transplantation

OR.20. Interleukin-7 Receptor Blockade Promotes Long-Term Allograft Acceptance

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We demonstrated for the first time the tolerogenic effects of IL-7R blockade by a mAb in murine allograft models. Anti-IL-7Rα mAb given from 3 weeks before graft to 4 weeks post-graft induced allograft tolerance in a pancreatic islet transplant model where C57BL/6 islets were grafted to BALB/c recipients previously rendered diabetic by streptozotocin. Tolerant mice developed donor-specific hyporesponsiveness as shown by MLR and IFNγ ELISPOT and had reduced donor-specific antibodies. The adoptive transfer of T cells isolated from these tolerant mice to BALB/c Nude mice having previously received an islet graft from B6 donors leaded to graft tolerance, whereas the same experiment using T cells from naïve BALB/c mice leaded to graft rejection. In a more stringent skin allograft model where C57BL/6 skin was grafted to BALB/c recipients, anti-IL-7Rα mAb given after T cell depletion by 2 injections of anti-CD4 and anti-CD8 mAbs doubled median graft survival to 58d compared to 30d with T cell depletion alone. IL-7R blockade following T cell depletion also synergized with suboptimal doses of tacrolimus to maintain long-term skin allograft survival of more than 90d in up to 80% of mice, a remarkable graft outcome since this model is highly immunogenic. IL-7R blockade following T cell depletion inhibits T cell reconstitution, decreases memory T cell number, decreases T cell cytokine secretion, abrogates both cellular and humoral alloimmune responses, and increases Treg/T effector ratio; the latter is an important contributing mechanism since Treg depletion shortened skin graft survival. IL-7R blockade has potential as a robust immunomodulatory treatment in transplantation.

OR.36. Massively Parallel Sequencing of Mixed Lymphocyte Culture Reveals a Broad and Stable Alloreactive T Cell Repertoire

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The cellular immune response is a major barrier to patient tolerance of allografts. Positive followed by negative selection in the thymus ensures that T cell populations have some binding affinity for self-HLA, but culls T cells with high specificity for peptides presented on self-HLA. However, in the context of a patient with an allograft, these processes also allow for mature T cells with off-target specificity for self-peptides presented on allo-HLA. Currently, the breadth of the alloreactive T cell repertoire is unknown, as well as whether the alloreactive T cell repertoire is stable over time. We have used high-throughput sequencing to characterize the alloreactive T cell repertoire in three pairs of healthy adults using mixed lymphocyte culture, analyzing 4 reactions per pair: duplicate MLR reactions at baseline followed by duplicate MLR reactions 3 months later. Our results indicate that thousands of T cell clones proliferate in mixed lymphocyte culture, suggesting a very broad alloreactive T cell repertoire. This repertoire is consistent across biological replicates and across a span of three months, and is dominated by relatively high-abundance T cell clones. Our results suggest the existence of a broad alloreactive T cell repertoire consisting mainly of expanded memory T cells which remain stable over time. The presence of a large, stable population alloreactive T cells should allow for long-term tracking of alloreactive clones in patients receiving allografts.

OR.44. Dual-reactive T Cells Accelerating Rejection of Allo-islet Graft Transplanted in Autoimmune Diabetic Recipients

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Multiple studies reveal that the presence of islet autoimmunity in T1D patients is associated with accelerated recurrence of hyperglycemia following islet transplantation. However, how autoreactive T cells recognize allo-graft cells expressing non-self MHC has been unknown. Here we show that “dual-reactive” T cells, which are not only autoreactive but also are allo-reactive,

are a major player exaggerating rejection. We transplanted diabetic NOD mice (H-2^{g7}) with an MHC-mismatched islet transplant from C3H mice (H-2^{k}). To test whether islet-autoreactive T cells infiltrate allo-islet grafts, we determined T cell receptor (TCR) repertoires in residual endogenous islets as well as in allograft-infiltrates by 454 high-throughput sequencing. A certain portion of TCRs were shared between the endogenous islets and islet allograft-infiltrating T cells. Of note, three TCRs studied that are most frequent in the graft were also detected in the endogenous islets. As expected, all the three T cell lines expressing these TCRs responded to NOD islet cells. Surprisingly, all of them also responded to spleen cells harvested from C3H mice. Responses of two of the three TCRs were suppressed by the anti-class I (H-$2D^{k}$) antibody, and the other by the anti-class II (I-A^{k}) antibody. These results suggest that dual-reactive T cells expand and infiltrate allo-grafts efficiently, resulting in the exacerbation of rejection. To our knowledge, this is the first demonstration of autoreactive T cells isolated from a rejected islet allograft that show cross-reactivity to allogeneic MHC molecules expressed by graft cells. Therapies targeting such dual-reactive T cells may improve islet transplantation in T1D patients.

W.17. CIITA Silencing in Human Endothelium Limits CD8+ Memory T Cell Alloresponses by Preventing CD4+ Memory T Cell Help

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Solid organ transplantation, curative of end-stage kidney, liver, lung and heart failure, is limited by immunological rejection. Acute allograft rejection correlates with both the frequency of circulating alloantigen-reactive memory T cells and CD8+ cytotoxic T lymphocyte (CTL) infiltrates. Human vascular endothelial cells (EC) express class I and II MHC, costimulatory molecules and cytokines and can activate CD8+ and CD4+ memory but not naïve T cells. Consequently, rejection can be initiated within the graft by recruitment and differentiation of alloreactive memory T cells and is not dependent on graft professional APCs ("passenger leukocytes"). Cultured human EC supplemented with exogenous IL-2 can induce CD8+ memory T cells to differentiate into CTL. We show that siRNA silencing of the MHC class II transactivator (CIITA) protein in human EC reduces class II MHC without affecting class I or co-stimulators and prevents EC activation of CD4+ but not CD8+ memory T cells. However, addition of CD4+ T cells (but not more CD8+ T cells) to CD8+ T cell/EC co-cultures enhances CD8+ T cell proliferation and increases perforin expression, a marker of CTL differentiation. The CD4+ T cell effect is ablated by CIITA silencing in EC. Addition of medium from activated CD4+ T cells, like IL-2, enhances CD8+ T cell proliferation to EC and but pretreatment fails to "license" EC. We conclude that activation and differentiation of CD8+ memory T cells by allogeneic human EC is enhanced by products of activated CD4+ memory T cells, suggesting that targeting CD4+ T cell/EC interactions may reduce allograft rejection.

W.66. Study of Nrp-1 Expression in a Murine Skin Transplant Model

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Regulatory T cells (Tregs) modulate the immune system, suppressing harmful immune responses for the body, thus they are essential for immunological self-tolerance and homeostasis. Searching for Tregs surface markers remains a priority in the field of cellular therapies. It has been described that Neuropilin-1 (Nrp1) is preferentially expressed by thymus-derived Treg cells (tTregs), which exhibit the surface phenotype CD4+CD25^{hi}Nrp1^{hi} and express Foxp3. In this work, we analyzed the *ex vivo* expression of Nrp1 in different tissues from Foxp3/GFP reporter mice. Using a model of skin allograft rejection, we investigated the expression of Nrp1 on CD4+ T cells present in graft-draining lymph nodes (dLN), spleen and skin grafts from both, syngeneic- and allogeneic-grafted mice. Our *ex vivo* data indicate that 50-80% of CD4+CD25^{hi}Nrp1^{hi} T cells express Foxp3, which allows us to consider this population as Tregs. Ten days post-surgery, we found an increase in number and proportion of dLN-resident Nrp1+CD4+ T cells in allogeneic-grafted mice versus controls (syngeneic group). However, there is a significant decrease in number and proportion of dLN-resident CD4+CD25^{hi}Nrp1^{hi} T cells from allogeneic-grafted mice,

which downregulate Eos expression. Our findings corroborate Nrp1 expression on Tregs. Regarding cellular dynamics during rejection, a decrease in Nrp1+ Tregs and loss of Eos expression suggests that these cells might be loosing phenotypic stability. (Funded by CONICYT 791100001, FONDECYT 11121309).

W.67. Long-term Allograft Survival in Rapamycin-treated Mice Receiving Autologous Marrow Transplants

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CD200 overexpression in transgenic recipients, or by tissue allografts, increases skin and cardiac graft survival in mice receiving rapamycin, in association with increased Foxp3+Treg graft infiltration. However, <50% of skin grafts persist beyond 25d. We investigated whether immune ablation using busulphan/cyclophosphamide, followed by autologous marrow reconstitution, would permit long-term survival in a greater percentage of recipients, and induce tolerance allowing for withdrawal of immunosuppressive drugs.C57BL/6 control or $CD200^{tg}$ mice received BALB/c skin grafts with rapamycin (1.5mg/Kg/36hr) for 7 days. Thereafter subgroups received busulphan/cyclophospamide for 6 days, followed by C57BL/6 marrow transplantation (BMTx). Control groups received no busulphan/cyclophosphamide, but were maintained on rapamycin. Beginning 7d post marrow transplantation all mice received rapamycin for a further 21d (to 42d post transplant) after which immunosuppression was withdrawn. Graft survival was monitored throughout, and MLCs (using PBL) performed for all groups at 60d post transplant. Gene expression was performed in grafts harvested at 80d. While control BL/6 mice rejected grafts by 16d, survival in $CD200^{tg}$ was 40% at 60d, with antigen-specific decreased MLC responses to BALB/c. After BMTx, survival in control mice was >35% at 60d, and >90% at 60d in $CD200^{tg}$. Long-term surviving grafts were infiltrated by Tregs as monitored by immunostaining/PCR at 80d.Conclusion: Autologous BMTx improves graft survival and promotes tissue tolerance.

W.68. Transplant Tolerance to Minor Antigen Mismatched Bone Marrow Grafts using PLG Particles

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An antigen-specific tolerance therapy is highly desirable for cell/organ transplantation to avoid toxicities from life-long immunosuppression. Using antigen-loaded (surface coupled and encapsulated) biodegradable PLG particles, we have investigated transplant tolerance in a histocompatibility Y chromosome antigen (Hya) model of bone marrow transplantation, where Dby and Uty are the respective CD4 and CD8 male-specific minor Hy antigens. C57BL/6 CD45.2 female recipient mice were irradiated (non-lethal dose) one day before transplant of C57BL/6 CD45.1 male bone marrow cells (Day 0). Surface-coupled or encapsulated particles (Dby, Uty, Dby+Uty) were injected i.v. into recipients on Day -7 and/or Day +1, where the timing of doses and dose amount was investigated. The CD4 male-specific Dby epitope induced tolerance to minor HY antigen mismatched bone marrow grafts, both when coupled to the surface of PLG particles and encapsulated within PLG particles. The percentage of engrafted donor bone marrow cells in tolerized recipients was approximately 30% by 12 weeks post transplant, and both two doses (Day -7 and Day +1) and a single particle dose (Day +1) induced tolerance. Isolated splenocytes from tolerized recipients showed reduced proliferation and IFN-γ production in response to the Dby peptide compared to sham tolerized mice. Conversely, the CD8 Uty epitope did not induce tolerance, indicating that CD4 T cells are the primary mediator of graft rejection in this model. Using PLG particles as a platform for inducing tolerance in minor HY antigen mismatched bone marrow transplantation is a key step in developing a translatable, donor-specific tolerance strategy for modern transplantation procedures.

W.69. SERA miR-338-5p Diderentially Expressed in Renal Transplantation Recipients and Negatively Related with Soluble Baff

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Objective: In our previous miRNA array assay, miR-338-5p was profiled from renal allograft tissues, and verified, and deep investigation suggested that miR-338-5p may participate in renal allograft antibody-mediated rejection. So this study will investigate the expression characteristics of sera miR-338-5p in renal transplant (RTx) recipients, and further analyse its

potential significance. Methods: Serum of RTx recipients (49 cases) were collected. Sera miR-338-5p were extracted and detected by qPCR, soluble B cell activating factor (sBAFF) was determined by ELISA (R&D), and the presence of circulating anti- HLA Ⅰ & Ⅱ &MICA was determined by single-antigen flow bead assays (One Lambda) on a Luminex platform. Healthy volunteers (20 cases) were controls. SPSS17.0 software was applied, and $P<0.05$ was considered to be significant. Results: Compared with healthy controls, sera miR-338-5p in RTx recipients significantly decreased and sBAFF significantly increased ($P<0.05$). For all subjects, sera miR-338-5p was negatively correlated with sBAFF (r=-0.51, $P<0.001$). Compared with RTx recipients in <3-year post-operation, sera miR-338-5p significantly down-regulated in RTx recipients in ≥3-year post-operation ($P<0.05$), respectively. Compared with <3-year group, ①the positive incidence of anti-MICA and anti-HLA&MICA antibody all significantly increased in ≥3-year group ($P=0.005$, 0.046, respectively); ②miR-338-5p negatively significantly correlated with anti-HLA and anti-MICA antibody (r=-0.423, -0.411; $P=0.04$, 0.046, respectively). Anti-MICA and anti-HLA&MICA antibodies were also found to be significantly correlated with sBAFF ($P<0.05$) in RTx recipients with MFI>1,000 group. Conclusions: miR-338-5p should involve in the procedure of immune response after RTx by indirectly or directly targeting BAFF signal.

W.70. Characterization of Myeloid-derived Suppressor Cells (MDSC) in Corneal Allograft Survival

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Myeloid-derived suppressor cell (MDSC)s are negative regulators of the immune response and are in part responsible for the inhibition of organ transplantation rejection. While MDSC have been demonstrated to participate in the induction of prolonged allograft survival in many allo-transplantation models, little is known about their function and characterization in corneal allograft. Here, we investigated the surface markers and cytokine expressions of MDSC by murine corneal allograft model. Standard protocols for murine orthotopic corneal transplantation were used as described previously. Briefly, donor center corneas (2-mm diameter) were excised from C57BL/6 mice and B6.CgTg(CAG-mRFP1)1F1Hadj/J mice. Then, the donor cornea was sutured on to the recipient graft beds prepared by excising a 2.0 mm site in the central cornea of BALB/c mice for allogeneic and B6 mice for syngeneic graft. Simultaneously, some BALB/c mice received syngeneic (BALB/c) grafts to control for the non-allospecific effects of surgery. All grafts were evaluated using slit lamp biomicroscopy at three-day intervals for two weeks and then weekly intervals thereafter for eight weeks. By flow analysis, $Gr1^{INT}CD11b^{+}$ cells were highly infiltrated in accepted allograft. However, $Gr1^{HI}CD11b+$ cells were not showed difference between accepter and rejecter. Corneal allograft specific MDSCs were appeared from postoperative day 5 and maximized their population in the graft stroma at 2weeks after surgery. In addition $Gr1^{INT}CD11b^{+}$ cells, not $Gr1^{HI}CD11b^{+}$, were expressed interferon-γ, IL-10, and TGF-β. To enhance allograft survival, chemoattraction of $Gr1^{INT}CD11b^{+}$ MDSC in early postoperative period may be a new strategy for modulating immunity in corneal transplantation.

W.71. Association of Polymorphisms -308 G/A of *TNF-α* and -383 A/C of *TNFR1* in Patients with Acute and Chronic Kidney Transplant Rejection in Western-Mexican Patients

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INTRODUCTION**.** Serum levels of TNF-α are elevated in patients with acute organ transplant rejection. Inflammatory effect of TNF-α is regulated by soluble receptors such as TNFR1. Polymorphisms -308 G/A of *TNF-α* and -383 A/C of *TNFR1* have been associated with inflammatory chronic diseases in diverse populations. AIM. To determinate the association of the polymorphisms -308 G/A of *TNF-α* and -383 A/C of *TNFR1* in Western-Mexican patients with episodes of kidney transplants rejection. MATERIALS AND METHODS. 85 cases of kidney transplant and 85 clinically healthy subjects were included, all from Western-Mexico and older than 18 years old. Genotyping of polymorphisms -308 G/A and -383 A/C of *TNF-α* gene were identified by PCR-RFLPs. Statistical analysis was made with program EPI-INFO 7.1.2, statistical level of p<0.05 RESULTS. Genotypic distributions of polymorphisms -308 G/A of TNF-α and -383 A/C of TNFR1 in healthy subjects were in Hardy-Weinberg's equilibrium. Genotypes G/G and A/C of polymorphisms -308 G/A and -383 A/C respectively, were found to be the most frequent. No significant statistic differences in genotypic and allelic frequencies of both study groups were found

(p>0.05). CONCLUSIONS. Allelic and genotypic frequencies of polymorphisms -308 G/A of *TNF-α* and -383 A/C of *TNFR1* were not associated with episodes of kidney transplant rejection.

W.72. Loss of Protein Tyrosine Phosphatase PTPN22 Increases Transplant Tolerance

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PTPN22 encodes for a protein tyrosine phosphatase that is involved in several immune responses to self and foreign antigens. Thus far, several conflicting reports have obscured how PTPN22 can serve as therapeutic target to harness autoimmunity and perhaps other immune-mediated diseases such as allograft rejection. Here we identified PTPN22 as a regulator of type 1 regulatory (Tr1) cell homeostasis and function with immediate implications for transplant tolerance. We found that in a mouse model of pancreatic islet transplantation, PTPN22 loss although increased the number of alloreactive T cells, it did not alter the kinetics of allograft rejection. Given that loss of PTPN22 results in an increased number of FOXP3 Treg cells, we monitored allograft rejection after Treg depletion. We found that PTPN22-deficient mice are resistant to allograft rejection in the absence of FOXP3 Tregs due to augmented frequency and number of Tr1 cells. Furthermore, we evaluated whether tolerance can be achieved in the absence of PTPN22 following an established tolerogenic treatment. Increased transplant tolerance could be achieved in PTPN22-deficient mice through the recompensed action of $FOXP3^+$ Treg and Tr1 cells. Moreover, Tr1 cells from tolerant PTPN22-deficient were more efficient at transferring transplant tolerance as compared to wild-type. Therefore, these findings establish PTPN22 as key regulator of allograft rejection and identify PTPN22 as possible candidate for promoting Tr1 cells for transplant tolerance.

W.73. Immune Mechanisms Underlying the Tolerogenic Effects of Intraperitoneal Injection of IDO-Expressing Skin Fibroblasts

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Introduction. We aimed to see whether the immune tolerance induced by intra-peritoneal (IP) injection of indolamine 2,3 dioxygenase (IDO)-expressing skin fibroblasts is based on generation of tolerogenic antigen presenting cells at the site of injection. Methods. Ten million C57BL/6 (B6) fibroblasts, B6 IDO-fibroblasts, B6 splenocytes and C3H/HeJ (C3H) fibroblasts were injected into the peritoneal cavity of C3H recipient mice. After 2 and 9 days, the peritoneal lavage (PL) cells were checked regarding the expression of co-stimulatory (CD80) and co-inhibitory molecules (PD-L1, PD-L2 and B7H4) on dendritic cells (DCs) and macrophages. PL cells of recipient mice were cultured and their ability to generate regulatory T cells (Tregs) in vitro was assessed. After 9 days, Tregs were evaluated within the spleen, mesenteric lymph node (MLN) and axillary lymph node (ALN) of recipient mice. Results. DCs and macrophages within the PL of fibroblast-treated groups expressed a significantly higher ratio of co-inhibitory/co-stimulatory molecules compared to splenocyte-treated and non-treated groups. In vitro, PL cells of fibroblast-treated groups significantly enhanced the percentage of Tregs. There was no significant difference between allogenic vs. syngenic and regular vs. IDO-expressing fibroblasts. However, the percentage of Tregs in MLN and ALN of recipient mice only increased in IDO-fibroblast-treated group. Conclusion. IP-injection of fibroblasts induces tolerogenic DCs and macrophages. While these cells are able to induce Tregs in vitro, presence of IDO is essential for Treg induction in vivo.

W.74. Nutrient Deprivation Contribute to Peri-Transplant Islet Death

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Islet transplantation is a minimally invasive curative therapy for type 1 diabetes, but often requires more than one donor to achieve insulin independence. One of the reasons for the need for multiple donors is peri-transplant islet death, leading to gradual attrition of the remaining islets due to overwork and exhaustion. In this study, we have investigated factors that contribute to islet death. We transplanted islets expressing a firefly luciferase under the control of the mouse insulin 1 promoter, so that functional islet mass could be monitored using bioluminescence imaging in the same animals over time. We

found that the density of transplanted islets in the kidney capsule graft bed was directly proportional to the extent of islet death in syngeneic recipients. High-density transplants resulted in 80-90% islet death within the first 5 days, whereas islets transplanted in wider areas led to significant improvement of islet survival with greater than 50% preservation of the islet mass. The impact of density on islet survival was also seen in in vitro cultures of mouse and human islets under normal oxygen tension, suggesting factors other than hypoxia contributed to islet death. We detected increased LC3+ autophagosomes in β cells cultured at high density, consistent with nutrient deprivation. Supplementation of glutamine in high-density cultures prevented islet death. These results demonstrate a previous unrecognized factor of nutrient deprivation exacerbated by high-density transplant procedures. Interventions that protect islets from nutrient deprivation may reduce the demand for high islet mass and improve therapeutic efficacy of islet transplantation.

W.75. Generation of Humanized Liver Mouse Model by Transplanted of Patient-derived Fresh Human Hepatocytes

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In the present, some research groups have produced immunodeficient mice with human liver tissue as a model system for the analysis of drug metabolism and liver regeneration. Mouse models are important for research and development of drugs and vaccines for viral infections. Recent progress in developing humanized mouse models permit studies of adaptive immune responses, innate host responses, and therapeutic approaches for several liver diseases of viral etiology. In this study, we generated a humanized liver mouse model by transplanted with fresh patient-derived hepatocytes (1 x 10^6 cells/mouse, intrasplenic injection) into pre-conditioned (50 mg/kg ganciclovir, intraperitoneal injection) TK-NOG mice. Successfully reconstitution of human hepatocytes in TK-NOG mouse liver tissues was observed with strong proliferation of human cells in a time-dependent manner, using cytokeratin 8/18 stain. Similarly, we detected significantly increased human albumin levels in TK-NOG mouse liver tissue and blood sera on immune staining and ELISA. Therefore, this humanized liver mouse model provides biomedical tool for studying human liver physiology, drug metabolism, and liver pathogenesis of viral etiology or liver regeneration.

W. 102 Functionally Impaired Maternally-Derived Memory T cell Engraftment in an Infant Harboring a Novel IL-2 Receptor Common Gamma Chain (CD132) Mutation

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X-linked severe combined immunodeficiency develops in roughly 1:50,000-100,000 births affecting males of virtually every ethnicity equally. The underlying defect is a functionally crippling mutation in the signal-transducing subunit (common gamma-chain) that is shared among immunoregulatory cytokines such as IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21. As IL-7 and IL-15 are required for the maintenance of peripheral T cells and NK cells, aberrant signaling results in a numerical deficit of these key lymphocyte subsets. We recently evaluated a 6 month old infant with a recent onset of respiratory and middle-ear infections that required intravenous antibiotics and ventilatory support at a community hospital. He also experienced persistent watery diarrhea for 5 weeks and a preliminary immunologic work up revealed barely detectable serum immunoglobulins. At our institution he received a full immunological work-up which confirmed his hypogammaglobulinemia and also revealed severely depressed NK cell numbers. Additionally we detected markedly discrepant frequencies of memory-phenotype T and B cell subsets in his peripheral blood (>90% of T displayed a memory phenotype while only 3% of his B cells expressed memory markers). Cytogenetic, molecular and functional analyses revealed the T cell subset to be entirely of maternal origin and unable to respond optimally to mitogenic stimuli. Gene sequencing uncovered a novel mis-sense mutation in the extracellular domain of the gene encoding CD132 (185 G>A). A stem-cell transplant was performed that led to the numerical and functional restoration of normal immunologic parameters and resolution of clinical symptoms.